The Possible Protective Role of Verapamil and Dantrolene in Experimentally Induced Early Acute Pancreatitis in Male Albino Rat

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Original Article

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ABSTRACT

Introduction: The aim of the present work was to elucidate the possible protective role of Verapamil (VPM) and Dantrolene (Da) in controlling experimentally induced early acute pancreatitis (AP) in male albino rats.

Materials and Methods: Thirty- two adult male wistar-albino rats were divided randomly into: Group I (Control group; n. 8). Group II ((AP) group; n.24): 8 for each experimental subgroup: Subgroup (IIa) untreated AP: each was injected intraperitoneal (IP) by 250mg L- arginine/100g body weight. Subgroup (IIb) AP treated with VPM: each was pre-treated with VPM (0.25mg/100g body weight) by IP injection an hour before L-arginine induction. Subgroup (IIc) AP treated with Da: each was pre-treated with Da (1mg/100g body weight) by IP injection an hour before induction. Twenty-four hours following L-arginine injection, the rats were sacrificed by cervical dislocation under anesthesia by IP injection of pentobarbital at dose of 50mg/Kg body weight. Serological assessment of serum lipase and amylase, histological, histochemical, immunohistochemical and morphometric studies were done. Biochemical Study for Malondialdehyde (MDA), catalase, trypsin and Tumor necrosis factor alpha (TNFα) assessment by quantitative polymerase chain reaction (qPCR) were performed. All studies were followed by statistical analysis.

Results: Subgroup IIa showed morphological changes indicating inflammation and degeneration that regressed in subgroups IIb & IIc with more obvious regression in subgroup IIb. The mean values of serum (s) amylase and lipase indicated a significant increase in AP subgroup compared to control and treated subgroups also a significant decrease was found in verapamil subgroup versus dantrolene subgroups. Morphometric and biochemical quantitative values were confirmative.

Conclusions: Verapamil and Dantrolene therapy proved definite protective effect, more obvious in response to verapamil, in controlling experimentally induced early AP in male albino rats.

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Key Words: Acute pancreatitis, COX2 marker, dantrolene, TNFα, verapamil.

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INTRODUCTION

Acute pancreatitis (AP) is an inflammatory potentially life-threatening disorder of the pancreas that is associated with major morbidity and mortality. Recognized causes of AP include; gallstones, alcohol, infections, hypercalcemia, endoscopic retrograde cholangio-pancreatography (ERCP) and different drugs trigger AP[1]. The occurrence of AP has increased worldwide in the last years, most patients with AP, who are discharged within days or weeks, have a mild and self-limited course. However, approximately 30% of patients will develop severe AP with extensive pancreatic necrosis and multiple organ failure, leading to mortality rates as high as 30%[2]. Acute pancreatitis is diagnosed by typical abdominal pain; serum amylase and/or lipase elevation three times more than the normal level; and imaging findings consistent with acute pancreatitis[3].

Pathological elevation of calcium ion (Ca2+) concentration in pancreatic acinar cells is a central event in acute pancreatitis that mediates cell death and pro-inflammatory pathways such as premature trypsinogen activation. Trypsin that results from premature trypsinogen activation causes auto-digestion of the acinar cells and finally causes acinar cell necrosis[4].

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There are at least two known types of intra-cytoplasmic Ca2+ channels in the acinar cell of the pancreas that regulate release of the Ca2+ from its stores: an apical inositol (1, 4, 5)-triphosphate sensitive channel receptors (IP3Rs) and a basal ryanodine sensitive channel receptor (RyRs). They amplify Ca2+ signals that are triggered by apical IP3Rs. The RyRs can be inhibited by dantrolene, a drug that is clinically used to treat malignant hyperthermia[5]. Recently, dantrolene is discovered to have a treatment role in corona virus disease 19 (COVID19) by its protective effects against virus cytotoxicity in host cells, cell or organ damage induced by hypoxia/ischemia, oxidative stresses, inflammation, and impaired autophagy function[6].

Voltage-gated calcium channels provide calcium entry in response to membrane depolarization in many different cell types and regulate cellular processes such as contraction, secretion, and neurotransmission. These channels can be blocked by calcium channel blockers like verapamil and diltiazem, but acinar cells do not possess voltage gated Ca2+ channels. The cytosolic Ca2+ signals that activate enzyme secretion are primarily from intracellular stores[7].

Although the protective mechanism of calcium channel blockers in AP is not clearly known, it is suggested that it is related to the stabilization of the membrane as increased pancreatic zymogen granule fragility is reported in conditions associated with pancreatic injury in the rats[8]. Another suggested mechanism is action on mast cells by blocking the slow calcium channels thus preventing the influx of calcium which is an essential step in the release of histamine from these cells. In addition, verapamil abolishes the synthesis and release of prostaglandins that are the main mediators in the process of inflammation[9]. Also, verapamil has anti-inflammatory and anti-fibrotic activity thus can be used in the treatment of inflammatory disorders resulting in remodeling of connective tissue into fibrotic plaque[10].

The aim of the present work was to elucidate the possible protective role of verapamil and dantrolene in controlling experimentally induced early acute pancreatitis in male albino rats, expressed by morphological changes and confirmed by serological, morphometric, biochemical, and quantitative polymerase chain reaction.

MATERIALS AND METHODS

Animals

Thirty- two adult male wistar-albino rats 12-weeks old, average body weight 200 grams were inbred in the Experimental Animal Unit of Kasr Al-Ainy, Faculty of Medicine after their local approval, under a 12 hours light/dark cycle and controlled temperature of 22 ± 2°C. Each subgroup was kept in a separate wire cage at room temperature, fed ad libitum and allowed for free water supply. Conventional size of cages was large enough to provide enough space for normal social behavior of each rat. Appropriate number of animals per cage was not exceeding 6 rats per cage with ambient humidity, ventilation and free access to food (Grains). All procedures were done in accordance with ethical guidelines approved by Faculty of Medicine- Helwan University- Research Ethics Committee 22-2020.

Experimental Design

The rats were divided randomly into:

Group I (Control group; n. 8): Each rat received 2ml of 0.9% normal saline intraperitoneal (IP).

Group II (Acute pancreatitis (AP) group; n.24): 8 for each experimental subgroup:

- Subgroup (II a) untreated AP: each rat was injected IP by a dose of 250mg/100g L-arginine (Otto Chemie PVT Ltd, India)[11]. A fresh solution was prepared on the day of injection and each rat received the dose in a 2 ml saline by IP injection using insulin syringe.
- Subgroup (II b) AP treated with Verapamil (Abbott Egypt Company, New Cairo, Egypt, ampoules): each rat was pre-treated with verapamil in a dose of 0.25mg /100g body weight (0.1 ml/ 100g body weight) by IP injection using insulin syringe an hour before L-arginine induction[8].
- Subgroup (II c) AP treated with Dantrolene (Chemipharm Pharmaceutical Company, Zamalik, Giza, Egypt, powder dissolved in distilled water): each rat was pre-treated with dantrolene (1mg /100g body weight) in a 1ml distilled water / 100g body weight by IP injection using insulin syringe an hour before L-arginine induction[12].

Twenty-four hours following L-arginine injection[13], the rats were sacrificed by cervical dislocation[14] under anesthesia by IP injection of pentobarbital at dose of 50mg/ Kg body weight[15]. They were subjected to the following studies:

Serological Study

Fasting blood samples were collected by cardiac puncture into plain tubes, and tubes containing ethylene diamine tetra-acetic acid (EDTA); for assessment of serum pancreatic amylase and lipase[16]. They were determined by enzyme-colorimetric method using Automated Hitachi Analyzer (Diagnostic Product Corporation, California, USA).

