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Article

Effect of pancreatin on acute pancreatitis resulting from L-arginine administration in mice, a morpho-histopathological and biochemical study

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Acute pancreatitis (AP) is a life-unpleasant situation with contradictory and inadequate treatments. In this regard, the present study evaluated the effect of the possible pretreatment of lipase-pancreatin on L-arginine-induced AP. Forty adult mice were selected and divided into five groups: I) control group, II and III) AP groups (i.p.) receiving L-arginine of 2×300 and 2×400 mg/100 g body weight (b.w.), IV) AP (2×300 L-arginine) group + pancreatin (mice were i.p. injected by 350 U-lipase), and V) AP (2×400 L-arginine) group + pancreatin (mice were i.p. injected by 350 U-lipase). All AP groups displayed a significant increase in serum levels of ALT, AST, TBARS, and TNF-alpha compared to the control group. Moreover, pancreatic tissue edema, inflammation, and vacuolization of acinar cells were significantly higher in the untreated L-arginine group compared to the control and pancreatin groups. Conversely, the diameter of pancreatic islets significantly declined after induction of pancreatitis compared with control and pancreatin groups. Pancreatin treatment can be used in pancreatic dysfunction, however, this medicine showed no protective effect against L-arginine-induced AP in the mouse model.

Keywords: Mice. Morphology. Biochemical parameters. Histopathology. Islands diameter.

Abbreviations: AP, acute pancreatitis; i.p., intraperitoneal; b.w., body weight; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TNF-alpha, tumor necrosis factor-alpha; TBARS, thiobarbituric acid reactive substances; U, unit; fipu, Fédération International Pharmaceutique Unit.

INTRODUCTION

AP is a serious health-threatening disease whose incidence rate has gradually increased worldwide (Abdelzaher *et al.*, 2020; Mederos, Reber, Girgis, 2021; Mirmalek *et al.*, 2016). Systemic inflammatory response and limb dysfunction syndrome resulting from AP eventually lead to fibrosis and subsequent organ failure (Abu-Hilal *et al.*, 2006; Melo *et al.*, 2010). Complexity in the pathophysiology of AP shows the involvement of numerous inflammatory pathways in the occurrence of this disease. Many reasons suggest the fundamental role of oxygen radicals in the pathophysiology of AP; however, some active sites of reactive oxygen species (ROS) are related to the pathogenesis of AP. ROS are overproduced in AP and inflammatory reactions through tissue necrosis pathways which may eventually lead to increased amylase and lipase activity and zymogen degranulation (Abu-Hilal *et al.*, 2006). Some studies have evaluated the

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relation between pancreatic inflammation and oxidative alterations. These findings show the incidence of pancreatic oxidative stress during the early stages of AP induction. Oxidative stress is severely elicited following the increase in the production of ROS and irregularity created in the antioxidant capacity balance (Abdelzaher *et al*., 2020; Sindhu *et al*., 2005; Vaziri *et al*., 2007).

It has been assumed that free-radical oxygen scavengers have a clinically protective effect and could be efficient in patients with AP (Fusco *et al*., 2020; Swentek, Chung, Ichii, 2021; Zhang *et al*., 2012). Oxidative stress caused by acute pancreatitis accelerates the inflammatory process and disturbs cellular metabolism regulation by activating more complex inflammatory processes, leading to cell death (Gukovsky *et al*., 2013; Pérez *et al*., 2015). Experimental investigations have shown the extensive production of pro-inflammatory mediators such as tumor necrosis factor (TNF) -α, nitric oxide (NO), adhesion molecules, and interleukin (IL) -1β, as inflammation precursors, during AP in addition to ROS (Abdel-Gawad, 2015). Inflammatory mediators amplify the inflammatory cytokine cascade and expand AP from localized disease to a problematic systemic disease (Bhatia *et al*., 2000; Makhija, Kingsnorth, 2002).

The possible *benefits* of the pancreatic enzyme supplementation (pancreatin) in managing exocrine pancreatic insufficiency, acute and chronic pancreatitis, pancreatectomy, pancreatic duct obstruction, and pancreatic cancer have been indicated (Ianiro *et al*., 2016; Johnson, Hillier, 2011).

The first L-arginine induction model in mice (2×4 g/1000 g, i.p.) was experimentally proposed by Dawra *et al*., (2007) for the development of AP. L-arginine (a type of amino acid) has been increasingly used as a model to induce AP due to its unique properties such as cost-effectiveness and non-invasiveness as well as a feasible injection for AP induction (Dawra, Saluja, 2012; Kui *et al*., 2014). L-arginine can stimulate the production of oxygen and nitrogen free radicals and eliminate the cell membrane of zymogen granules, thus increasing the secretion of digestive enzymes and the rate of inflammatory mediators (El-Ashmawy *et al*., 2018).

Stereology is the first selective method capable of predicting three-dimensional information of structural values (volume, surface, diameter, length, and number) in different tissue components (Fatahian Dehkordi, Hamid, 2015; Mayhew, 1991; Mayhew, 1992). Qualitative variables such as hypoplasia or hypertrophy and atrophy can be studied through stereological techniques and expressed as quantitative data. Therefore, the values obtained from the stereological evaluation can be explored to provide information.

Regarding the recently documented effect of pancreatic enzyme supplements on the outcome of acute pancreatitis (Kahl *et al*., 2014), the present study is aimed to investigate the effect of pancreatin on L-arginine-induced AP by determining the levels of oxidative stress parameters (TBARS, AST, and ALT) along with the assessment of pancreatic morpho-histopathological changes.

