

Original Article

Effects of diclofenac sodium and octreotide on treatment of caerulein-induced acute pancreatitis in mice

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Abstract: Background: Research continues to develop novel therapeutic modalities that particularly focus on the pathogenesis of acute pancreatitis. This study aimed to assess the effects of diclofenac sodium and octreotide, alone or in combination, on pancreatic enzymes, pancreatic myeloperoxidase activity, histopathology and apoptosis of pancreas cells, using a model of experimentally induced acute pancreatitis. Objectives: We aimed to demonstrate effects of diclofenac sodium, octreotide and their combined use on pancreatic enzymes, activity of pancreatic myeloperoxidase (MPO) activity, histopathology and apoptosis of pancreas on treatment of caerulein-induced experimental acute pancreatitis. Materials and methods: Caerulein-induced acute pancreatitis model was created using a total of 58 male BALB-C mice of 25 gr in seven groups. Serum amylase, lipase levels and pancreatic myeloperoxidase activity were examined as well as apoptotic values in pancreatic acinar cells through TUNNEL method. Histopathology of pancreas was evaluated for presence of edema, hemorrhage, parenchymal necrosis, fat necrosis, leukocyte infiltration, and fibrosis. Results: In the diclofenac sodium group, apoptotic values in the pancreatic acinar cells were found to be statistically lower than in the acute pancreatitis group in terms of parenchymal necrosis and hemorrhage scores ($P = 0.007$, $P = 0.002$, and $P = 0.052$, respectively). No statistically significant differences were found in serum level of amylase, lipase, pancreatic myeloperoxidase activity and the other histopathological scores ($P > 0.05$). Conclusion: Diclofenac sodium, a cost-effective agent with a favorable side-effect profile, may represent a novel therapeutic agent for the treatment of acute pancreatitis. Findings of this study suggest a better efficacy for diclofenac sodium monotherapy as compared to octreotide alone or octreotide/diclofenac combination.

Keywords: Diclofenac sodium, octreotide, experimental acute pancreatitis

Introduction

Acute pancreatitis (AP) is inflammation of the pancreatic tissue clinically characterized by abdominal pain and elevation of the pancreatic enzymes in the blood. Currently, there is no clear opinion on pathogenesis of the acute pancreatitis, direct relationships between its etiological factors and pathogenesis, and its treatment although it has been defined more than one hundred years ago and numberless clinical and experimental studies have been conducted so far. Furthermore, it is one of the most serious problems in the medicine because of its high morbidity and mortality rates [1-3]. As

no clear opinions exist currently on treatment of acute pancreatitis, studies on new therapeutic approaches directed to its pathogenesis are still in progress. Furthermore, clinical and pathological characteristics in human acute pancreatitis are similar independent of the initiating events. Thus, despite limitations of the animal models, this supports the view that the events have similar cascade independent of the cause initiating acute pancreatitis [4].

Caerulein is an analogue of cholecystokinin. It causes some changes in the acinar cells and this, in turn, leads to formation of big amounts of free oxygen radicals [5].

Octreotide is a synthetic analogue of somatostatin. It exhibits its pharmacological effects by inhibiting release of several hormones (Growth hormone [GH], thyroid stimulating hormone [TSH], insulin, glucagon and all gut hormones), exocrine secretions (gastric acid, pancreatic enzymes) and their re-absorption from the intestines [6]. There are many clinical and experimental studies in which octreotide were used for treatment of acute pancreatitis. Results of these studies are mixed [7-14].

Diclofenac sodium is derivative of phenylacetic acid as analgesic, anti-inflammatory and antipyretic. In recent clinical trials, healing effects of especially the use of rectal or parenteral diclofenac sodium on treatment of post ERCP pancreatitis are shown [15-20].

We created caerulein induced experimental acute pancreatitis and aimed to show that effects on pancreatic enzymes, pancreatic myeloperoxidase activity, histopathology and apoptosis of pancreas of diclofenac sodium and octreotide together or alone on treatment of caerulein induced acute pancreatitis. Thus, we aimed to open new horizons about the pathogenesis and treatment of acute pancreatitis.