Histological Study

Half of the pancreatic specimens were fixed in 10 % formol saline. Sections of 5 µm thickness were subjected to:

1. Hematoxylin and Eosin (H&E) staining[17].
2. Histochemical staining using Von Kossa's stain for demonstration of calcium (Ca) deposits in pancreas[18].

3. Immunohistochemical staining[19] for cyclooxygenase2 (COX2) inflammation marker[20]. It's a rabbit monoclonal IgG antibody SP21 (In*vitro*gen, Thermo Fisher, USA, MA5-14568).
4. Toluidine blue stain for demonstration of mast cell[21].
5. Morphometric assessment: Using Leica Quin 500 LTD image analyzer system to assess:
    a. Area of dark nuclei and area of interstitial zymogen granules.
    b. Area % of calcium (Ca) deposits.
    c. Area % of COX 2 immunoexpression (IE).
    d. Optical density of COX 2 immunoreactivity (IR).
    e. Area % of degranulation of mast cells.

H&E, Von Kossa's, COX2 and Toluidine blue stains (Sigma Chemical CO., P.O. Box 14508 St. Louis, USA).

Biochemical Study

The other half of pancreatic specimens were homogenized for assessment of:

1. Malondialdehyde (MDA)[22], catalase[23] and trypsin[24] (Sigma Chemical CO., P.O. Box 14508 St. Louis, USA).
2. Tumor necrosis factor alpha (TNFα)[25] by quantitative polymerase chain reaction (qPCR)[26]:

Total RNA extraction was performed (Thermo Fisher Scientific Inc. Germany), (Gene JET, Kit, #K0731). PCR kit was provided by Vivantis, ViPrime PLUS One Step Taq qPCR Green Master Mix I with ROX (SYBR Green Dye) (cat no #QLMM14-R). The TNF α primer using the 5′-oligonucleotide primer -5′ ACCACGCTCTTCTGTCTACTG -3′ and 3′-oligonucleotide primer 5′-CTTGGTGGTTTGCTACGAC -3′ (mixture was prepared in each tube: 5µg total ribonucleic acid (RNA) and 3 µl random hexamers).Prepared reaction mix samples were applied in PCR (Applied Biosystem, Foster city, USA). Kit was compatible at 55° C for 10 min as one cycle, enzyme activation at 95° C for 8 min as one cycle, denaturation occurs at 95° C for 10 s and annealing and extension at 60 ° C for 60s for 40 cycles. The target gene is quantified by normalization against house-keeping gene according to calculation of delta-delta Ct (ΔΔCt).

Statistical Study

Statistical study was carried out using the statistical package of social science (SPSS) version 25 (SPSS Inc., USA). All data were reported as mean ± standard deviation (SD) using (ANOVA) and Post hoc tests. p *values* ≤ 0.05 were regarded significant (sig)[27].

RESULTS

2 rats showed signs of distress, were immediately euthanized to prevent against animal suffering by cervical dislocation under anesthesia by an IP injection of pentobarbital at dose of 50mg /kg body weight. They were compensated. Lethargy was noticed in AP group that improved in verapamil and dantrolene groups.

Serological Results: The mean values of serum (s) amylase and lipase indicated a sig increase in AP subgroup compared to control and treated subgroups. On the other hand, a sig decrease was found in verapamil subgroup versus dantrolene subgroup and a sig increase versus control group (Table 1).

Histological Results

Hematoxylin and Eosin (H&E) Stained Sections

Subgroups (Ia, Ib and Ic), revealed normal histological architecture, demonstrating pale stained islets surrounded by darkly stained acini (Figure1a), central acidophilic zymogen granules (ZGs), basal basophilia and pale basal nuclei in acini (Figure 1b).

AP subgroup showed multiple congested vessels and distended ducts in some fields (Figure 2a), homogenous material in blood vessels and in interstitium, besides congested vessels in multiple fields (Figure 2b). Other fields demonstrated widespread vacuolation among acinar cells (Figure 2c). Other sections revealed more numerous disorganized acini with multiple interstitial ZGs, dark nuclei of multiple acinar cells and mononuclear infiltrating cells (Figure 2d).

Subgroup IIb demonstrated apparently normal acini in many fields (Figure 3a), pale nuclei, apical acidophilia, basal basophilia of acini and apparently normal islets (Figure 3b). Few fields contained a distended vessel containing lymphocytes in between apparently normal acini (Figure 3c) and some acini containing few dark nuclei (Figure 3d).

Subgroup IIc revealed apparently normal acini in some fields (Figure 4a), pale nuclei, apical acidophilia and basal basophilia of acini (Figure 4b). Other fields demonstrated few leaked ZGs, some dark nuclei of acinar cells, homogenous material in blood vessels and in interstitium in some fields (Figure 4c). Dark nuclei of some acinar cells, and ZGs were noticed among islets and surrounding acini (Figure 4d).

Von Kossa's stained sections

Group I recruited no Ca deposits (Figure 5a), in AP subgroup, multiple large Ca deposits (Figure 5b), in verapamil subgroup multiple small Ca deposits (Figure 5c) and in dantrolene subgroup few small Ca deposits were found among the acini (Figure 5d).

Immunohistochemical stained sections using COX2

Group I showed –ve IE (Figure 6a), in subgroup IIa, +ve IE was seen among multiple acini in some fields

(Figure 6b), among fewer acini in other fields (Figure 6c) and dense + ve IR was evident among few acini and in surrounding CT in some other fields (Figure 6d). Subgroup IIb showed + ve IE among few acinar cells (Figure 6e), While, + ve IE was observed in some acinar cells in subgroup IIc (Figure 6f).

Toluidine blue stained sections

Group I showed few mast cells in connective tissue (CT) and near blood vessels in few fields (Figure 7a). In subgroup IIa some degranulated mast cells (Figure 7b), in subgroup IIb few mast cells (Figure 7c) and in subgroup IIc, few partially degranulated mast cells were observed in CT (Figure 7d).

Morphometric Results

Mean area of dark nuclei indicated sig (P<0.05) increase in subgroup IIa versus all subgroups. Mean area of interstitial ZGs denoted sig increase in subgroup IIa versus subgroup IIc (Table 2). Mean area% of Ca deposits recorded a sig increase in subgroup IIa versus all subgroups and sig increase in subgroup IIb versus control and subgroup IIc. Mean area % of degranulation indicated sig increase in subgroup IIa versus subgroup IIc (Table 3). Mean area% of IE and the optical density of COX2 IR reported sig increase in subgroup IIa versus all subgroups. In subgroup IIb, sig decrease was noted versus subgroups IIa and IIc (Table 4).

Biochemical Results

The mean MDA values indicated sig increase in subgroup IIa versus all subgroups while the mean catalase values indicated sig decrease in subgroup IIa versus all subgroups. While sig decrease of mean MDA values and increase of mean catalase values were noticed in subgroup IIb versus subgroups IIa and IIc (Table 5).

The mean trypsin values indicated sig decrease in subgroup IIc versus subgroup IIa (Figure 8a). The mean values of PCR for TNFα indicated a sig increase in subgroup IIa versus all subgroups and sig decrease in subgroup IIb versus subgroups IIa and IIc, IIa (Figure 8b).

