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ORIGINAL ARTICLE

Comparing the Pancreatic and Pulmonary Protective Effects of Adropin and Dexamethasone on L-arginine Induced Acute Pancreatitis in Rats

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ABSTRACT

Background: Acute pancreatitis is a dangerous disease that may be complicated by multi-organ failure. Adropin is a metabolic hormone expressed in many tissues including endothelium and pancreas. Dexamethasone has protective effects against acute pancreatitis.

Aim: To assess the potential protective effect of adropin on pancreatic and pulmonary changes in L-arginine induced acute pancreatitis in rats, in comparison to that of dexamethasone.

Materials and Methods: Rats were randomly separated into equal groups including control, acute pancreatitis (AP), acute pancreatitis pretreated with adropin (AP+Adro), and acute pancreatitis pretreated with dexamethasone (AP+Dexa) groups. In AP group, acute pancreatitis was induced on the 5th day of the start of study by two intraperitoneal (i.p.) injections of L-arginine. In AP+Adro and AP+Dexa groups, rats received a single daily i.p. dose of adropin (34-76) (2.1 µ g/kg) and of dexamethasone (2 mg/kg), respectively for the first 5 days. In the 5th day, acute pancreatitis was induced as in AP group, then the rats were left for 24 hours then sacrificed and blood, lungs, and pancreas were collected.

Results: In AP group, there was a significant increase in; serum (amylase, lipase, interleuken-1 beta & tumor necrosis factor alpha), pancreatic (MDA, NO & Bcl-2) and pulmonary (MDA, NO & Bcl-2), with a significant decrease in; serum adropin, pancreatic TAC and pulmonary TAC. Additionally, there were strong positive immunoreactions for; NF-κB (in pancreas and lung), and TNFα (in lung). Marked histological alterations were observed in both pancreatic and lung tissues. On pretreatment by either adropin or dexamethasone, the changes were ameliorated as compared to the AP group.

Conclusion: Acute pancreatitis had a detrimental influence on exocrine pancreatic function and lung structure, which may be preserved by adropin as well as dexamethasone therapy through their anti-inflammatory, antioxidant, and pro-apoptotic activities. Thus, adropin and dexamethasone may be used to prevent acute pancreatitis and the associated lung injury.

Key Words: Acute pancreatitis; L-arginine; Lung injury; Adropin; Proinflammatory cytokines

INTRODUCTION

Acute pancreatitis (AP) is a common worldwide acute reversible inflammatory non-infectious disease [1]. Causes of AP include gallstones, medications, blunt trauma to the abdomen and alcohol [2]. AP may be self-limited, mild, or severe complicated by multi-organ failure. The pathogenesis of the disease needs more declaration. Zhu, et al. [3] stated that AP is either localized to the pancreas or may spread to adjoining tissues. Wu, et al. [4] declared that in AP, activated leukocytes synthesized and released cytokines that increase pancreatic damage and systemic inflammatory response. Yenicerioglu, et al. [5] reported that in AP, the oxygen free radicals and pro-inflammatory cytokines as tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) were responsible for the pancreatic tissue damage and various organ failures.

Acute lung injury (ALI) was found to be the most important cause of death in patients with AP [6]. The fundamental mechanisms behind the increase of lung injury are complex and not completely understood. Zhu, et al. [3] reported that ALI occurs at an early stage of severe acute pancreatitis and is accompanied by activated neutrophils sequestration in the pulmonary microvasculature.

Adropin is a metabolic hormone that is expressed by the energy homeostasis associated (Enho) gene in the rat brain, endothelium, pancreas, liver, kidney, heart, skeletal muscle and small intestine [7]. Adropin is considered to be related to endothelial function regulation [8]. Ayrancı, et al. [7] reported that plasma adropin levels were significantly high in acute pulmonary embolism patients while, Ganesh-Kumar, et al. [9] detected adropin deficiency in myeloperoxidase specific-antineutrophil cytoplasmic antibody-related lung injury.

Closa, et al. [10] confirmed that AP increased pancreatitis-associated protein gene expression in pancreas. Kandil, et al. [11] found that dexamethasone has an anti-inflammatory effect in acute pancreatitis via regulating expression of that gene.

As scarce data were reported about the effect of adropin on pancreatic and pulmonary changes in L-arginine induced acute pancreatitis in rats, thus this study was done to clarify that effect in comparison to that of dexamethasone and the possible mechanism involved.