Materials and methods

The current study was conducted using a total of 58 male BALB-C mice of 25 gr after taking approval from the local ethical committee of Research and Application Center for the Experimental Animals of Necmettin Erbakan University. The experimental groups are seven in number and as follows: the control group (saline): Group 1; acute pancreatitis control group: Group 2; (with intraperitoneal [IP] caerulein); octreotide control group: Group 3; diclofenac sodium control group: Group 4; group of octreotide treatment in acute pancreatitis: Group 5; group of diclofenac sodium treatment in acute pancreatitis: Group 6, and group of octreotide and diclofenac sodium treatment in acute pancreatitis: Group 7. A total of 58 experimental animals were used: 6 in the Group 1, 9 in the Group 2; 6 in the Group 3; 6 in the Group 4; 9 in the Group 5; 10 in the Group 6, and 12 in the Group 7. Standard laboratory foods and drinking water were given ad libitum, and 12:12 hours of light and dark cycles with temperature of 21-24°C were provided (the mice not used previously in any experiment received care for 2

days prior to the experiment). The mice could drink water freely but were starved for 12 hours prior to beginning of the experiment. Entire experimental procedure was conducted under anesthesia and the experiment was terminated 6 hours after the last injection by scarifying the animals.

Experimental protocol

One day before the experiment, blood samples were taken from the animals under anesthesia (for anesthesia, 7.5 mg of ketamin per 100 gr body weight and 2.5 mg of xylazine per 100 gr body weight were injected intramuscularly (i.m.) in 200 µL saline and about 0.2 ml of blood was taken from retroorbital part of the animal). On the day of experiment, the mice in the groups in which acute pancreatitis would be induced received a total of seven intraperitoneal injections of 50 µg/kg caerulein in 50 µL of 0.9% NaCl on hours 0 to 6 with intervals of one hour under anesthesia (the same as the previous anesthesia). The mice in the octreotide group received seven intraperitoneal injections of 250 µg/kg caerulein on hours 0 to 6 with intervals of one hour. The mice in the diclofenac sodium group received single injection of 15 mg/kg (one hour after the first injection of caerulein). All mice were killed under high dose of anesthesia after taking 1-2 ml of blood from their hearts through a syringe 6 hours after the last caerulein injection, and then their abdomen was opened and their pancreatic tissue was removed immediately. Pancreatic tissue was divided into two and one of them was placed in 10% formaldehyde and the other one was wrapped in an aluminum folio and kept at -20°C for investigation of myeloperoxidase activity, and then the experiment was terminated.

Measurement of amylase and lipase

Sera were separated from the blood samples taken from the mice into the protective-free biochemistry tubes after centrifuging the blood samples at 3000 G for 5 minutes. Serum levels of amylase and lipase were U/L measured in Abbott C16.000 (IL, USA) using original Abbott kits (Abbott Clinical Chemistry).

Detection of myeloperoxidase level

Tissue samples kept at -20°C until the day of experiment were homogenized in 0.5 ml of cold

phosphate buffer (100 mM KH_2PO_4 - K_2HPO_4 , pH: 7.4) using a homogeniser (Ika T10 basit ultra-turrax). The analyses were performed in clear supernatants obtained after centrifuging the homogenates at 13.000 G and +4°C for 20 minutes. Myeloperoxidase level was measured using EastBiopharm operated by ELISA method (cat. nr: CK-E20262). Its operational mode and principle are briefly as follows:

Anti-myeloperoxidase antibody labeled with standards, tissue homogenates and biotin were incubated in the wells covered by mice anti-myeloperoxidase antibody. Following wash-out, they were incubated with the chromogen solution A and B at 37°C for 10 minutes. The reaction was terminated after the incubation by adding acidic solution. Absorbance of the yellow color occurring in this way was measured spectrophotometrically at wavelength of 450 nm. Absorbance is directly proportional to myeloperoxidase concentration. Standard curve was drawn with the absorbance values corresponding to the standard myeloperoxidase concentrations. Using this standard curve, myeloperoxidase concentrations in the tissue homogenates were calculated in ng/ml. The results were expressed as ng/mg protein.