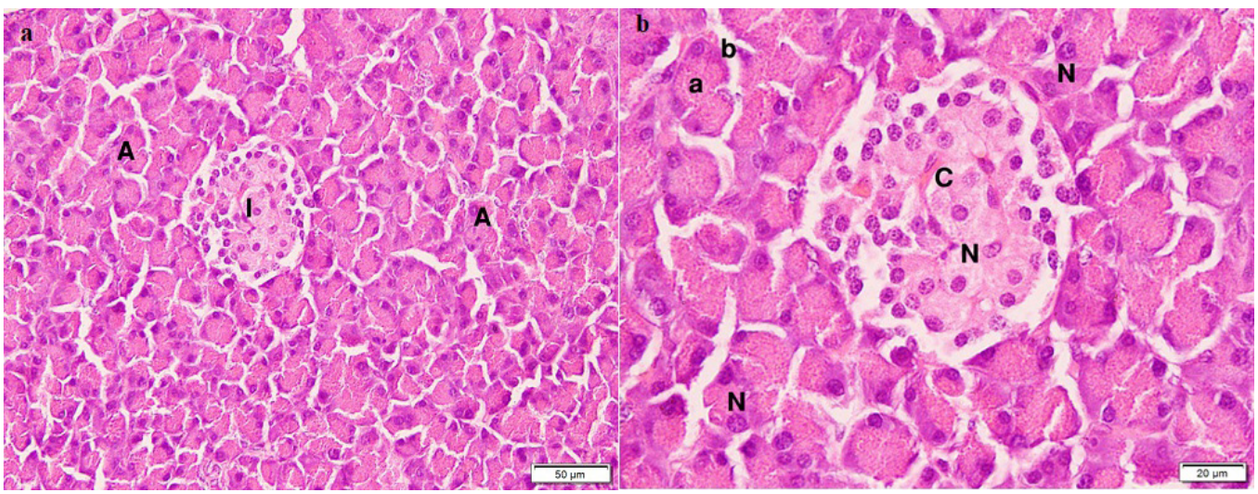

Fig. 1: Sections in pancreas of control (H&E). showing: (a) average sized islet (I) surrounded by acini (A) (x200). (b) pale nuclei (N) of islet cells, capillaries (C), central acidophilic (a) ZGs, basal basophilia (b) and pale nuclei (N) of acinar cells (x400).

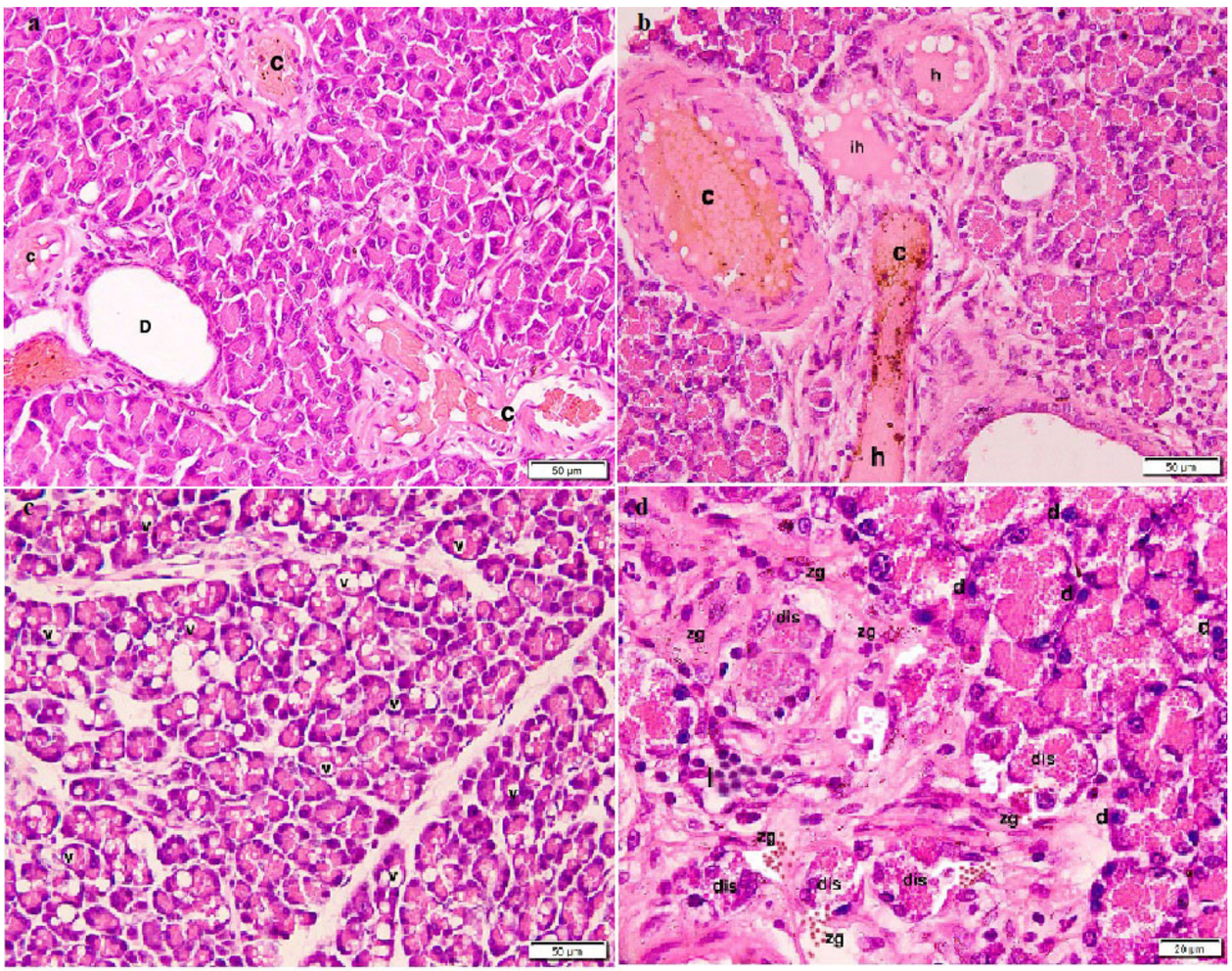

Fig. 2: Sections in pancreas of subgroup IIa (H&E). showing: (a) multiple congested vessels (c) and a distended duct (D) (x 200). (b) homogenous material (h) in blood vessels, in interstitium (ih) and congested vessels (c) (x 200). (c) widespread vacuolation (v) among acinar cells (x 200). (d) multiple disorganized acini, multiple interstitial ZGs (zg), dark nuclei (d) of multiple acinar cells and mononuclear infiltrating cells (I) (x 400).