MATERIALS AND METHODS

32 healthy, adult male Wistar albino rats weighing 140- 170 g, obtained from Zagazig Faculty of Veterinary Medicine Animal House. Animals were housed in hygienic steel wire cages (50x30x20 cm), with four rats per cage, and fed a standard food supplied from the Zagazig Faculty of Agriculture. All of the animals were given unlimited access to water and were kept at a suitable temperature (20 to 24ºC) and a normal light-dark cycle. The rats were accommodated to conditions of Physiology Department Research Lab, Zagazig Faculty of Medicine, for 2 weeks before the experiment. Zagazig University's Physiology Department Committee and Institutional Animal Care and Use Committee approved the experimental protocol (ZU-IACUC/3/F/42/2021).

Experimental Design:

Rats (N=32) were randomly separated into 4 equal groups (8 rats/group) including control, acute pancreatitis (AP), acute pancreatitis pretreated with adropin (AP+Adro) and acute pancreatitis pretreated with dexamethasone (AP+Dexa).

Induction of acute pancreatitis: it was induced on 5th day of the beginning of study by two intraperitoneal (i.p.) injections with one-hour interval of 2.5 g/kg L-arginine for each injection [12]. After that, animals were left for 24 hours before scarification. Success of induction of acute pancreatitis was confirmed by increased serum amylase and lipase, with loss of pancreatic acinar cells observed in microscopic image [13]. L-arginine (Sigma Aldrich, USA, catalog # A5006) was obtained by liquifying powder in 0.9% saline to reach concentration of 0.5 g/ml. The pH was adjusted at 7 by using 5 N HCl [2].

In AP+Adro group, rats received a single daily i.p. dose of adropin (34-76) (2.1 µ g/kg, Phoenix Pharmaceuticals, USA, catalog # 032-35) dissolved in saline [14] for the first 5 days. Adropin (34-76) is a synthetic polypeptide formed of 76 amino acids with disulfide bond between Cys^{34}-Cys^{56} [15]. 1 hour after the final dose of adropin, in the 5th day, acute pancreatitis was induced as in AP group, then rats were left for 24 hours then sacrificed. In AP+Dexa group, rats received daily i.p. injection of dexamethasone (2 mg/kg, purchased from Amriya Pharmaceuticals, Egypt, as ampoules, each was 8 mg/2 ml) [11] for the first 5 days. 1 hour after the final dose of dexamethasone, in the 5th day, acute pancreatitis was induced as in AP group, then rats were left for 24 hours then sacrificed. In control and AP groups, daily i.p. injection of saline (1 ml/rat) for the first 5 days. In control group, 1 hour after the final dose of saline, another two i.p. injection of saline (1 ml/rat) separated by an hour interval was done, then rats were left for 24 hours then sacrificed. In AP group, the last dose of saline was given one hour prior to induction of acute pancreatitis.

Samples collection: By the end of experiment, animals were decapitated under thiopental (50 mg/kg) injection [16]. The animals were anesthetized after night fasting. Blood samples were collected, left for half an hour at room temperature, and then centrifuged at 5000 rpm for ten min. The sera were collected and kept at −80°C until used for assay.

Biochemical analysis: For measurement of serum amylase and lipase, colorimetric assay kits (abcam.com, catalog #ab102523 and #ab102524, respectively) were used. Also, commercial kits were purchased for measurement of Interleukin-1β (IL-1β, LifeSpan BioSciences, USA, catalog # LS-F23554), tumor necrosis factor alpha (TNFα, Sigma Aldrich, USA, catalog # RAB0479), adropin (MDA, MyBioSource.com, catalog # MBS2533588), malondialdehyde (MDA, MyBioSource.com, catalog # MBS268427), total antioxidant capacity (TAC, Mybiosource.com, Catalog # MBS8243210), nitric oxide (NO, Creative-diagnostics.com, catalog # DEIA-

BJ2206) and B-cell leukemia/lymphoma-2 (Bcl-2, Mybiosource.com, Catalog # MBS2881713).

Analysis of pancreatic and lung homogenates:

Just after sacrificing of animal, both pancreas and lung were removed. One half of pancreatic and lung tissues of each animal was used for homogenization, while the other half was processed for histopathological examination. The pancreatic and lung samples were kept at −80°C and then homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The proportion of tissue weight to homogenization buffer was 1:10 [2]. The homogenate was centrifuged at 5000 rpm for 10 minutes at 4°C. The obtained supernatant was used for measuring MDA, TAC, NO and Bcl-2.

Histopathological examination:

The half of pancreatic and lung tissues were collected and selected randomly from different parts of each organ, then rapidly fixed in 10% formal saline, then samples were washed by tap water and processed for paraffin embedding. Sections of 5µm were obtained and stained with Hematoxylin and Eosin (H&E) [17]. Each slide was examined by light microscopy (LeicaICC50W) at Anatomy Department Imaging Unit.