MATERIAL AND METHODS

Animals

Forty adult male BALB/C mice weighing 28–32 g was used in this study. The animals were fed by commercial mouse food and tap water, and in a standard temperature (around 21 °C) and humidity conditions (21–33%) with a 12–12 h light-dark-cycle were maintained. 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.

Material

L-arginine hydrochloride powder (Sigma, UT, USA) was dissolved in 0.9% saline and set at a pH=7.4 with 0.1 M sodium hydroxide (NaOH) solution. Before each injection, the fresh solution was prepared from L-arginine. The mouse model of acute pancreatitis was developed by intraperitoneal injections (i.p.) of L-arginine hydrochloride. Two different doses of 2×300 and 2×400 mg/100 g of L-arginine were established for administration to mice via i.p. injection (at an interval of 2 h). Control animals were injected i.p. as a placebo with normal saline. Several samples were selected and

after tissue slide preparation, pancreatitis was confirmed by a pathologist microscopically.

After experimental induction of pancreatitis (72 h), the pancreatin (amylase 6500 fipu + lipase 8000 fipu+ protease 450 fipu) was used, 350 units of lipase-pancreatin (0.1 mg/g) 1% and injected twice a day by i.p.

Grouping

The mice (8/group) were randomly divided into the following groups:

Group I: the mice were injected (i.p.) with saline as a placebo (control group).
Group II: the exposed group with L-arginine, 2×300 mg/100g (at an interval of 2 h).
Group III: the exposed group with L-arginine, 2×400 mg/100g (at an interval of 2 h).
Group IV: treatment group of L-arginine 2×300 mg/100g along with pancreatin (350 U-lipase).
Group V: treatment group of L-arginine 2×400 mg/100g along with pancreatin (350 U-lipase).

At the end of the experiment and 6 hours after the last treatment, blood samples were collected from the heart under general anesthesia. Blood sampling was performed to determine levels of TNF-alpha, ALT, AST, and TBARS. The samples were centrifugated for 10 minutes at 3000 rpm at 4 °C using a Universal centrifuge (Hettich, Tuttlingen, Germany) set. The obtained clear sera were stored at -70 °C until use. In addition, pancreatic tissue was removed by laparotomy after dissection of surrounding tissues. Specimens trimmed and fixed in 10% neutral buffered formalin solution for histopathological examinations and the microscopic serially slides were stained with H&E. Finally, the pathological analysis was performed under light microscopy by an expert pathologist.

TNF-α assay

The serum TNF-alpha concentration was determined by enzyme-linked immunosorbent assay using Mouse/Rat TNF-α ELISA Kit (Diaclone SAS, mAbexperts group Company - France). The kit includes an assay range of 31.25 pg/ml-1000 pg/ml and a highly sensitive 25 pg/ml.

Diameter of pancreatic islets histologically

Preparation of tissue sections was performed using morphological techniques. Thus, the quantitative technique was based on the serial sections and according to our previous study (Amiri *et al*., 2018) on the random selection protocol from sections to measure the diameter of pancreatic islets. The mean diameter of the islets was measured by tracing the cross-sectional boundary of the pancreatic islets with the pancreatic exocrine glands. Since the islands lacked a regular geometric shape, the mean of the smallest and largest diameter was considered as average diameter, and then measured diameter was introduced as the final diameter.

Pathology examinations

Pancreatic histology slides were prepared according to the process of procurement the tissue sections and stained with hematoxylin and eosin. Pancreatic sections were evaluated by an intervening pathologist according to the experiment protocol. The extent of the injuries was interpreted based on edema, inflammation and histopathological changes.

ALT activity assay:

ALT activity was measured by using Pars Azmoon kit (Tehran, Iran). The reaction is evaluated based on the transferring of an amino group from alanine to α-ketoglutarate resulting in the production of glutamate and pyruvate. Then, in parallel reaction, the measurement of the absorbance change of NADH concentration at 340 nm is assayed based on the pyruvate reaction with lactate dehydrogenase (LDH). ALT activity in serum samples was measured as U/L.

AST activity assay:

AST activity was measured by using Pars Azmoon kit (Tehran, Iran). The reaction is based on the reversible

transamination between aspartate and α-ketoglutarate to form glutamate and oxaloacetate. AST activity is assayed by monitoring the rate of NADH oxidation at 340 nm in the presence of oxaloacetate and malate dehydrogenase (MDH). AST activity was measured as U/L.

Thiobarbituric Acid Reactive Substances Assay

The measurement of Thiobarbituric Acid Reactive Substances (TBARS) was performed by the method of Olatosin *et al.*, (2014). Briefly, a stock solution of trichloroacetic acid (TCA), thiobarbituric acid (TBA) and Hydrochloric acid (HCl) containing 15g TCA, 0.375g TBA and 0.25N HCl was prepared. 2ml of TCA-TBA-HCl mixture was added to the 1 ml of serum. The mixture was placed in boiling water for 50 min, cooled to room temperature and centrifuged at 1000 rpm for 10 min. Thereafter, the absorbance of the supernatant was read at the wavelength of 535 nm against blank reference. TBARS levels were expressed as nmol/ml.

Statistical analysis

To compare data between treatment and control groups, SPSS software version 23 was used. Data analysis was performed using One-Way Analysis of Variance (ANOVA), followed by LSD test as a post-hoc test and were calculated as means ± SD. Significant differences were observed between the groups at a 95% probability level with a significant level of $P<0.05$.