Detection of myeloperoxidase activity

Tissue samples kept at -20°C until the day of experiment were homogenized in ice water for 30 seconds in 9 mL of phosphate tampon (50 mM KH_2PO_4 - K_2HPO_4 , pH: 6, 0.5% hexadecyltrimethylammonium bromide (Sigma, USA)) per gram of wet tissue using a homogeniser (Ika T10 basit ultra-turrax). The homogenates underwent sonication in ice water for 10 seconds. The analyses were performed in clear supernatants obtained after centrifuging the homogenates at 13.000 G and +4°C for 20 minutes. The assay is based on kinetic measurement of absorbance of yellow-orange complex occurring as a consequence of oxidation of o-dianisidine by MPO in the presence of H_2O_2 at wavelength of 460 nm [21]. Phosphate buffer (50 mM, pH: 6.0) was prepared containing the measurement reagent: 0.167 mg/mL o-dianisidine dihydrochloride, 0.0005% H_2O_2 (30%). 15 µl of sample was mixed with 285 µl of measurement reagent. Blind reading was done with 15 µl of distilled water. After pipetting was done into the microplates, absorbance increase was measured at wavelength of 460 nm against

blind for 5 minutes in the ELISA microplate reader. Calculations were made on the graphic of reaction on which linear activity increase was observed. One unit of MPO activity was defined as amount of enzyme destroying 1 µmol of H_2O_2 at 25°C. Specific tissue MPO activity was calculated by dividing MPO levels determined as U/L to protein values as g/mL. The results were expressed as U/µg protein.

Protein amount in the samples was detected by BCA protein assay kit (Boster Biological Technology, cat. nr: AR0146). Bovine serum albumin was used as standard. Protein amount in the homogenates was calculated in mg/L.

Histopathological examination

A) Preparing the tissues: All tissues were fixed in 10% formaldehyde solution for 24 hours, and then underwent procedure of tissue following in the auto-technicon instrument (Leica ASP300). The tissues of which procedure of tissue following was completed in about 16 hours were embedded in paraffin blocks. Two sections of 5 µm were taken from these paraffin blocks onto the slides with lysine using a microtome device. One of the sections was stained with hematoxylin-eosin (H&E) dye and the other one with immune-histochemical apoptosis dye (Tunnel method).

B) Histopathological evaluation: H&E stained preparations were evaluated by a single experienced pathologist using Olympus BX51 light microscope in a blinded fashion. Edema, hemorrhage, parenchymal necrosis, fat necrosis, leukocyte infiltration and fibrosis were rated using previously described criteria. Scores ranged between 0 (no pathology) and 3 (most severe) in magnification field per 40 points for each parameter [22].

Apoptosis

Number of the stained cells was detected in the preparations undergoing staining with immune-histochemical apoptosis dye (Tunnel method) (Chemicon International, S7101, CA, USA Apop Peroxidase in situ apoptosis detection kit). The counting procedure was as follows: total number of cells on 3 separate areas on which staining was detected with the microscope's objective magnification of ×20 were averaged. Values were noted for each subject.