NF-κB and TNFα Immuno-histochemistry staining:

Immunohistochemical staining was processed using rabbit polyclonal antibodies (NF-κB, Protein tech company, USA, catalog #10409-2-AP) used at a concentration of 1/200; which is considered as an inflammatory reaction marker, and TNFa IHC antibody (polyclonal, Abbiotec, USA) diluted 1:80 in phosphate buffered saline (PBS). slides were processed for deparaffinized and then washed in PBS. Endogenous peroxidases were reduced by using a peroxidase blocking solution (3% H_2O_2 in methanol) for 15 min then washed by tris buffer saline (TBS). normal goat serum was used to block nonspecific binding of IgG, diluted 1: 50 in 0.1% bovine serum albumin with TBS for half an hour. Primary antibodies for anti-NF-κB and anti-TNFα were added on slides and left overnight at room temperature. Then, slides were subjected for washing in buffer and incubated for further 30 min with biotinylated goat anti-rabbit secondary antibodies diluted 1: 1000, followed by several washing. The slides incubated for 30 min with vectastain Avidin, Biotinylated horse radish peroxidase Complex kits and washed for 10 min, the substrate, diaminobenzidine tetra hydrochloride in distilled water was applied for 5–10 min. Slides were stained by hematoxylin as a counter stain to obtain a clear morphology of cells. the mouse spleen and human mammary cancer sections were used as positive control for anti-NF-κB and TNFα successively. For negative control slides, we repeated the previous steps without addition of 1ry antibody [18].

Morphometric analysis: To evaluate degree of histological alteration in the pancreatic tissue; edema, infiltration of leukocyte, and degeneration were scored by grading according to Binker, et al. [19] 0 equal absent, 1 equal mild, 2 equal moderate, 3 equal severe, and 4 equal overwhelming. The grade of scoring was evaluated quantitatively and was obtained from each rat as the mean of five random non overlapped fields within each section (3 sections/ rat, H&Ex400). H&E-stained lung slides were subjected to estimating interalveolar septum thickening. Mean of ten measuring in five non overlapped fields in each slide (x400). A computerized image analyzer (Leica Imaging System Ltd., England) at Anatomy and Embryology Department Imaging Unit, Zagazig Faculty of Medicine, was used. The area percent of positive expression for NF-κB stained sections in both pancreatic acini and islets cells in all studied groups as well as lung parenchyma was measured. Additionally, area percent of TNFα positive reaction of lung tissue was estimated at magnification X 400 by using image J 22 program (Version 1.50).

Statistical Analysis:

The data obtained were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Tukey HSD for post hoc multiple comparisons were used to compare means for statistical significance. The software, IBM SPSS Statistics (Version 26 Software for Windows), was used for that purpose. Significance was considered with P value ≤ 0.05.

RESULTS

Changes in serum amylase and lipase: (table-1)

In comparison to control group, there was a significant increase in serum levels of both amylase and lipase in AP, AP+Adro, and AP+Dexa groups. These changes were improved in AP+Adro and AP+Dexa groups.

Changes in inflammatory markers: (table-1)

In comparison to control group, there was a significant increase in serum IL-1β, serum TNFα, pancreatic and pulmonary NO in AP, AP+Adro, and AP+Dexa groups. These changes were ameliorated in AP+Adro and AP+Dexa groups.

Changes in oxidative stress markers: (table-1)

In comparison to control group, there was a significant increase in pancreatic and pulmonary MDA, with a significant decrease in pancreatic and pulmonary TAC in AP, AP+Adro, and AP+Dexa groups. These changes were reversed in AP+Adro and AP+Dexa groups.

Changes in pancreatic and pulmonary Bcl-2: (table-1)

In comparison to control group, there was a significant increase in pancreatic and pulmonary Bcl-2 in AP, AP+Adro, and AP+Dexa groups. These changes were improved in AP+Adro and AP+Dexa groups.

Changes in serum adropin levels: (table-1)

In comparison to control group, there was a significant reduction in serum adropin levels in AP and AP+Dexa groups. This change was ameliorated in AP+Adro.

Histopathological results:H&E

Pancreatic specimen

The control pancreatic tissue showed normal pancreatic structure, appeared as lobules separated by thin interlobular septa. The pancreatic lobules composed of acini, ducts and islets of Langerhans. The acini appeared basophilic in its base and acidophilic apically with vesicular nuclei. Well-formed ducts lined with cuboidal epithelium were observed. The islets of Langerhans cells had lightly stained β cells, darkly stained α cells and intervening blood capillaries (Fig. 1a). In AP group, the architecture appeared disturbed, interlobular septa were wide, congested intralobular and interlobular blood vessels, and inflammatory cellular infiltration around dilated ducts. few acini appeared disturbed with loss of their apical acidophilic and basal basophilic portions with cytoplasmic vacuolations. Islets of Langerhans showed reduction of cellularity with cytoplasmic vacuolations (Fig. 1b & 1c). In AP+Adro (Fig. 1d) and AP+Dexa (Fig. 1e) groups, most of histological alterations were alleviated. The pancreatic lobules were more as control with less widened interlobular septa, inflammatory cell infiltration and edematous areas. Most of pancreatic acini restored their normal structure. Islets of Langerhans revealed more cellularity.