RESULTS AND DISCUSSION

Diameter of Pancreatic islets

In this investigation, the mean diameter of islets (µm) showed a statistically significant difference among the groups as shown in Table I. L-arginine groups (2×300 and 400) significantly reduced the mean diameter of islets by 103.97±24.8 and 121.89±23.6 respectively compared to the control group 149.24±23.5 (*P<0.05*). Whereas, experimental groups treated with pancreatin (350 units, groups IV and V) exhibited higher diameter of islets compared with untreated pancreatitis groups (groups II and III); however, there was no statistically significant difference between groups IV and V compared to groups II and III (*p>0.05*) (Figure 1).

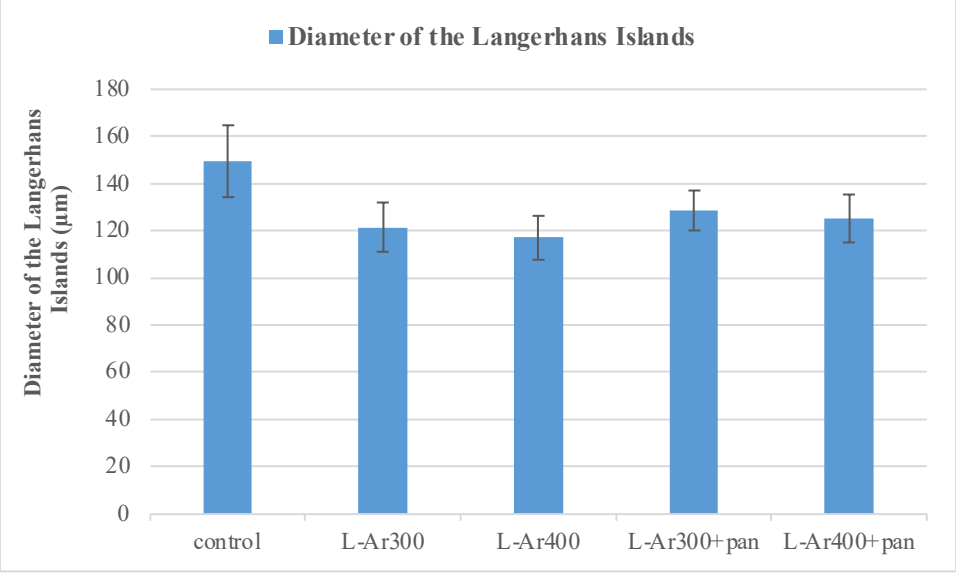

FIGURE 1 - Diameter of the langerhance islands in pancreas spacimens of control, pancreatitis and pancreatine-treated pancreatitis groups.*Significant difference with the control group at p level less than 0.05. L-Ar300: L-Arginine 2×300 mg/100; L-Ar400: L-Arginine 2×400 mg/100; pan: pancreatine.

Histopathological changes

As shown in figures 2 and 3, histopathological appraisal of the pancreatic tissue from treated and untreated L-arginine groups (groups II to V) showed fluid accumulation, disturbance of tissue architecture, vacuolization of acinar cells, edema and infiltration of inflammatory cells (neutrophils). The observations also showed that the pancreatic islets appeared to be smaller (shrinkaged) compared to the control group. Besides, there were mild histopathology changes in the pancreas in L-arginine plus pancreatin-treated groups.