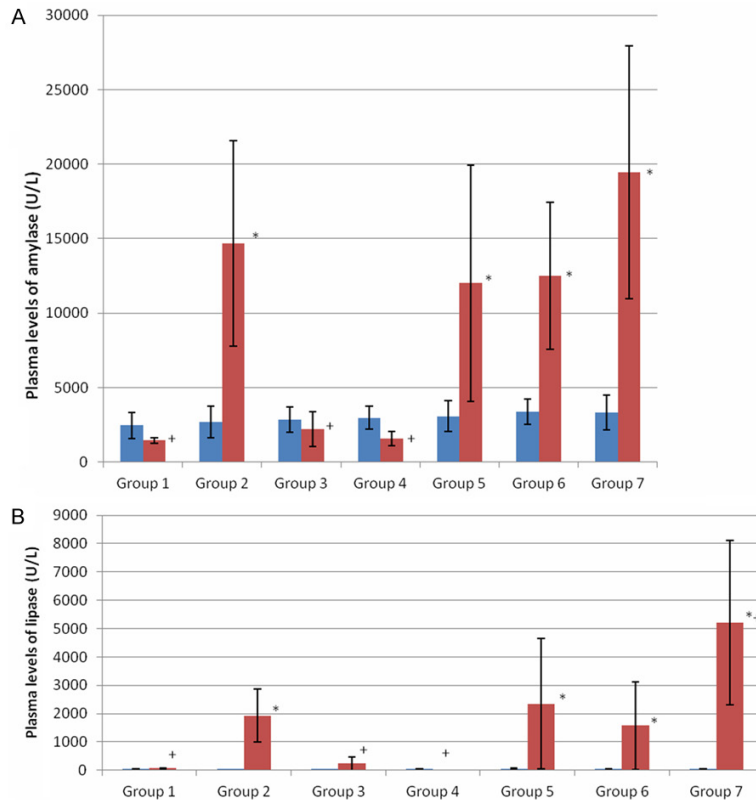


Figure 1. A. Plasma levels of amylase (U/L). Effects of octreotide and diclofenac sodium on acute-pancreatitis induced hyperamylasemia. ■: Represents baseline amylase levels for each group; ■: Represents post-treatment amylase levels. *P < 0.05 for comparison with the control group; +P < 0.05 for comparison with acute pancreatitis group. B. Plasma levels of lipase (IU/L). Effects of octreotide and diclofenac sodium on acute-pancreatitis induced hyperamylasemia. ■: Represents baseline lipase levels for each group; ■: Represents post-treatment lipase levels. *P < 0.05 for comparison with the control group; +P < 0.05 for comparison with acute pancreatitis group.

Statistical analysis

SPSS v.19.0 software was used for analyzing the statistical data and statistical significance level was chosen as $P < 0.05$. Arithmetical mean and standard deviation of the numerical data were calculated. Kruskal-Wallis and Mann-Whitney U tests were used in comparing the numerical data. For the categorical data, frequency and percentage were calculated and Pearson's Chi-square test and Kruskal-Wallis tests were used for statistical analyses. Mann-Whitney's U test was used in dual comparisons following Kruskal-Wallis test and statistical significance level was chosen as $P < 0.05$.

Results

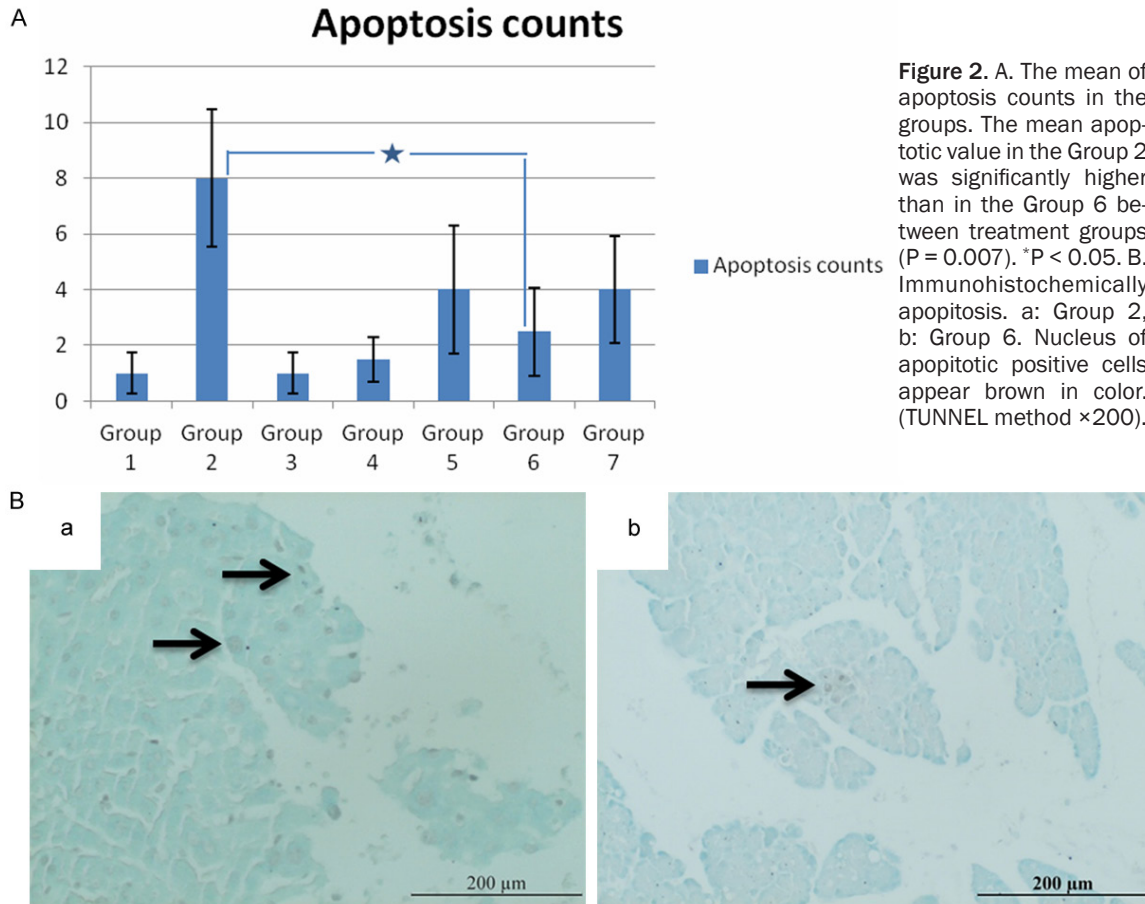
Group 1 was saline control group; Group 2 acute pancreatitis (AP) group; Group 3 octreo-