Lung specimen

Lung sections of control group showed; normal thin-walled alveoli opening into alveolar sacs, terminal bronchioles and blood vessels. The alveoli were lined with flat pneumocyte I and cuboidal pneumocyte II. Terminal bronchioles were lined with columnar ciliated epithelium, while the blood vessels appeared with thin walls (Fig. 3a & 3b). In AP group, lung sections revealed thick interalveolar septa with marked extravasation of RBCs and hemosiderin deposition within alveolar cavities. Blood vessels appeared congested and thickened. Bronchial walls showed heavy inflammatory infiltrate and disturbed epithelial lining with vacuolated cells (Fig. 3c, 3d &3e). In AP+Adro and AP+Dexa groups, there was marked restoration of lung parenchyma with moderate thickening of interalveolar septa, mild inflammatory infiltrate and extravasation of RBCs within alveoli and bronchioles (Fig. 4a, 4b, 4c & 4d).

Immuno-histochemical results for NF-κB expression:

Pancreatic specimens; control group showed negative NF-κB expression in both pancreatic acini and islets cells (Fig. 2a) while, AP group displayed strong diffuse positive immunoreactivity (dark brown stained) in the previous mentioned areas (Fig. 2b). AP+Adro and AP+Dexa groups showed moderate positive immunoreactivity in both; islets cells and pancreatic acini (Fig. 2c & 2d).

Lung tissue; control group showed weak NF-κB expression in lung parenchyma (Fig.5a) while, AP group displayed strong diffuse positive immunoreactivity (Fig. 5b). AP+Adro and AP+Dexa groups showed moderate positive immunoreactivity (Fig. 5c & 5d).

Immuno-histochemical results for TNFα expression of lung:

Control group showed negative TNFα expression in lung parenchyma (Fig. 5e), whereas AP group displayed strong diffuse positive immunoreactivity (Fig. 5f). AP+Adro and AP+Dexa groups showed moderate to weak positive immunoreactivity (Fig. 5g & 5h).

Morphometric results (Table-2):

Regarding the histological scoring; degeneration, inflammatory cell infiltration and edema of pancreas were significantly increased in AP group. These changes were significantly improved in AP+Adro and AP+Dexa groups.

Interalveolar septa in AP group's lung sections showed significant thickening which significantly improved in AP+Adro and AP+Dexa groups. There was a significant increase in NF-κB area fraction of both pancreas and lung parenchyma in AP group which significantly decreased in AP+Adro and AP+Dexa groups. There was a significant increase in TNFα immune positive area percent within lung parenchyma in AP group which significantly decreased in AP+Adro and AP+Dexa groups.

Table-1: Biochemical changes among different studied groups (number of rats in each group= 8)

Group / Parameter	Control	AP	AP+Adro	AP+Dexa
Serum amylase (U/L)	**13.45±0.23**	**44.07±2.16[a]**	**21.9±1.37[a&b]**	**16.63±0.88[a,b&c]**
Serum lipase (U/L)	**484.2±14.7**	**1482.4±14.7[a]**	**806.4±18.9[a&b]**	**632.4±13.1[a,b&c]**

Group Parameter	Control	AP	AP+Adro	AP+Dexa
Serum IL-1β (pg/ml)	**12.37±0.42**	**24.85±0.43[a]**	**18.26±0.26[a&b]**	**15.04±0.18[a,b&c]**
Serum TNFα (pg/ml)	**17.36±0.38**	**28.6±1.38[a]**	**21.22±0.56[a&b]**	**18.04±0.48[b&c]**
Serum adropin (ng/ml)	**35.45±0.55**	**21.08±0.21[a]**	**36.11±0.49[b]**	**30.33±0.72[a,b&c]**
Pancreatic MDA (pg/mg tissue)	**51.81±1.43**	**161.57±2.99[a]**	**90.84±2.51[a&b]**	**72.04±2.24[a,b&c]**
Pancreatic TAC (µM/mg tissue)	**89.29±1.29**	**21.53±0.67[a]**	**51.34±1.38[a&b]**	**78.72±1.37[a,b&c]**
Pancreatic NO (µM/mg tissue)	**1.81±0.09**	**5.82±0.11[a]**	**3.64±0.09[a&b]**	**2.48±0.09[a,b&c]**
Pancreatic Bcl-2 (ng/mg tissue)	**1.05±0.06**	**2.39±0.07[a]**	**1.73±0.08[a&b]**	**1.37±0.11[a,b&c]**
Pulmonary MDA (pg/mg tissue)	**71.4±2.34**	**221.26±7.79[a]**	**95.11±4.04[a&b]**	**82.16±3.07[a,b&c]**
Pulmonary TAC (µM/mg tissue)	**74.73±1.48**	**43.03±2.97[a]**	**60.42±2.36[a&b]**	**69.67±1.53[a,b&c]**
Pulmonary NO (µM/mg tissue)	**3.73±0.09**	**6.86±0.09[a]**	**4.52±0.09[a&b]**	**4±0.11[a,b&c]**
Pulmonary Bcl-2 (ng/mg tissue)	**4.29±0.06**	**7.11±0.12[a]**	**5.6±0.18[a&b]**	**4.81±0.11[a,b&c]**