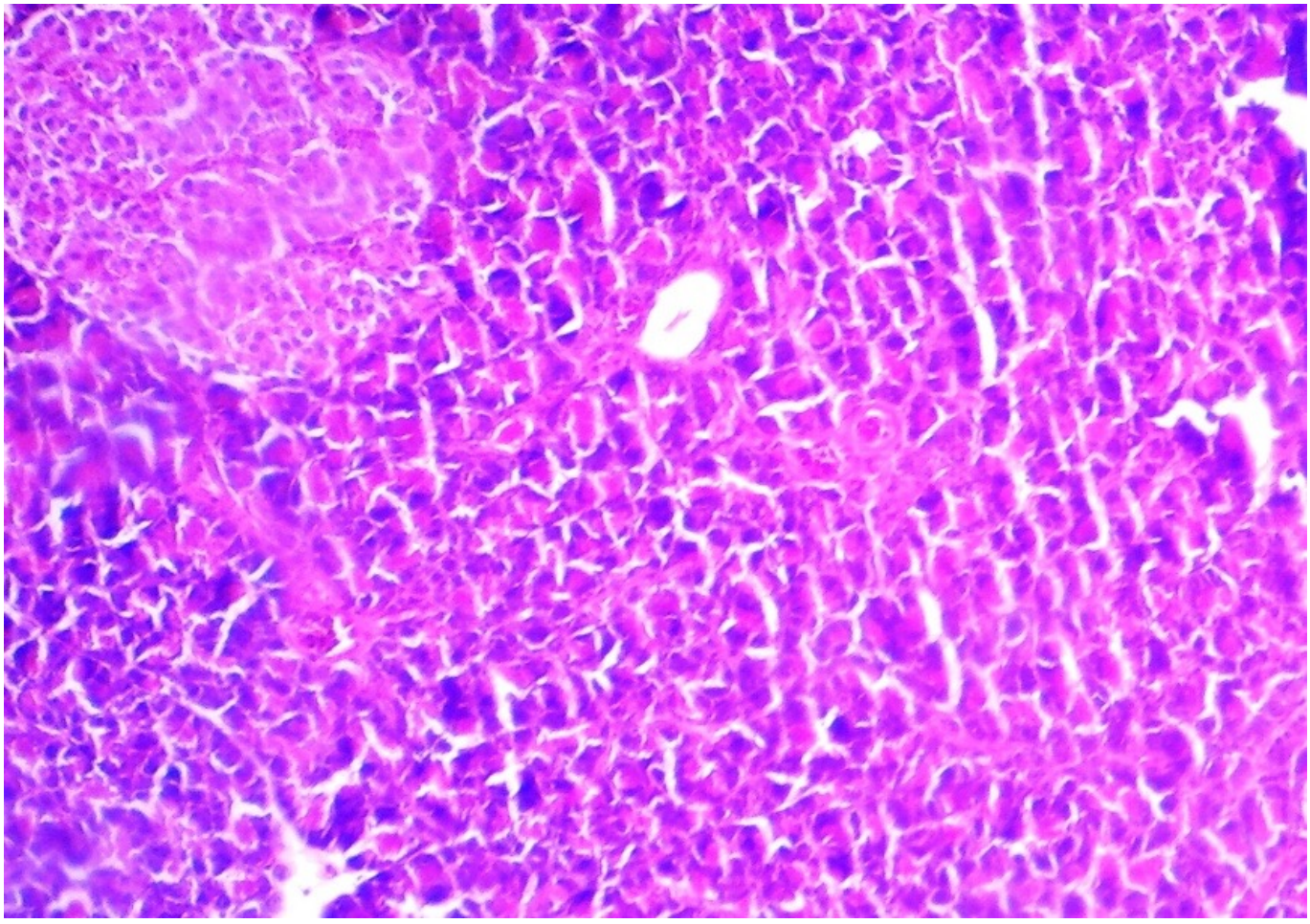

FIGURE 2 - Pancreas photomicrograph in the control group. It shows a normal slide of pancreatic islands and acini without pathologic alterations; (H&E "hematoxylin and eosin", magnification was 20×).

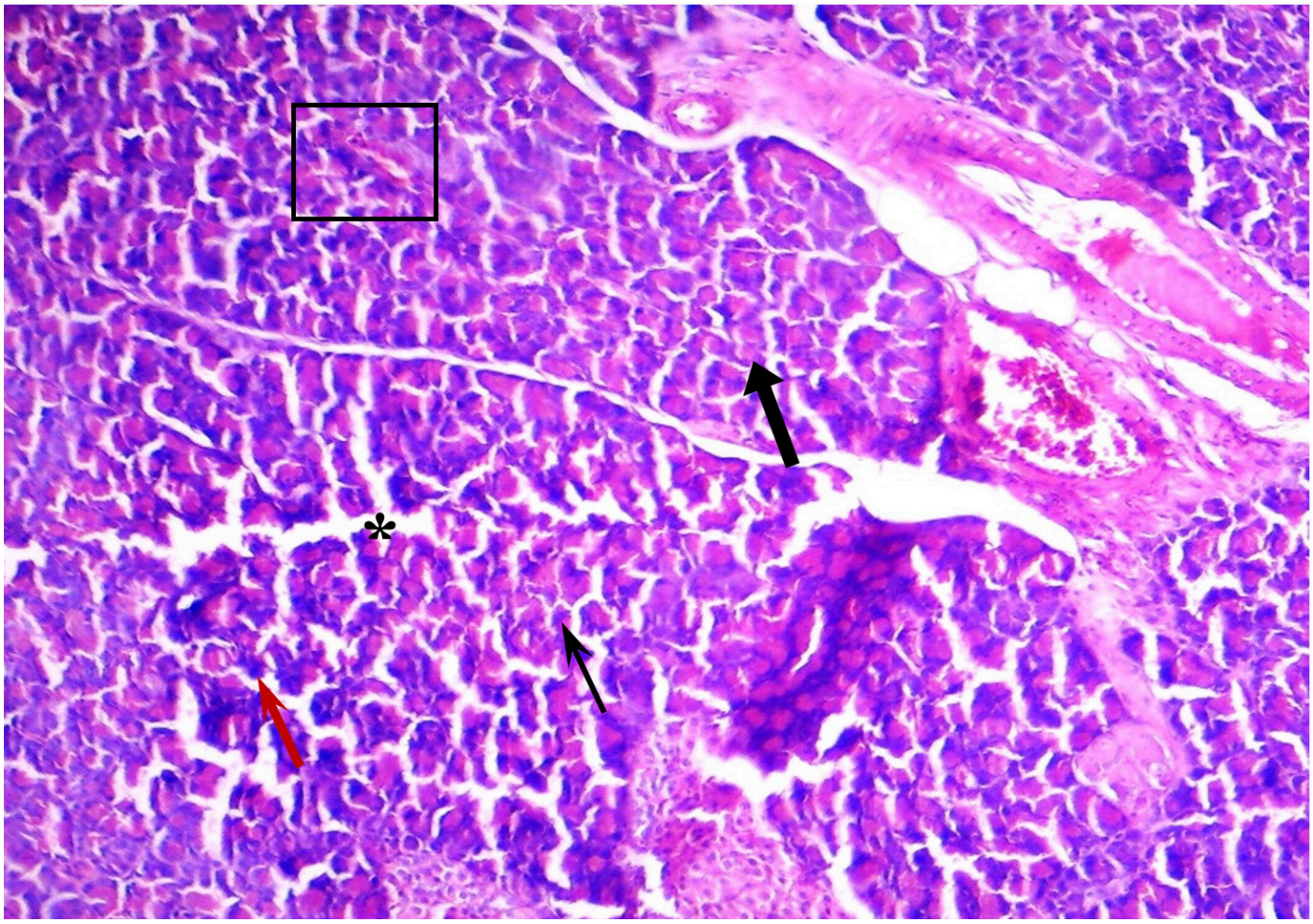

FIGURE 3 - Pancreas photomicrograph in L-arginine-induced pancreatitis group. A slide showing pancreatitis in the form of a disorder in the architecture of the pancreas, meaningful interstitial edema marked with a black asterisk, necrosis of the acinar cell marked with the black arrow, extensive infiltration of inflammatory cells marked with red arrows, vacuolation of acinar cells marked by bold arrows, small areas of bleeding marked with a square; (H&E "hematoxylin and eosin", magnification was 20×).