tide control (OCT) group; Group 4 diclofenac sodium (DS) control group; Group 5 octreotide + acute pancreatitis (AP+OCT) group; Group 6 diclofenac sodium + acute pancreatitis (AP+DS) group, and Group 7 was octreotide + diclofenac sodium + acute pancreatitis (AP+OCT+DS) group.

Amylase levels

Mean baseline amylase levels in the groups 1 to 7 were 2465 ± 865 U/L, 2711 ± 1063 U/L, 2865 ± 837 U/L, 2986 ± 775 U/L, 3089 ± 1024 U/L, 3386 ± 823 U/L and 3318 ± 1165 U/L, respectively. No statistically significant difference was found in baseline amylase levels among the groups ($P = 0.811$). Post-treatment amylase levels in the groups were 1456 ± 200 U/L, 14671 ± 6883 U/L, 2212 ± 1177 U/L, 1563 ± 483 U/L, 12009 ± 7945 U/L, 12521 ± 4934 U/L, and 19455 ± 8487 U/L, respectively. Statistically significant differences were found in post-treatment mean amylase levels among the groups ($P = 0.001$). When Mann-

Whitney U test was applied after Kruskal-Wallis test in multiple comparisons to find source of the significance, post-treatment mean amylase level was found to be significantly lower in Group 1 than in the Groups 2, 5, 6, and 7 ($P = 0.001$, $P = 0.004$, $P = 0.002$, $P = 0.001$, respectively). There was no significant difference between Group 1 and Groups 3 and 4 ($P = 0.360$ and $P = 0.583$, respectively). Post-treatment mean amylase level in the Group 2 was found to be significantly higher than in the Groups 1, 3, and 4 ($P = 0.001$, $P = 0.003$, and $P = 0.003$, respectively). Statistically significant difference was not found between the Group 2 and the Groups 5, 6, and 7 ($P = 0.556$, $P = 0.501$, and $P = 0.210$, respectively). Based on these results, it may be concluded that the best decline in mean post-treatment amylase levels was in the octreotide group and the worst one



was in octreotide + diclofenac sodium group. But the difference was not significant ($P > 0.05$) (Figure 1A).

Lipase levels

Baseline lipase levels in the groups 1 to 7 were 46 ± 14 U/L, 55 ± 4 U/L, 51 ± 10 U/L, 45 ± 12 U/L, 56 ± 13 U/L, 48 ± 12 U/L, and 48 ± 16 U/L, respectively. There were no statistically significant differences in baseline lipase levels among the groups ($P = 0.907$). Post-treatment lipase levels in the groups 1 to 7 were 69 ± 23 U/L, 1928 ± 932 U/L, 237 ± 245 U/L, 33 ± 4 U/L, 2350 ± 2402 U/L, 1578 ± 1649 U/L, and 5210 ± 2892 U/L.

Distribution of post-treatment mean serum lipase levels in the groups showed statistically significant difference ($P = 0.001$). When Mann-Whitney U test was applied after Kruskal-Wallis test in multiple comparisons to find source of the significance, post-treatment mean lipase level was found to be significantly lower in

Group 1 than in the Groups 2, 5, 6, and 7 ($P = 0.001$, $P = 0.004$, $P = 0.002$, and $P = 0.001$, respectively). There was no significant difference compared to the groups 3 and 4 ($P = 0.357$ and $P = 0.129$, respectively). Post-treatment mean lipase level was found to be significantly higher in Group 2 than in the Groups 1, 3, and 4 ($P = 0.001$, $P = 0.003$, and $P = 0.003$, respectively). No significant difference was found compared to the Groups 5 and 6 ($P = 0.814$ and $P = 0.178$, respectively). Post-treatment serum lipase level in the Group 7 was found to be statistically significantly higher than in the Group 2 ($P = 0.003$). Based on these results, it may be concluded that the best decline in mean post-treatment lipase levels was in the diclofenac sodium group and the worst one was in octreotide + diclofenac sodium group (Figure 1B).