[a]P<0.05 compared with control group. [b]P<0.05 compared with AP group. [c]P<0.05 compared with AP+Adro group. **AP**, acute pancreatitis; **AP+Adro**, acute pancreatitis pretreated with adropin; **AP+Dexa**, acute pancreatitis pretreated with dexamethasone; **IL-1β**, interleukin 1 beta; **TNFα**, tumor necrosis factor alpha; **MDA**, malondialdehyde; **TAC**, total antioxidant capacity; **NO**, nitric oxide; **Bcl-2**, B-cell leukemia/lymphoma-2.

Table-2: Comparison of different studied group regarding histological scoring of pancreas, interalveolar septa thickness (µm), area percent (%) of NF-κB immune positive in pancreas and lung and area percent (%) of TNFα immune positive in lung

Group Parameter	Control	AP	AP+Adro	AP+Dexa
Pancreatic histological scoring	**0.528 ±0.1948**	**1.913 ± 0.480[a]**	**0.944± 0.2018 [b]** NS[a]	**0.833±0.298[b]** NS[a&c]
Interalveolar thickness (µm)	**0.773± 0.2552**	**2.649±0.7005[a]**	**1.460±0.4572[a&b]**	**1.131±0.329[a,b&c]**
Area percent of NF-κB immune positive in pancreas	**2.304±1.247**	**54.55±7.791[a]**	**30.73±1.942[a&b]**	**27.58±1.102[a&b]** NS[c]
Area percent of NF-κB immune positive in lung (%)	**11.15 ± 1.329**	**40.69 ± 2.637[a]**	**21.02 ± 1.458 [a&b]**	**18.13±1.187[a,b&c]**
Area percent of TNFα immune positive in lung (%)	**1.014±0.6196**	**30.22±2.586[a]**	**23.06±1.64 [a&b]**	**18.92 ± 1.597[a,b&c]**

[a]P<0.05 compared with control group. [b]P<0.05 compared with AP group. [c]P<0.05 compared with AP+Adro group. NS[a] non-significant compared with control group. NS[c] non-significant compared with AP+Adro group. **AP**, acute pancreatitis; **AP+Adro**, acute pancreatitis pretreated with adropin; **AP+Dexa**, acute pancreatitis pretreated with dexamethasone

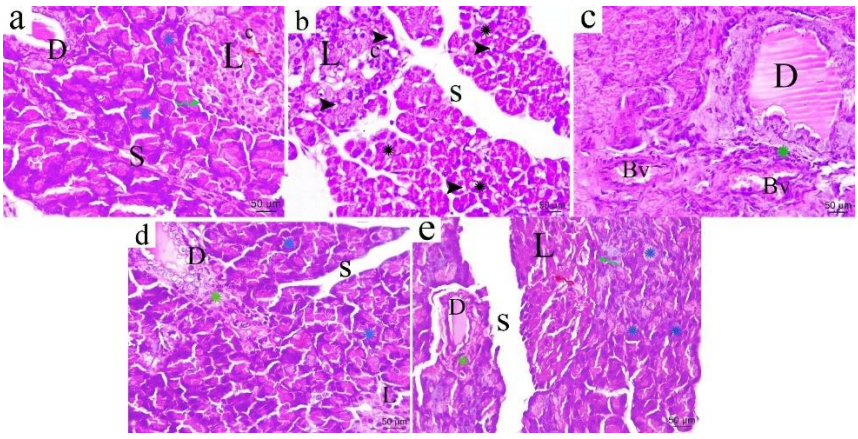

Fig. 1: Photomicrograph of pancreatic tissue; (a) control group, (b & c) AP group, (d) AP+Adro, (e) AP+Dexa. (L) Islets of Langerhans, (D) duct, (Bv) blood vessel, (c) blood capillary, (S) interlobular septa, (blue asterisk) well-structured acini, (black asterisk) disturbed acini, (black arrowhead) vacuolation, (green asterisk) inflammatory cells, (red zigzag arrow) β cells, (green zigzag arrow) α cells (H&E, X400).