TNF-alpha level

The progression of primary injury to the exocrine pancreas occurs in the local and systemic inflammatory response, resulting in AP (Hegyi *et al.*, 2011). Hence, the serum level of pancreatic TNF-α was determined using ELISA in all experimental groups. It was shown that the blood serum TNF-α level of mice (Figure 4) with AP (groups II and III) were significantly increased in comparison with the mice in the control group ($p<0.05$). Pancreatin in treated groups (the last two groups) decreased the serum TNF-α level of mice with AP, although, these differences were not significant (P>0.05). In addition, it was found that there was a significant difference between the pancreatitis groups treated with pancreatin compared to the control group ($P<0.05$).

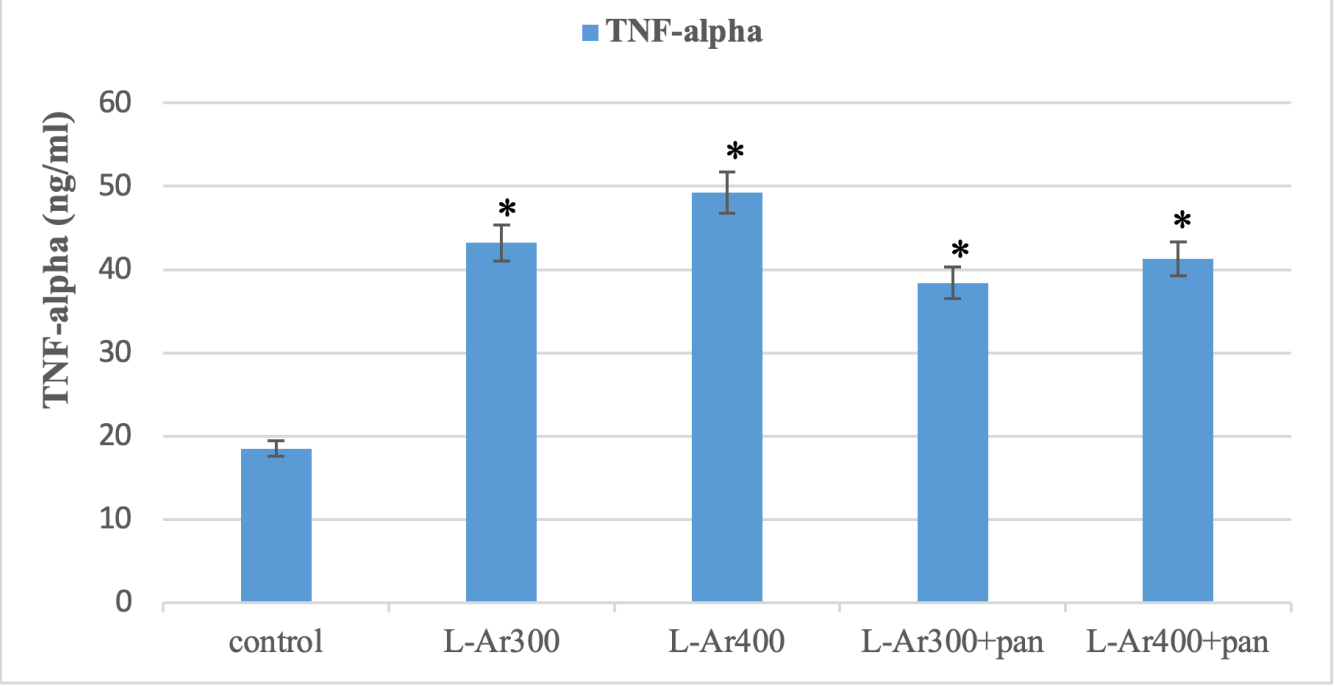

FIGURE 4 - TNF-α level in serum specimens of control, pancreatitis and pancreatine-treated pancreatitis groups.*Significant difference with the control group at p level less than 0.05. L-Ar300: L-Arginine 2×300 mg/100; L-Ar400: L-Arginine 2×400 mg/100; pan: pancreatine.

ALT and AST

According to Table I, comparison of the mean serum levels of ALT and AST between the different groups showed that parameters mentioned were significantly increased in the L-arginine groups 2×300 and 400 (group II and III) compared to the other groups (*P<0.05*). Nevertheless, the levels of ALT and AST parameters when were compared among the groups, showed a decrease non-significant in treated pancreatitis groups by pancreatin than to untreated L-arginine groups (*P>0.05*). Although the pancreatin-treated groups showed a decrease compared to the untreated pancreatitis groups, however, increasing significance in the pancreatin-treated group compared with the control group was quite evident (*P<0.05*), in fact, pancreatin could not show a healing effect.

TABLE I - Effect of pancreatin on ALT and AST level (IU/L) in L-arginine-induced pancreatitis mice

	Groups (mg/100g B.W.)	ALT	AST
I	control	21.3±2.8[a]	32.5±3.3[a]
II	L-Arginine (2×300)	48.7±4.5[b]	61.1±2.0[b]
III	L-Arginine (2×400)	50.8±3.7[b]	64.4±3.1[b]
IV	L-Arginine (2×300) + pancreatin (350 U)	47.4±2.6[b]	57.2±2.7[b]
V	L-Arginine (2×400) + pancreatin (350 U)	48.4±3.2[b]	60.2±3.4[b]

[a, b, c] Values with different superscripts in each parameter differed significant at p<0.05 than to control.