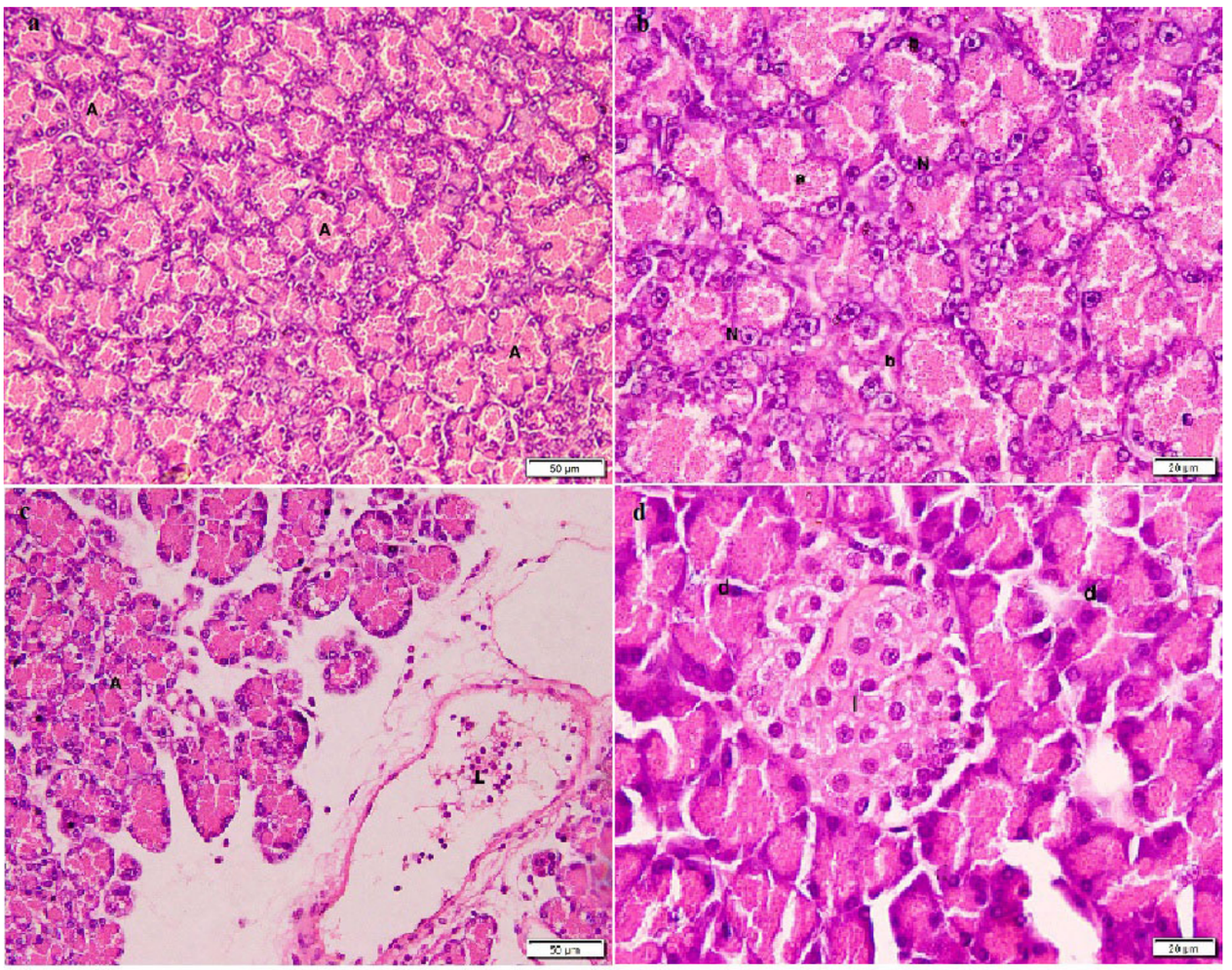

Fig. 3: Sections in pancreas of subgroup IIb (H&E) showing: (a) apparently normal acini (A) (x 200). (b) pale nuclei (N), apical acidophilia (a) and basal basophilia (b) of acini (x400). (c) distended vessel containing lymphocytes (L) in between apparently normal acini (A) (x 200). (d) some acini containing few dark nuclei (d) and apparently normal islet (I) (x 400).

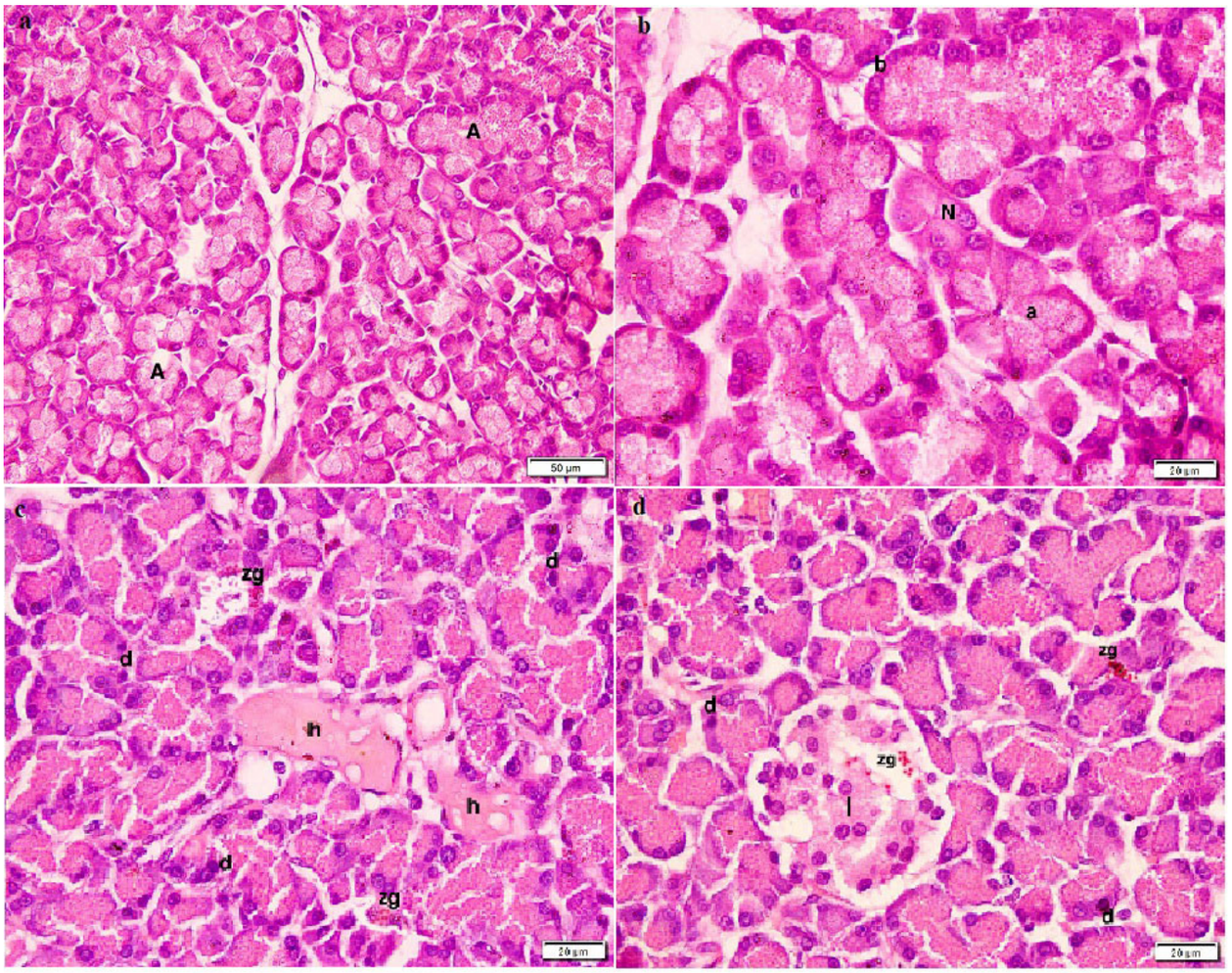

Fig. 4: Sections in pancreas of subgroups IIc (H&E) showing: (a) apparently normal acini (A) (x 200). (b) pale nuclei (N), apical acidophilia (a) and basal basophilia (b) of acini (x 400). (c) few leaked zymogen granules (zg), some dark (d) nuclei of acinar cells and homogeneous material (d) (x 400). (h) dark (d) nuclei of some acinar cells, zymogen granules (zg) among acini and islet. (x 400).