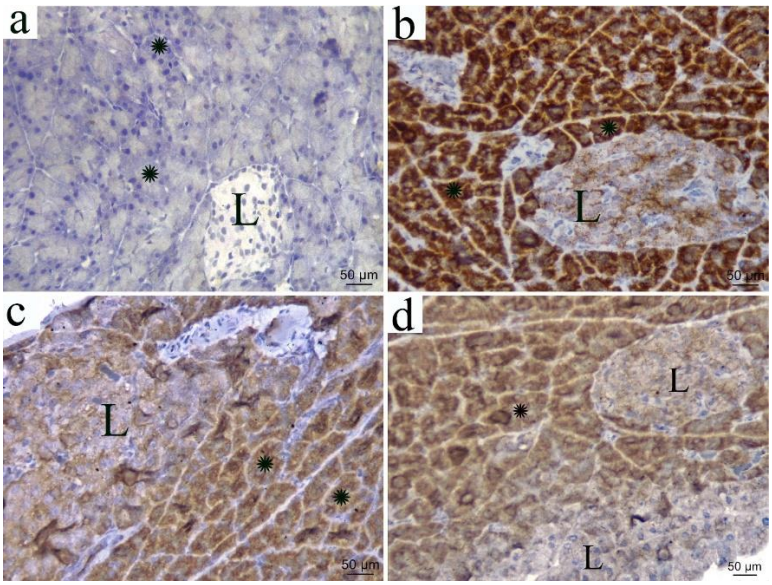

Fig. 2: Photomicrograph of **NF-κB** immune-stained pancreatic sections; (a) control group, (b) AP group, (c) AP+Adro and (d) AP+Dexa groups. (L) Islets of Langerhans, (asterisk) acini (**NF-κB,** X400).

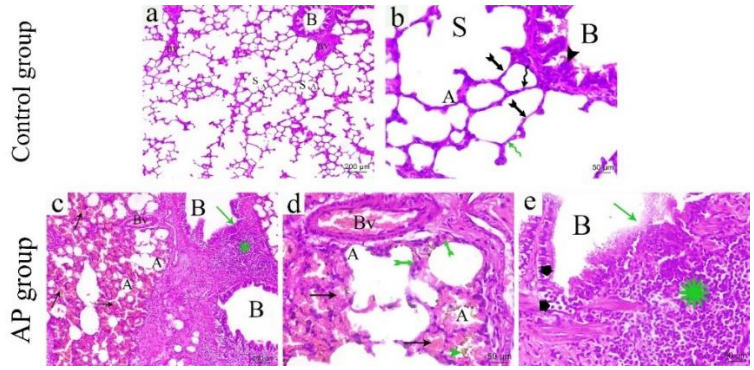

Fig. 3: Photomicrograph of a control and AP group lung sections; (B) bronchiole, (arrowhead) columnar ciliated epithelium, (A) alveoli, (S) alveolar sac, (black tailed arrow) thin interalveolar septum, (black zigzag arrow) pneumocyte I, (green zigzag arrow) pneumocyte II, (Bv) blood vessel, (green arrow) disturbed epithelial lining, (green tailed arrow) thick interalveolar septum, (thick arrow) vacuolated cells, (black arrow) RBC's, (green arrowhead) hemosiderin, (green asterisk) inflammatory cells infiltrate (H&E, X100 & X400).

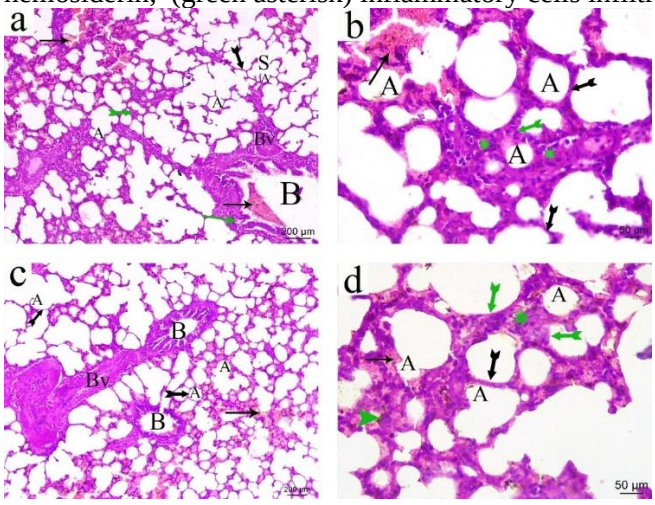

Fig. 4: Photomicrograph of lung sections; (a & b) AP+Adro and (c & d) AP+Dexa groups; (B) bronchiole, (green arrow) disturbed epithelial lining, (A) alveoli, (black tailed arrow) thin interalveolar septum, (green tailed arrow) thick interalveolar septum, (black arrow) RBC's, (green arrowhead) hemosiderin, (Bv) blood vessel, (green asterisk) inflammatory cell infiltrate (H&E, X100 & X400).