TBARS

Table II, displays that in serum isolated from the untreated pancreatitis mice (groups II and III), TBARS values were significantly higher than the control group and those from pancreatin-treated mice (groups IV and V). Whereas, pancreatin treatment decreased TBARS values non-significantly in serum from the treated pancreatitis mice compared with the untreated pancreatitis mice ($P>0.05$). Despite the decreasing effects of pancreatin treatment groups in TBARS values compared to L-arginine-induced pancreatitis groups, however, there was still a significant increase in TBARS values in pancreatin treatment group compared to the control group ($P<0.05$).

TABLE II - Effect of pancreatin on TBARS level (μm) in L-arginine-induced pancreatitis mice

TBARS		L-Arginine 2×300 (group II)	L-Arginine 2×400 (group III)	L-Arginine 2×300 + pancreatin (group IV)	L-Arginine 2×400 + pancreatin (group V)
Control (*n=8*) (group I)	3.32[a] (3.20-3.47)				
Treatments (n=32)		5.47[b] 5.22-5.73	6.14[b] 6.08-6.23	5.12[b] 4.87-5.23	6.05[b] 5.95-6.23

[a, b] Values with different superscripts differed significant at $p<0.05$.

AP is an invasive inflammation of the pancreas following pancreatic dysfunction. Regarding its increasing incidence, AP is considered common acute damage (El Morsy, Ahmed, 2020; Mirmalek *et al.*, 2016). In this regard, remarkable attention has been paid to cell biology, pathophysiology, and cell damage mechanisms in AP. The present experimental study histopathologically and biochemically evaluated the effect of lipase-pancreatin to show the possible treatment of pancreatin on L-arginine-induced pancreatitis.

The generation of Reactive Oxygen Species (ROS) and as a result, oxidative stress is one of the most important challenges in AP (Pasari *et al.*, 2019). The present study showed a significant difference in serum TBARS, ALT, AST, and TNF-alpha levels in the L-arginine-induced pancreatitis groups as compared with the controls and a non-significant difference in pancreatin treatment groups. As observed in numerous studies, excessive concentrations of amino acids (often L-arginine) can seriously damage the structure of the pancreas through an unknown mechanism (Biczó *et al.*, 2011; Kui *et al.*, 2014). Nevertheless, L-arginine-induced pancreatitis is considered a well-defined pattern in the development of pancreatitis that resembles those of AP in humans (Zhang *et al.*, 2019). L-arginine induces AP as a nitric oxide donor through activation of nitric oxide synthetase, leading to endoplasmic reticulum stress and free radical-induced oxidative stress (Abdelzaher *et al.*, 2020; Aziz, Kamel, Rifaai, 2017). These mechanisms are in line with the findings of other researchers, who introduced pancreatitis-induced oxidative stress as the basis of AP disease (Aziz, Kamel, Rifaai, 2017; El Morsy, Ahmed, 2020; Mirmalek *et al.*, 2016). The pathophysiology of AP is highly complex involving damage to the intracellular and extracellular structure; the generation of free radicals results in the development of AP (Affourtit, Jastroch, Brand, 2011; Hernández *et al.*, 2011). The pancreatic tissue is vulnerable to oxidative stress due to the poor expression of antioxidant enzymes in pancreatic islet cells, and hence its poor antioxidant capacity (Miki *et al.*, 2018). Following AP, the oxygen-free radicals disrupt pancreatitis acinar cell damage and play a key role in pancreatic dysfunction (Abdelzaher *et al.*, 2020).

The intraperitoneal injection of L-arginine solution to intact mice showed an increase in serum levels of TBARS, ALT, AST, and TNF-alpha; however, the intraperitoneal administration of pancreatin led to a partial reduction in

the mentioned parameters (*P>0.05*). Collectively, it does not seem that pancreatin can even partially control the destructive effects of pancreatitis; thus, it does not play a significant role in reducing the effects of antioxidant disruptors in this study. Following the induction of AP, microvascular failure in the pancreas leads to ischemia and inflammation (Yenicerioglu *et al.*, 2013). With the increase of the inflammation severity, the accumulation of toxic mediators becomes noticeable in the pancreatic tissue. Monocytes and macrophages begin to secrete cytokines with the spread of inflammation. Reactive oxygen intermediates (ROI) generated by macrophages also contribute to the increased oxidative stress, an additional destructive intermediary in the increasing aggressiveness of pancreatitis (Shrivastava, Bhatia, 2010). Therefore, plasma levels of some cytokines are elevated in patients suffering from severe AP (Hu *et al.*, 2020).

Given this anatomical relationship and the proximity of the bloodstream, the distribution of oxygen free radicals in pancreatitis enters the liver, triggering the process of liver damage (Abdelzaher *et al.*, 2020). According to these findings, it can be inferred that the development of inflammation leads to an increase in the level of AST, and ALT, as well as, an increment in pro-inflammatory cytokines (TNF-α). TIBARS, a marker of lipid peroxidation, was also enhanced in L-arginine-treated mice. Lipid peroxidation produced by steps of the free-radical mediated chain reaction impairs the functional and structural integrity of membranes (Biradar, Veeresh, 2012). This could be due to the accumulation of L-arginine-produced free radicals. Hernández *et al.*, (2011) in their study showed that in severe AP there are high degrees of lipid peroxidation.