Myeloperoxidase activity

Detection of MPO activity in the pancreatic tissue is used as biochemical marker of neutro-

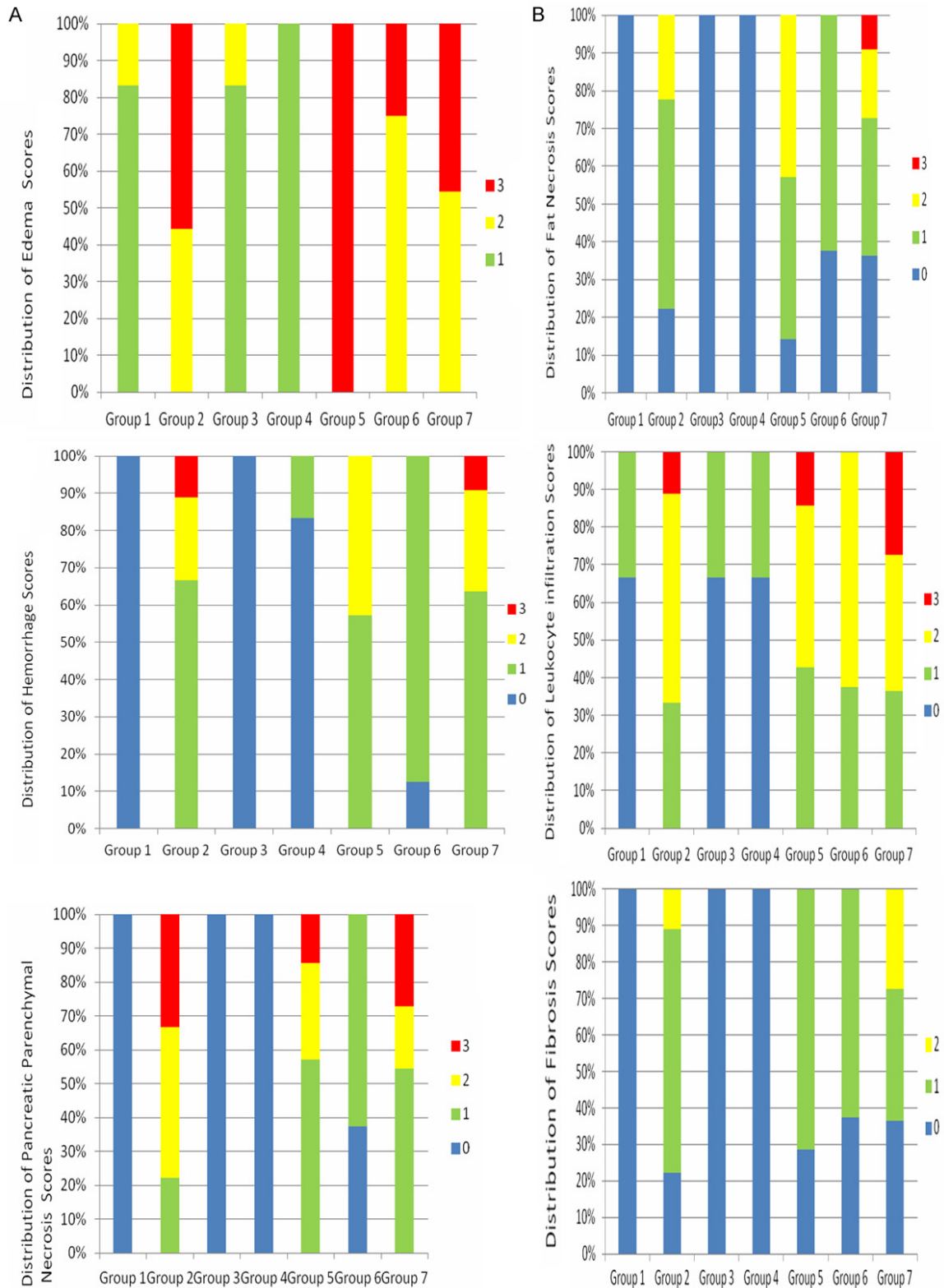


Figure 3. A. Distribution of histopathological scores for edema, hemorrhage and parenchymal necrosis in the pancreatic tissue among the groups. B. Distribution of histopathological scores for fat necrosis, leukocyte infiltration and fibrosis in the pancreatic tissue among the groups.