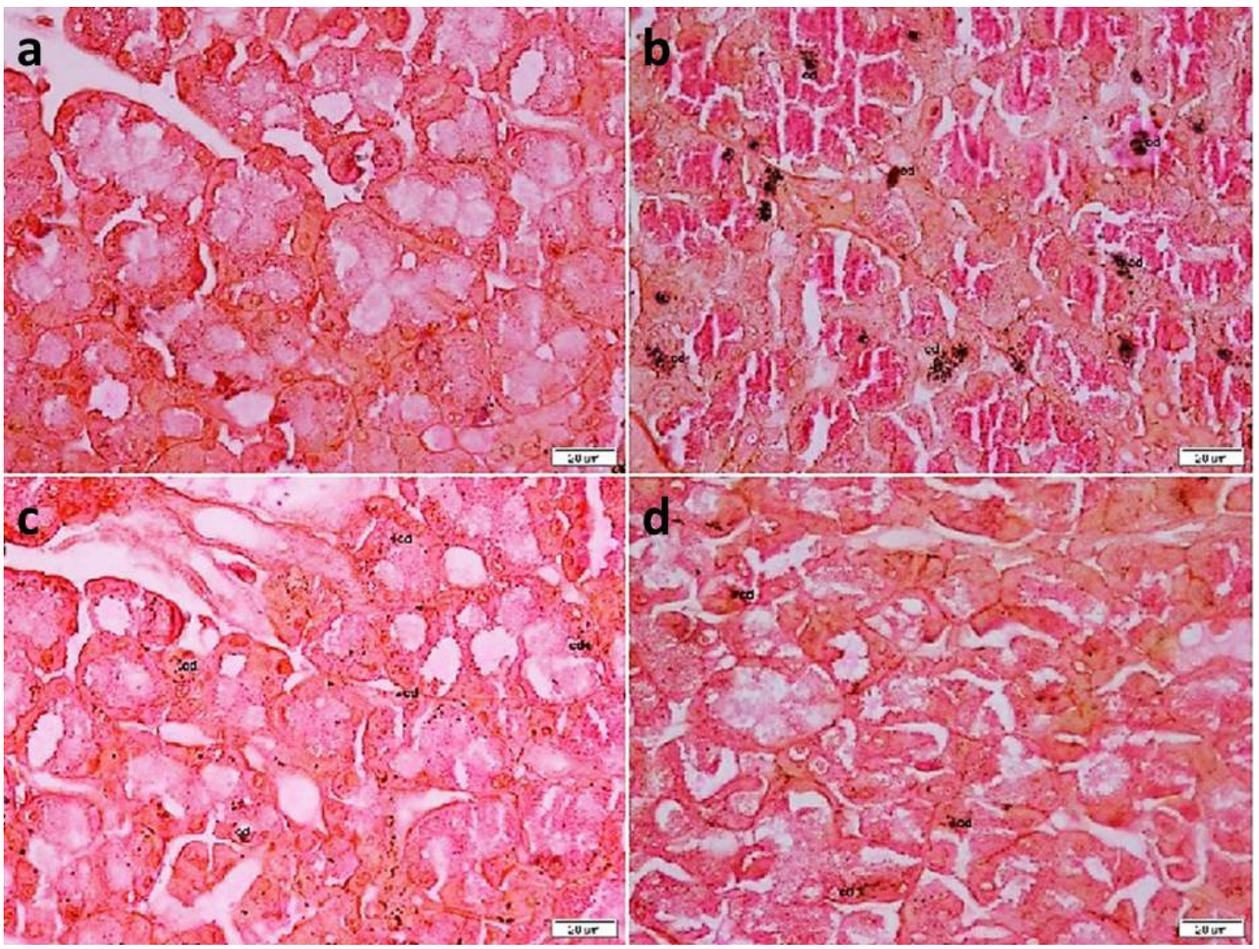

Fig. 5: Sections in pancreas (Von Kossa's stain x 400) showing: (a) no Ca deposits in control. (b) multiple large Ca deposits (cd) in subgroup IIa (c) multiple small Ca deposits (cd) in subgroup IIb. (d) few small Ca deposits (cd) in subgroup IIa.

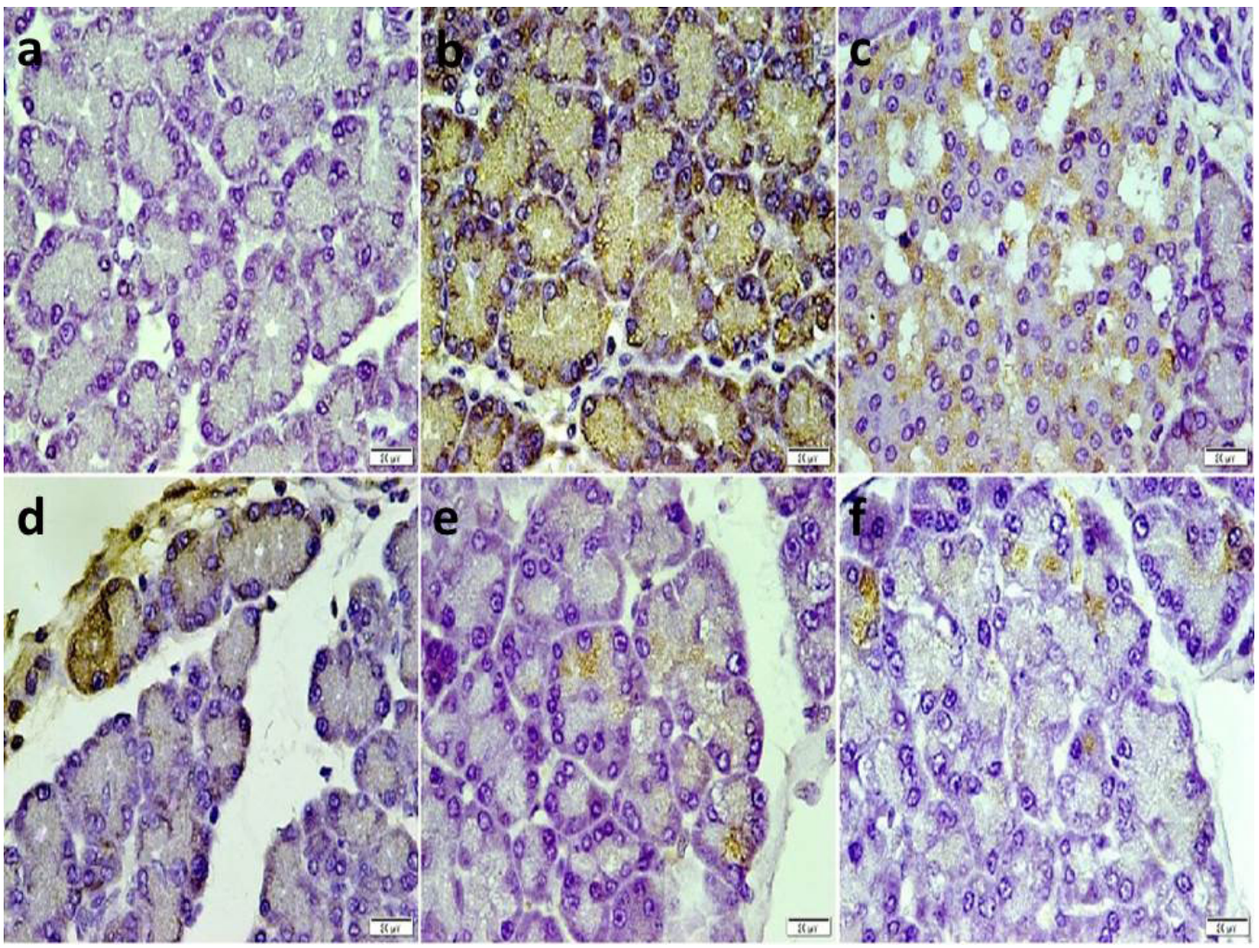

Fig. 6: COX2 immunostained sections in pancreas x 400 showing: (a)–ve IE in control. (b) +ve IE among multiple acini (c) +ve IE among fewer acini (d) dense + ve IR among few acini in subgroup IIa. (d) + ve IE among few acinar cells in subgroup IIb. (f) + ve IE in some acinar cells in subgroup IIc.