The other parameter measured was the elliptical and Feret's diameters for each of the pancreatic islets. The elliptical diameter was a representation of an elliptical model from the diameter of the islands. The Feret's diameter in a structure was the longest distance (largest distance) from a given origin (Dražić, Sladoje, Lindblad, 2016). In the current work, morphometrical assessment of the diameter of pancreatic islets showed a significant reduction in L-arginine-induced pancreatitis groups compared to the control group. L-arginine induced a dramatic decrease in islet diameter in a dose-dependent mode. The pancreatic islets appear to be shrunken and this may be the main reason for the decrease in the diameter of the islets in L-arginine-induced pancreatitis groups. Therefore, L-arginine negatively affects the structure of the pancreas. These findings were accordant with the results obtained from Wahba *et al.*, (2016) investigations, which showed that the diameter of pancreatic islets after L-arginine-induced pancreatitis had reduced. Abdelzaher *et al.*, (2021) also found a significant decrease in the mean diameter of pancreatic islets and concluded that the degeneration of the pancreatic islets is one of the reasons for the decrease in the diameter of the islands.

CONCLUSION

This study was carried out on mice suffering from AP induced by L-arginine and pancreatin administration. The results indicated that pancreatin did not play an impressive role in protecting the pancreas of mice with AP. However, pancreatin showed some remarkably subtractive effects on the evaluated parameters in contrast to the L-arginine-induced pancreatitis group. Administration of pancreatin as an enzymatic-gastrointestinal treatment of pancreatitis requires several clinical trials at different doses and for various durations. In any case, L-arginine administration significantly changed the histopathology evaluation and biochemistry parameters of the pancreas.

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ETHICAL APPROVAL

The experimental method was confirmed by the Institutional Animal Ethical Committee, Faculty of Veterinary Medicine, University of Shahrekord.

CONSENT TO PARTICIPATE

All authors voluntarily agree to participate in this research study.

CONSENT TO PUBLISH

All authors agree to publish this article.

AUTHORS CONTRIBUTIONS

RF conceived and designed research. RGH, RA and HM conducted experiments. BK contributed new reagents or analytical tools. RF and IK analyzed data. RF wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

COMPETING INTERESTS

'Not applicable'

All authors stat that there was not any "conflict of interest" for this article. All authors agree with the addition, removal or rearrangement of this article. The submitted manuscript has not been published previously in full form or partially, and is not under consideration for publication elsewhere.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Abdel-Gawad SK. Therapeutic and protective effect of wheat germ oil on l-arginine induced acute pancreatitis in adult Albino rats. J Cell Sci Ther. 2015:1-7.

Abdelzaher WY, Ahmed SM, Welson NN, Marraiki N, Batiha GE-S, Kamel MY. Vinpocetine ameliorates L-arginine induced acute pancreatitis via Sirt1/Nrf2/TNF pathway and inhibition of oxidative stress, inflammation, and apoptosis. Biomed Pharmacother. 2020; 133: 110976.

Abu-Hilal M, McPhail M, Marchand L, Johnson CD. Malondialdehyde and superoxide dismutase as potential markers of severity in acute pancreatitis. JOP. 2006;7:185-192.

Affourtit C, Jastroch M, Brand MD. Uncoupling protein-2 attenuates glucose-stimulated insulin secretion in INS-1E insulinoma cells by lowering mitochondrial reactive oxygen species. Free Radic Biol Med. 2011;50:609-616.

Amiri A, Dehkordi RAF, Heidarnejad MS, Dehkordi MJ. Effect of the zinc oxide nanoparticles and thiamine for the management of diabetes in alloxan-induced mice: a stereological and biochemical study. Biol Trace Elem Res. 2018;181:258-264.

Aziz N, Kamel M, Rifaai R. Eff ects of hemin, a heme oxygenase-1 inducer in L-arginine-induced acute pancreatitis and associated lung injury in adult male albino rats. Endocr Regul. 2017; 51: 20-30.

Bhatia M, Brady M, Shokuhi S, Christmas S, Neoptolemos JP, Slavin J. Inflammatory mediators in acute pancreatitis. J Pathol. 2000;190:117-125.

Biczó G, Hegyi P, Dósa S, Shalbuyeva N, Berczi S, Sinervirta R, et al. The crucial role of early mitochondrial injury in L-lysine-induced acute pancreatitis. Antioxid Redox Signal. 2011;15:2669-2681.

Biradar S, Veeresh B. Screening of natural antioxidants by using L-arginine induced acute pancreatitis model. Int J Drug Dev Res. 2012;4:284-297.

Dawra R, Saluja AK. L-arginine-induced experimental acute pancreatitis. Pancreapedia. 2012;6:1-8.

Dawra R, Sharif R, Phillips P, Dudeja V, Dhaulakhandi D, Saluja AK. Development of a new mouse model of acute pancreatitis induced by administration of L-arginine. Am J Physiol Gastrointest Liver Physiol. 2007;292:G1009-G1018.

Dražić S, Sladoje N, Lindblad J. Estimation of Feret's diameter from pixel coverage representation of a shape. Pattern Recognit Lett. 2016;80:37-45.

El-Ashmawy NE, Khedr NF, El-Bahrawy HA, Hamada OB. Anti-inflammatory and antioxidant effects of captopril compared to methylprednisolone in L-arginine-induced acute pancreatitis. Dig Dis Sci. 2018;63:1497-1505.

El Morsy EM, Ahmed MA, Carvedilol attenuates l-arginine induced acute pancreatitis in rats through modulation of oxidative stress and inflammatory mediators. Chem-Biol. Interact. 2020;327:109181.

Fatahian Dehkordi RA, Hamid M, Stereological estimation and morphological assessment of the endocrine pancreatic components in relation to sex in hen. Vet Res Forum. 2015;6:49-54.