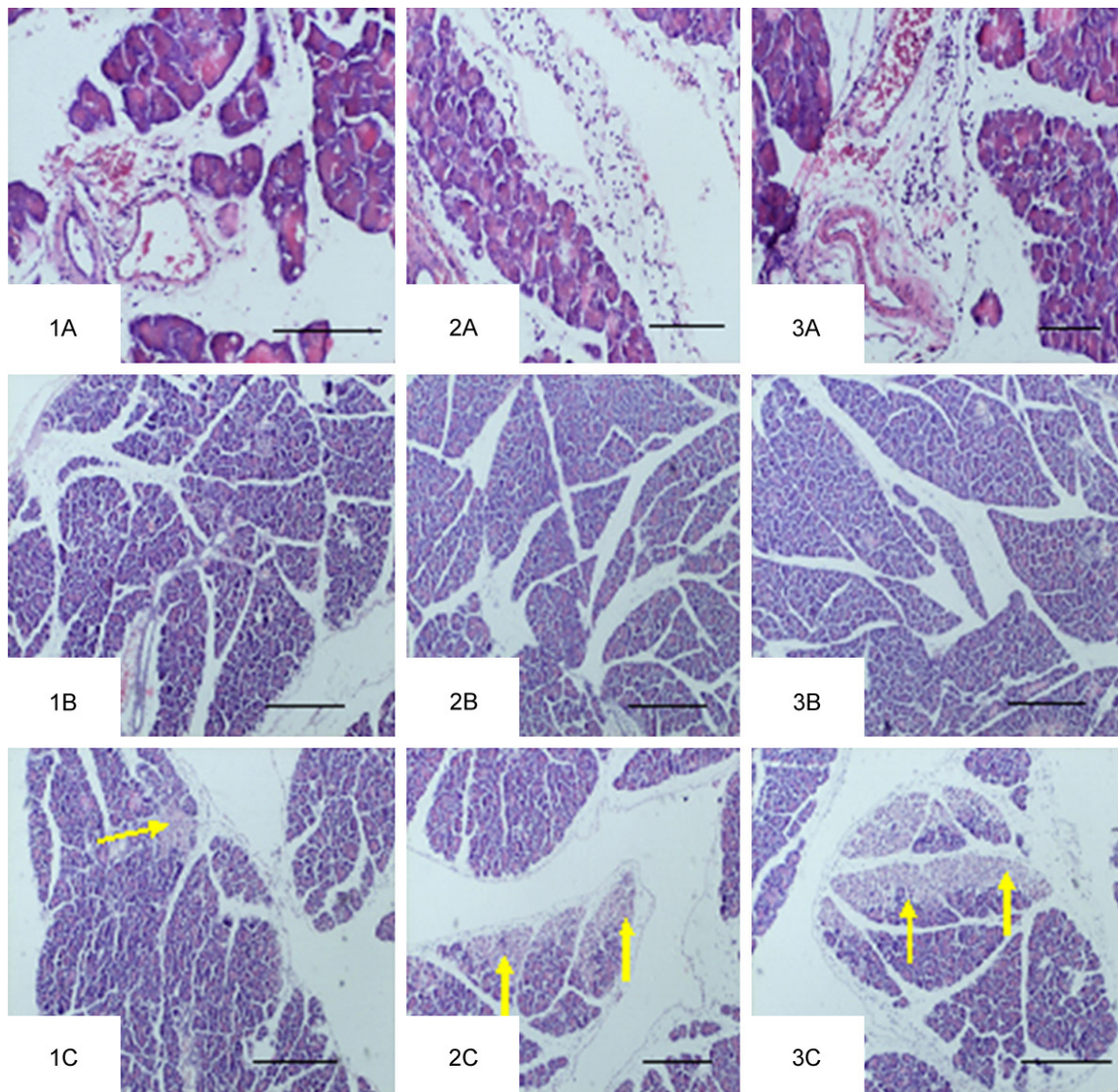


Figure 4. Group 6 (1), Group 5 (2), Group 7 (3) groups of hemorrhage and inflammation (A), edema (B), parenchymal necrosis (C-yellow arrows). (A. Hematoxylin & Eosin $\times 100$, Bar = 100 μm ; B, C. Hematoxylin & Eosin $\times 40$, Bar = 40 μm).