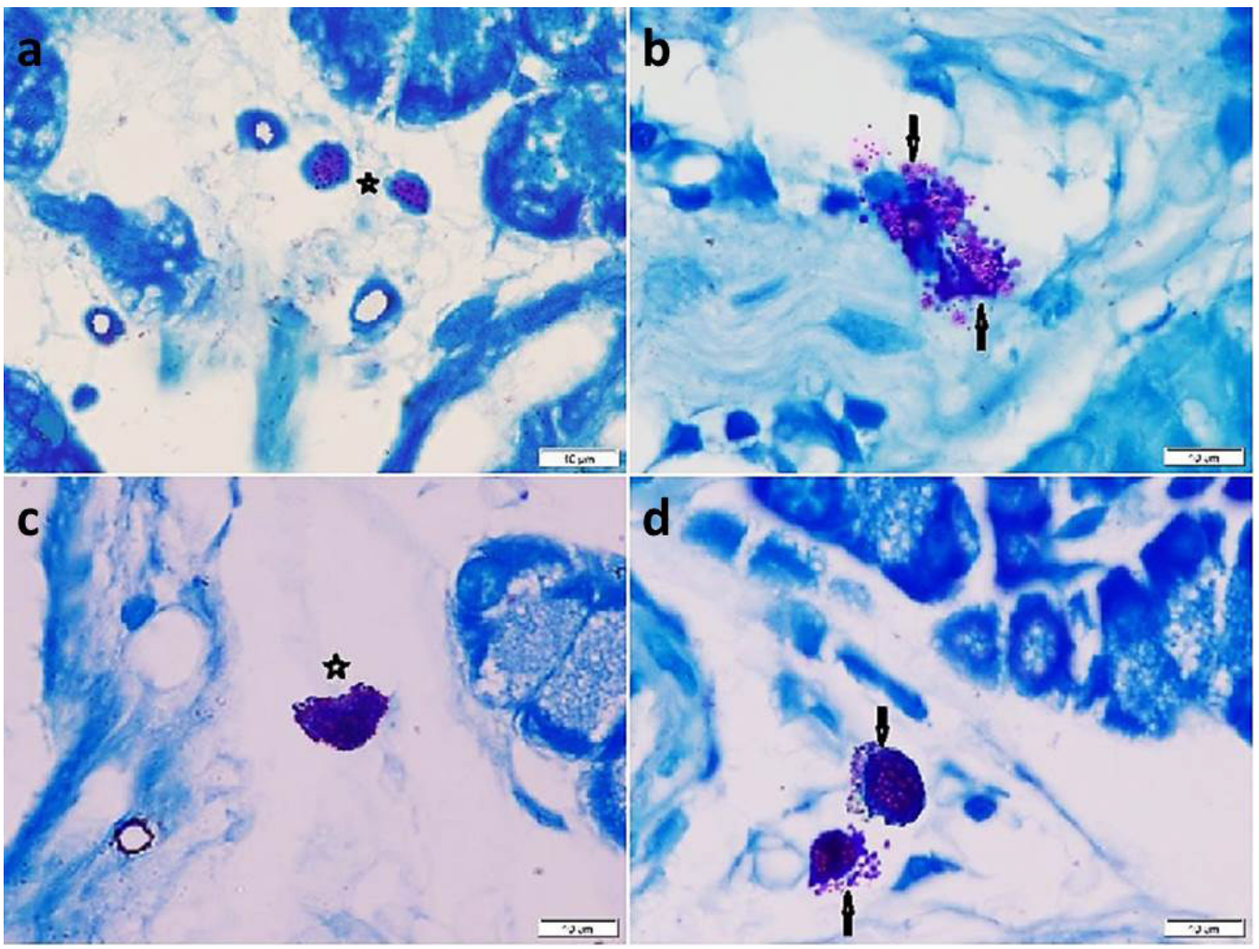

Fig. 7: Sections in pancreas (Toluidine blue x 1000) showing:(a) 2 mast cells (star) in control. (b) degranulated mast cells (arrows) in subgroup IIa. (c) a mast cell (star) in subgroup IIb. (d) 2 partially degranulated mast cells (arrows) in subgroup IIc.

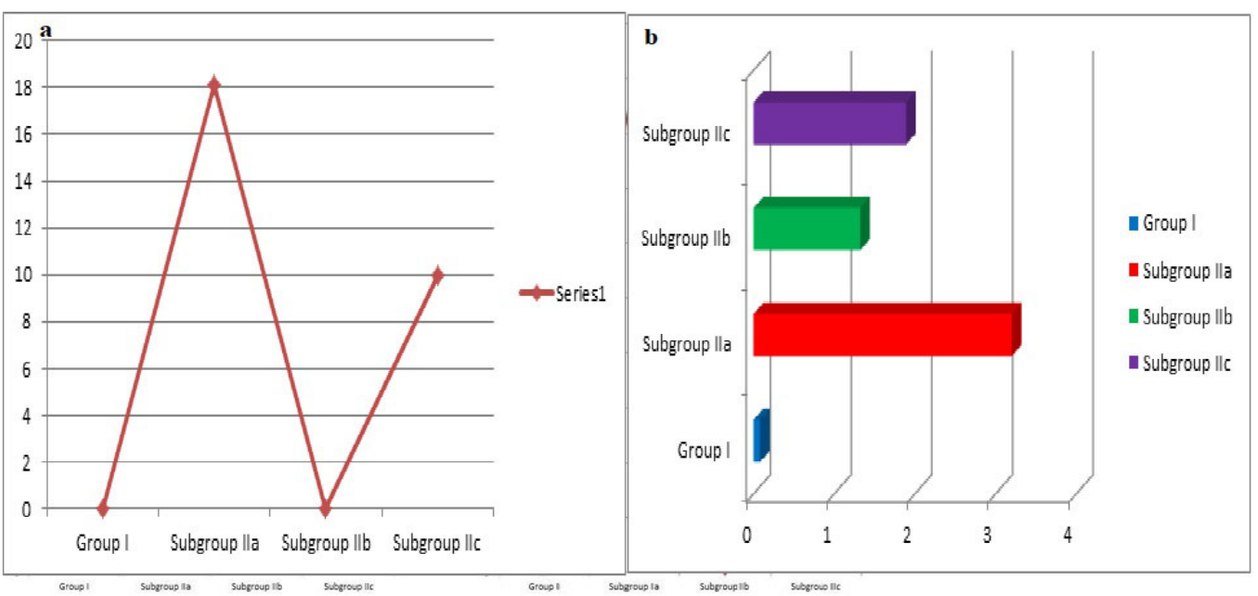

Fig. 8: Histograms of (a) The mean trypsin values show a sig. decrease in subgroup IIc versus subgroup IIa. (b) The mean values of PCR for TNFα show a sig. increase in subgroup IIa versus all subgroups and a sig. decrease in subgroup IIb versus subgroups IIa and IIc, IIa.