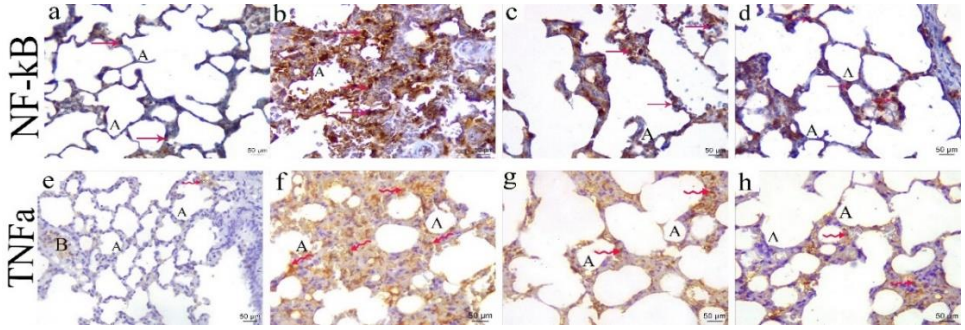

Fig. 5: Photomicrograph of **NF-κB** and **TNFα** immune-stained lung sections; (a) control group, (b) AP group, (c) AP+Adro and (d) AP+Dexa groups; (A) alveoli, (red arrow) positive **NF-κB** immune-stained cells, (red zigzag arrow) positive TNFα immune-stained cells ((**NF-κB & TNFα,** X400).

DISCUSSION

The current work used a high dosage of L-arginine to generate acute pancreatitis in rats, which is the most widely used model because it is highly reproducible and induces biochemical and pancreatic alterations that are comparable to those seen in humans [20].

In terms of exocrine pancreatic function, serum amylase and lipase were high, with loss of pancreatic acinar cells observed in AP microscopic image which was in agreement with Li, et al. [13] and Yildar, et al. [21] who owed these changes to production of hydrolytic enzymes in AP causing acinar cell necrosis.

Also, serum proinflammatory cytokines IL-1β and TNFα were significantly elevated in AP group which was supported by Yildar, et al. [21] who referred their elevation to be either produced by macrophages and monocytes that were activated as a result of peritoneal macrophage stimulation, or severe pancreatic injury following intraperitoneal L-arginine injection.

In addition, AP group showed a significant pancreatic expression of NF-κB, the transcription factor of proinflammatory cytokines, implying that NF-κB plays a pro-inflammatory role in the development of AP which was in line with Yu, et al. [22]. Scuteri and Monfrini [23] stated that first stage of acute pancreatitis showed intra-pancreatic enzyme activation, which attracts neutrophils and initiates pancreatic inflammation.

AP group showed enhanced pancreatic anti-apoptotic Bcl-2 which was supported by Liu, et al. [24]. This could be explained by increased pancreatic NF-κB which increases transcription of anti-apoptotic genes as described by Liu, et al. [25].

NO levels were higher in pancreatic tissue of AP group which might be linked to cytokines' capacity to increase the production or expression of the pancreatic enzyme inducible nitric oxide synthase (iNOS) as reported by Al-Malki [26]. Abdelzaher, et al. [27] stated that iNOS increases conversion of L-arginine to NO, producing oxidative stress in pancreas that plays a key role in pancreatic damage. This observation is consistent with arterial dilatation and congestion seen in AP group's microscopic image. In agreement with current results, Kosekli, et al. [28] observed microscopic signs of acute pancreatic parenchymal necrosis, bleeding, and inflammatory infiltration. Also, Yassien and El-ghazouly [29] reported that rats with AP showed; loss of normal lobular architecture, edema with inflammatory response, and vascular congestion which could be related to buildup of NO in pancreatic acini [21].

The oxidative stress in pancreatic tissue of AP group was confirmed by increased MDA, a lipid peroxidation marker, and decreased TAC. This might be due to excessive generation of reactive oxygen species by damaged cells which promote lipid peroxidation of polyunsaturated fatty acids in cell membranes, and consume antioxidant enzymes [30]. This oxidative stress could also explain the inflammatory reaction observed in AP group [12, 31].