Fusco R, Cordaro M, Siracusa R, D'Amico R, Genovese T, Gugliandolo E, et al. Biochemical evaluation of the antioxidant effects of hydroxytyrosol on pancreatitis-associated gut injury. Antioxidants. 2020;9:781.

Gukovsky I, Li N, Todoric J, Gukovskaya A, Karin M. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144:1199-1209. e1194.

Hegyi P, Pandol S, Venglovecz V, Rakonczay Z. The acinar-ductal tango in the pathogenesis of acute pancreatitis. Gut. 2011;60:544-552.

Hernández V, Miranda M, Pascual I, Sánchiz V, Almela P, Añón R, et al. Malondialdehyde in early phase of acute pancreatitis. Rev Esp Enferm Dig. 2011;103:563.

Hu F, Lou N, Jiao J, Guo F, Xiang H, Shang D. Macrophages in pancreatitis: Mechanisms and therapeutic potential. Biomed Pharmacother. 2020;131:110693.

Ianiro G, Pecere S, Giorgio V, Gasbarrini A, Cammarota G, Digestive enzyme supplementation in gastrointestinal diseases. Curr Drug Metab. 2016;17:187-193.

Johnson M, Hillier K. Pancreatin. 2011. In S. J. Enna and D. B. Bylund (eds), xPharm: The Comprehensive Pharmacology Reference. Elsevier Inc. p. 1-3

Kahl S, Schütte K, Glasbrenner B, Mayerle J, Simon P, Henniges F, et al. The effect of oral pancreatic enzyme supplementation on the course and outcome of acute pancreatitis: a randomized, double-blind parallel-group study. JOP. J Pancreas. 2014;15:165-174.

Kui B, Balla Z, Végh ET, Pallagi P, Venglovecz V, Iványi B, et al. Recent advances in the investigation of pancreatic inflammation induced by large doses of basic amino acids in rodents. Lab Invest. 2014;94:138-149.

Makhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. J Hepatobiliary Pancreat Surg. 2002;9:401-410.

Mayhew T. The new stereological methods for interpreting functional morphology from slices of cells and organs. Exp Physiol. 1991;76:639-665.

Mayhew TM. A review of recent advances in stereology for quantifying neural structure. J Neurocytol. 1992;21:313-328.

Mederos MA, Reber HA, Girgis MD. Acute Pancreatitis: A Review. JAMA. 2021;325:382-390.

Melo CM, Carvalho KMMB, de Sousa Neves JC, Morais TC, Rao VS, Santos FA, et al. α, β-amyrin, a natural triterpenoid ameliorates L-arginine-induced acute pancreatitis in rats. World J Gastroenterol. 2010;16:4272.

Miki A, Ricordi C, Sakuma Y, Yamamoto T, Misawa R, Mita A, et al. Divergent antioxidant capacity of human islet cell subsets: A potential cause of beta-cell vulnerability in diabetes and islet transplantation. PLoS One. 2018;13:e0196570.

Mirmalek SA, Gholamrezaei Boushehrinejad A, Yavari H, Kardeh B, Parsa Y, Salimi-Tabatabaee SA, et al. Antioxidant and anti-inflammatory effects of coenzyme Q10 on L-arginine-induced acute pancreatitis in rat. Oxid Med Cell Longev. 2016;2016:1-8.

Olatosin TM, Akinduko DS, Uche CZ. Antioxidant capacity of Moringa oleifera seed oil against CCl4-induced hepatocellular lipid peroxidation in wistar albino rats. J Exp Biol. 2014;4:514-518.

Pasari LP, Khurana A, Anchi P, Saifi MA, Annaldas S, Godugu C. Visnagin attenuates acute pancreatitis via Nrf2/NFκB pathway and abrogates associated multiple organ dysfunction. Biomed Pharmacother. 2019;112:108629.

Pérez S, Pereda J, Sabater L, Sastre J. Redox signaling in acute pancreatitis. Redox biology. 2015;5:1-14.

Shrivastava P, Bhatia M. Essential role of monocytes and macrophages in the progression of acute pancreatitis. World J Gastroenterol. 2010;16:3995.

Sindhu RK, Ehdaie A, Farmand F, Dhaliwal KK, Nguyen T, Zhan C-D et al. Expression of catalase and glutathione peroxidase in renal insufficiency. Molecular Cell Research. 2005;1743:86-92.

Swentek L, Chung D, Ichii H. Antioxidant Therapy in Pancreatitis. Antioxidants. 2021;10:657.

Vaziri ND, Bai Y, Ni Z, Quiroz Y, Pandian R, Rodriguez-Iturbe B. Intra-renal angiotensin II/AT1 receptor, oxidative stress, inflammation, and progressive injury in renal mass reduction. J Pharmacol Exp Ther. 2007;323:85-93.

Wahba NS, Shaban SF, Kattaia AA, Kandeel SA. Efficacy of zinc oxide nanoparticles in attenuating pancreatic damage in a rat model of streptozotocin-induced diabetes. Ultrastruct Pathol. 2016;40:358-373.

Yenicerioglu A, Cetinkaya Z, Girgin M, Ustundag B, Ozercan IH, Ayten R, et al. Effects of trimetazidine in acute pancreatitis induced by L-arginine. Can J Surg. 2013;56:175.

Zhang X, Jin T, Shi N, Yao L, Yang X, Han C, et al. Mechanisms of pancreatic injury induced by basic amino acids differ between L-arginine, L-ornithine, and L-histidine. Front Physiol. 2019;9:1922.

Zhang Z, Wang Y, Dong M, Cui J, Rong D, Dong Q. Oxymatrine ameliorates L-arginine-induced acute pancreatitis in rats. Inflammation. 2012;35:605-613.

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