Table 1: Mean values (± SD) of serum amylase and lipase in control and experimental groups.

Group and Subgroups	Serum Amylase (U/ml)	Serum Lipase (U/ml)
Group I (Control)	2010.24±15.22	45.92±3.93
Subgroup IIa (AP subgroup)	9786.32±8.50*	1390.07±5.57*
Subgroup IIb (Verapamil subgroup)	4005.58±15.08^•	426.31±9.01^•
Subgroup IIc (Dantrolene subgroup)	5581.56±16.03º#	650.57±12.03º#

* $P \leq 0.05$ increase versus control and subgroups IIb and IIc.
^ $P \leq 0.05$ increase versus control.
• $P \leq 0.05$ decrease versus subgroups IIa and IIc.
º $P \leq 0.05$ increase versus control and subgroup IIb.
$P \leq 0.05$ decrease versus subgroup IIa.

Table 2: Mean area (μ2) (± SD) of dark nuclei and interstitial ZGs.

Group and Subgroups	Area of dark nuclei	Area of interstitial ZGs
Group I	0	0
Subgroup IIa	15.09±2.48*	9.89±1.54*
Subgroup IIb	3.15±0.61^	0
Subgroup IIc	3.99±0.58^	2.05±0.33^

* $P \leq 0.05$ increase versus control and treated subgroups.
^ $P \leq 0.05$ decrease versus subgroup IIa.

Table 3: Mean area% (± SD) of Ca deposits and degranulation of mast cells.

Group and Subgroups	Area% of Ca deposits	Area% of degranulation of mast cells
Group I	0	0
Subgroup IIa	6.32±0.1.01*	5.84±1.22*
Subgroup IIb	3.02±0.05^•	0
Subgroup IIc	1.35±0.08º#	2.13±0.13@•

* $P \leq 0.05$ increase versus control and treated subgroups.
^ $P \leq 0.05$ increase versus control and subgroup IIc.
• $P \leq 0.05$ decrease versus subgroup IIa.
º $P \leq 0.05$ increase versus control
$P \leq 0.05$ decrease versus subgroups IIa and IIb.
@ $P \leq 0.05$ increase versus control and subgroup IIb.

Table 4: Mean area% (± SD) of COX2 IE and mean optical density of COX2 IR.

Group and Subgroups	Area% of COX2 IE	Optical density of COX2 IR
Group I	0	0
Subgroup IIa	20.51±3.92*	0.98±0.14*
Subgroup IIb	2.89±0.51^	0.09±0.01^
Subgroup IIc	5.08±1.34#	0.45±0.08#

* $P \leq 0.05$ increase versus control and treated subgroups.
^ $P \leq 0.05$ decrease versus subgroups IIa and IIc.
# $P \leq 0.05$ decrease versus subgroup IIa.

Table 5: Mean values (± SD) of MDA and Catalase.

Group and Subgroups	MDA (nM/gm)	Catalase (U/gm)
Group I	22.04±3.10	31.45±1.93
Subgroup IIa	77.08±4.32*	9.97±0.68**
Subgroup IIb	35.02±4.08^	27.61±4.01^^
Subgroup IIc	55.16±6.00#	19.37±2.43##

* $P \leq 0.05$ increase and
** $P \leq 0.05$ decrease versus control and treated subgroups.
^ $P \leq 0.05$ decrease and
^^ $P \leq 0.05$ increase versus subgroups IIa and IIc.
# $P \leq 0.05$ decrease and
$P \leq 0.05$increase versus subgroup IIa.

DISCUSSION

In the present study, mean serum amylase and lipase values indicated sig increase in untreated acute pancreatitis (AP) subgroup. On contrary, sig decrease was found in AP treated with verapamil subgroup versus AP and AP treated with dantrolene subgroups. In agreement, the levels of serum amylase and lipase were detected to evaluate whether the AP model was successfully constructed[28]. Similarly, AP patients show elevated values of serum lipase or amylase three times the normal limit[29]. However, dantrolene and verapamil reduced the levels of serum lipase and amylase significantly[8].

Untreated AP subgroup showed multiple congested vessels, homogenous material in vessels and interstitium, also, mononuclear cellular infiltration. The previous results confirmed the incidence of AP. In accordance, congested vessels were found among the parenchyma in AP rat model[30]. Pancreatic tissues showing exudation and inflammatory cell infiltration proved that AP model rats were successfully established[31].

Untreated AP subgroup revealed distended ducts. Concomitantly, duct with stagnant secretion was described experimentally[22]. Multiple disorganized acini and multiple ZGs were seen, confirmed by sig increase in area of interstitial ZGs. The previous findings may precipitate intrapancreatic activation of digestive enzymes. Going with, acinar cell autophagy was proved experimentally[32], acinar cell loss[33] and intrapancreatic trypsin activity[34] were recorded in AP mouse models.

Widespread vacuolation and multiple dark nuclei were seen among acinar cells, confirmed by sig increase in mean area of dark nuclei in untreated AP subgroup. The previous findings indicated L-arginine induced inflammation that can progress to degeneration. In support, it was confirmed that the previous changes were found in AP rat model after nine hours[35].

Acute pancreatitis treated with verapamil subgroup demonstrated apparently normal acini, few distended vessels containing lymphocytes, few dark nuclei and

sig decrease in mean area of dark nuclei. The previous findings denoted obvious amelioration of morphological changes, indicating inflammation and degeneration, that developed in AP. In accordance, Elberry *et al*[36] found fewer inflammatory cells with less congestion and few lymphocytes as local immunity cells. Ghandi *et al*[37] added that renal tubulointerstitial inflammation and degeneration were downregulated by verapamil therapy. Recently, verapamil exerted definite antioxidant activity[38].

Acute pancreatitis treated with dantrolene subgroup revealed apparently normal acini, few leaked ZGs and some dark nuclei. The latter findings were proved by sig decrease in mean area of dark nuclei and sig decrease mean area of interstitial ZGs. The previous findings denoted amelioration of morphological changes less obvious than in subgroup IIb. Concomitantly, dantrolene was found to significantly reduce congestion seen in inflammation.[39] In addition, Dantrolene was proved to reduce cytoplasmic vacuolation and pyknotic nuclei in rats. However, single treatment with dantrolene at high dose led to the increase in histopathological scores[40].

Untreated AP subgroup showed multiple large Ca+2 deposits, AP treated with verapamil subgroup demonstrated multiple small Ca+2 deposits and AP treated with dantrolene subgroup revealed few small Ca+2 deposits among the acini. Area % of Ca+2 deposits values denoted sig increase in untreated AP subgroup and in AP treated with verapamil versus AP treated with dantrolene subgroup. In support, Pallagi *et al*[41] stated that activation of ryanodine sensitive channel receptor (RyRs) on endoplasmic reticulum (ER) leads to sustained influx of Ca+2 and sustained elevated intracellular Ca+2 levels. Gryshchenko *et al*,[42] considered elevated Ca+2 levels the hallmark of AP pathogenesis, leading to premature trypsinogen activation, blocking physiological apical exocytosis of ZGs, redirecting to basolateral membrane and activating acinar cell injury.

Inhibition of RyRs is being actively investigated as strategies for minimizing intrapancreatic trypsinogen activation and amelioration of pancreatitis response[43]. Recently, RyRs are inhibited by antagonists, such as dantrolene, where induced Ca+2 release was found to be significantly reduced[44]. It is now well established that pancreatic acinar cells are electrically non-excitable, as they can't fire action potentials, and don't possess voltage activated Ca+2 channels. Therefore, RyRs are not blocked by voltage gated Ca+2 channels as verapamil[45].