In AP group, a significant decrease in serum adropin was observed. But, adropin is considered to be related to endothelial function regulation and it is claimed to be an endothelial dysfunction marker [8]. This could indicate that L-arginine may cause acute pancreatitis via downregulating adropin levels. This was supported by Kumar and Bhatia [32] who declared that lipid peroxidation products damage the membranes of blood capillary endothelial cells, resulting in an increase in vascular permeability, which leads to inflammatory infiltration and edema of the pancreatic interstitium.

Thus, it was proposed that L-arginine's inflammatory, oxidative, anti-apoptotic, and adropin down regulation actions might be the fundamental processes causing pancreatic damage in AP.

Lung injury was observed in AP group as evidenced by increased pulmonary MDA, NO, and Bcl-2, with a significant decrease in pulmonary TAC which was supported by Sowjanya, et al. [33]. Lung histopathological changes in AP rats

confirmed lung damage as there was thickened interalveolar septa with marked extravasation of RBCs within alveolar cavities with hemosiderin deposition. Also, blood vessels appeared congested and thickened. In addition, bronchial wall showed heavy inflammatory infiltrate and disturbed epithelial lining with vacuolated cells. These findings were supported by the significant increase in immune expression of both NF-κB and TNFα in AP group's lung tissue. Adropin deficiency in AP, might also be the cause of lung damage which was in line with Gao, et al. [34] and Ganesh-Kumar, et al. [9] who detected adropin deficiency in myeloperoxidase specific-antineutrophil cytoplasmic antibody-related lung injury. Also, the activation of peritoneal and alveolar macrophages, which release systemic cytokines and inflammatory mediators, local pulmonary endothelial cell injury caused by neutrophil-generated oxygen radicals, and the specific effects of pancreatic enzymes are some of the mechanisms underlying acute lung injury associated with AP [2].

The exocrine pancreatic function was ameliorated on pretreatment of AP with adropin (in AP+Adro group), as evidenced by improvement in; biochemical findings [decreased serum (amylase, lipase, IL-1β and TNFα), decreased pancreatic (MDA, NO, Bcl-2), increased pancreatic TAC, decreased pulmonary (MDA, NO, Bcl-2), and increased pulmonary TAC], and histological examination. The histological improvement manifested itself as a return to normal architecture, reduced inflammatory cell infiltration and edema, and acini that were almost normal. This could be related to the protective role of adropin on endothelium [9]. In addition, the improvement observed with adropin treatment could be partly due to decreased immunoexpression of NF-κB which resulted in increased apoptosis that is helpful in treatment of acute pancreatitis as it can remove old, unneeded, and damaged cells [35, 36]. As a result, the use of apoptotic inducers may aid in the treatment of pancreatitis and the reduction of cellular and systemic damage [37]. Also, pretreatment with adropin could improve pancreatic and pulmonary changes by its antioxidant activity which was supported by Liu, et al. [38] and Gao, et al. [39] who declared that antioxidants were effective in inhibiting NF-κB activity as well as reducing proinflammatory cytokines TNFα and IL-1β. This is consistent with the significantly decreased expression NF-κB and TNFα in the lung parenchyma of AP+Adro group. Both pancreatic and pulmonary changes were improved after pretreatment of AP with dexamethasone (in AP+Dexa group), as proved by chemical and histological and immunohistochemical findings. According to the results of this study, dexamethasone protects pancreas and lung by its anti-inflammatory, antioxidant, and by reducing pancreatic and pulmonary NF-κB expression. Also, it prevents marked decrease in serum adropin level as compared to AP rats. These findings were in line with Zhao, et al. [40] and Kandil, et al. [11] who reported that pretreatment of AP with dexamethasone significantly reduced serum amylase, and improved both local and systemic inflammatory parameters and correlated with upregulation of pancreatitis associated protein gene expression. On comparing effects of dexamethasone therapy with that of adropin therapy, it was observed that dexamethasone has more powerful protective effects. Study limitations include small sample size, brief pretreatment period, and it is experimental on animal.

CONCLUSION

Acute pancreatitis had a detrimental influence on exocrine pancreatic function and lung structure, which may be preserved by adropin as well as dexamethasone therapy through reduction of pancreatic and pulmonary expression of NF-κB, as well as their anti-inflammatory, antioxidant, and pro-apoptotic activities. In addition, the protective effects of dexamethasone may be related to its adropin regulating action observed in this study. Thus, adropin and dexamethasone may be used to prevent acute pancreatitis and the associated lung injury. Future work is recommended to assess the ability to utilize adropin therapy to prevent acute pancreatitis and the associated lung injury in high-risk patients.

Conflict of Interest: Nothing.

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