In untreated AP subgroup, +ve IE was observed among multiple acini and dense + ve IR was evident among few acini and in CT, AP treated with verapamil subgroup showed + ve IE among few acinar cells and among some acinar cells in AP treated with dantrolene subgroup. Area % of IE and optical density of IR of COX2 values reported a sig increase in untreated AP subgroup versus all subgroups and in AP treated with dantrolene subgroup versus AP treated with verapamil subgroup.

Higher expression of COX2 (relevant in prostaglandin synthesis) in brain of copper-treated rats proved its vital role in inflammation[46]. Accumulated prostaglandins stimulate inflammatory cells to release proinflammatory cytokines such as TNF-α[47]. Slight repressive effects of verapamil lead to strong TNF-α suppression, indicating anti-inflammatory effects[48]. Dantrolene has been demonstrated to suppress plasma and tissue concentration of TNF-α and inflammatory cytokines. Consequently, dantrolene inhibited ER-mediated Ca+2 release and ameliorated ER stress[6].

In untreated AP subgroup some degranulated mast cells and in AP treated with dantrolene subgroup, few partially degranulated mast cells were observed. Area % of degranulation of mast cells values indicated sig increase in untreated AP subgroup versus AP treated with dantrolene subgroup. In pancreas, sig increase in mast cell degranulation was observed after cerulein injection versus sham group[49].

Calcium channel blockers inhibit Ca+2 entry in mast cell, reduction of Ca+2 leads to inhibition of eicosanoids and leukotrienes. Stabilization of cell membrane integrity (by inhibiting Ca+2 influx) was proved to prevent tissue injury and inflammation in rat[9]. Ig E-antigen complexes bind the high-affinity receptors and causes the release of Ca+2 from ER through RyRs of mast cell. Calcium needed for degranulation comes from intracellular stores and extracellular Ca+2 influx[50]. Consequently, dantrolene as RyRs blocker can reduce mast cell degranulation.

As regard the mean MDA values indicated sig increase in untreated AP subgroup versus all subgroups while the mean catalase values indicated sig decrease in untreated AP subgroup versus all subgroups. While sig decrease of mean MDA values and increase of mean catalase values were noticed in AP treated with verapamil subgroup versus untreated AP and AP treated with dantrolene subgroups. Similarly, L-arginine induced AP in a rat model produced a high increment in MDA content[51]. Verapamil was proved as an efficient therapeutic agent for neuroprotection during cell oxidation stress as proved by Jangholi *et al*,[52] who recorded sig increase in catalase activity and dramatic inhibition of increased MDA. Román *et al*,[53] recorded dantrolene reducing MDA level and augmenting endogenous antioxidant defenses in rat.

The mean trypsin values indicated sig decrease in AP treated with dantrolene subgroup versus untreated AP subgroup, while verapamil treated AP subgroup didn't record any intrapancreatic trypsin value. Uyumlu *et al*,[8] found reduced trypsin activities by verapamil therapy in rat referred to stabilization of ZGs membrane. However, dantrolene has little benefit on preventing early protease activation.

The PCR values of TNFα indicated sig increase in untreated AP subgroup. On the other hand, a sig decrease was recorded in AP treated with verapamil subgroup versus AP treated with dantrolene subgroup. In support,

Ma *et al*,[54] performed TNF-α analysis by RT-qPCR that confirmed increased expression in AP mouse model. On the other hand, it was concluded that verapamil reduced TNF-α value in rat testis[55]. It was reported that dantrolene resulted in sig suppression of TNF-α) in mouse liver[56].

CONCLUSION

It can be concluded that morphological findings confirmed by morphometric, serological and biochemical quantitative values proved definite protective effect of verapamil and dantrolene in controlling experimentally induced early AP in rats. The protective effect was more pronounced by verapamil therapy.

Ethics approval and Consent to participate

The housing and handling of rats follow ethical guidelines approved by the Animal Ethics Committee of Faculty of Medicine- Helwan University- Research Ethics Committee 22-2020. Animals are monitored daily for infection and other illnesses by trained animal technicians. Only trained laboratory personnel and animal technicians were allowed to handle laboratory animals.

CONFLICT OF INTERESTS

There are no conflicts interest.

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الملخص العربى

الدور الوقائي المحتمل للفيراباميل والدانترولين في التهاب البنكرياس الحاد المبكر المحدث تجريبيا في ذكر الجرذ الأبيض

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الخلفية: الهدف من هذا البحث: هو تقييم الدور الوقائي المحتمل للفيراباميل والدانترولين في التحكم في التهاب البنكرياس الحاد المبكر في ذكر الجرذ الابيض المحدث تجريبيا

المواد والطرق: تم تقسيم اثنين وثلاثون ذكر جرذ ابيض بالغ عشوائيا إلى المجموعة ١ (المجموعة الضابطة عدد ٨). المجموعة ٢ (مجموعة الالتهاب الحاد عدد ٢٤): ٨ جرذان لكل مجموعة فرعية تجريبية : المجموعة الفرعية (٢ ا) مجموعة الاتهاب الحاد بدون علاج : تم حقن الجرزان ب ٢٥٠ مجم ل- أرجنين /١٠٠ جم وزن في التجويف البريتوني . المجموعة الفرعية (٢ ب) مجموعة الاتهاب المعالجة بالفيراباميل : تم حقن الجرزان ب ٠,٢٥ مجم فيراباميل /١٠٠ جم وزن في التجويف البريتوني ساعة قبل حقن ل-أرجنين . المجموعة الفرعية (٢ ج) مجموعة الاتهاب المعالج بالدانترولين : تم حقن الجرزان ب ١ مجم دانترولين/١٠٠ جم وزن في التجويف البريتوني ساعة قبل حقن ال ل-أرجنين .بعد أربع وعشرين ساعة من حقن ل-أرجنين ، تم التضحية بالفئران عن طريق خلع الفقرات العنقية تحت التخدير عن طريق الحقن في التجويف البريتوني من بنتوباربيتال بجرعة ٥٠ مجم / كجم من وزن الجسمتم تقييم الليباز والاميليز في المصل كما تم عمل دراسات نسيجية ،نسيجية كيميائية ، نسيجية كيميائية مناعية وقياسية . كما تم قياس المالون دايالدهايد والكاتاليز والتريبسين و TNF الفا عن طريق تفاعل البلمرة الكمي. كما تم عمل تحليل احصائي .

النتائج: اظهرت المجموعة الفرعية ٢ أ تغيرات تشكلية تدل علي حدوث التهاب وتفسخ . وشوهد تراجع في التغيرات في المجموعة الفرعية ٢ ب اكثر وضوحا عن التراجع المسجل في المجموعة الفرعية ٢ج. كما اشارت القيم المتوسطة إلى زيادة ملحوظة لمصل الأميليز والليباز في المجموعة الفرعية لاتهاب البنكرياس الحاد مقارنة بالجموعة االضابطة والمجموعات الفرعية المعالجة. , كما تم العثور على انخفاض ملحوظ لمصل الأميليز والليباز في مجموعة فرعية (فيراباميل) مقابل المجموعة الفرعية (دانترولين). كانت القيم الكمية المورفومترية والكيميائية الحيوية مؤكدة.

الاستنتاج : اثبتت المعالجة بالفيراباميل والدانترولين اثر وقائي مؤكد اكثر وضوحا في المجموعة الفرعية للفيراباميل في التحكم في التهاب البنكرياس الحاد المحدث تجريبيا في ذكر الجرذ الابيض