phil infiltration. Distribution of mean values for myeloperoxidase (MPO) activity in the pancreatic tissue showed statistically significant differences among the groups ($P = 0.029$). Mean values for the pancreatic MPO activity in the Groups 1 to 7 were 0.5 ± 0.1 U/ μg , 2 ± 1.7 U/ μg , 0.5 ± 0.1 U/ μg , 0.5 ± 0.2 U/ μg , 0.9 ± 0.2 U/ μg , 1 ± 0.4 U/ μg , and 0.9 ± 0.4 U/ μg , respectively. Statistically significant differences were found in mean values for MPO activity among the groups ($P = 0.029$). Mean values for pancreatic MPO activity in the treatment groups (Groups 5, 6, and 7) were lower than in the acute pancreatitis group (Group 2). When Mann-Whitney U

test was applied after Kruskal-Wallis test in multiple comparisons to find source of the significance, post-treatment mean values for myeloperoxidase (MPO) activity in the pancreatic tissue was found to be significantly lower in Group 1 than in the Groups 2 and 5 ($P = 0.019$ and $P = 0.014$, respectively). No significant difference was found in Group 1 compared to the Groups 3, 4, 6 and 7 ($P = 1.000$, $P = 0.773$, $P = 0.062$, and $P = 0.217$, respectively). When the Group 2 was compared with other groups, it was found that the value in the Group 2 was statistically significant higher than in the Groups 1, 3, and 4 ($P = 0.019$, $P = 0.019$, and P

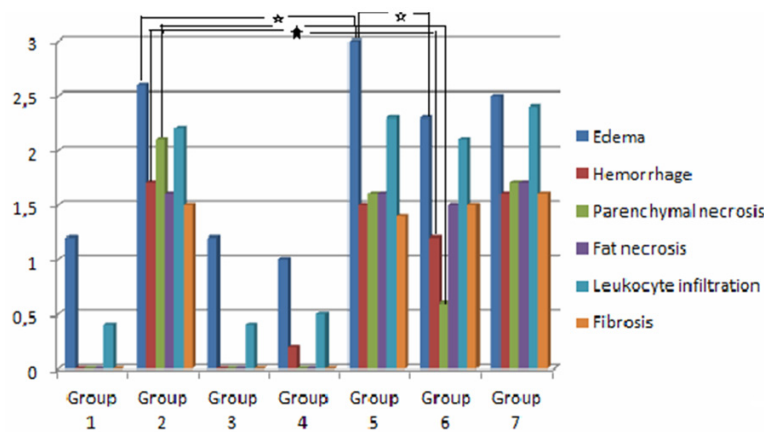


Figure 5. The mean of histopathological scores. It was observed that edema was statistically significant higher in the Group 5 compared to Group 2 ($P = 0.049$) Edema scores were significantly lower in the diclofenac group when compared to the octreotide group ($P = 0.025$). When the Group 2 was compared to Group 6 in terms of presence of hemorrhage, it was found that hemorrhage was significantly lower in the Groups 6 ($P = 0.052$). When the Group 2 was compared to the other groups, parenchymal necrosis was significantly lower in the Groups 6 ($P = 0.002$) * $P < 0.05$.

= 0.019, respectively). There was no significant difference relative to the Groups 5, 6 and 7 ($P = 0.361$, $P = 0.366$, and $P = 0.126$, respectively).

Apoptotic values for the pancreatic tissue

Mean pancreatic apoptotic values of the groups 1 to 7 were 1.00 ± 0.75 , 8.00 ± 2.47 , 1.00 ± 0.75 , 1.50 ± 0.81 , 4.00 ± 2.28 , 2.50 ± 1.58 , and 4.00 ± 1.91 , respectively. Distribution of mean apoptotic values of the pancreatic tissue in the groups showed statistically significant differences ($P = 0.001$) (Figure 2A, 2B). When Mann-Whitney U test was applied after Kruskal-Wallis test in multiple comparisons to find source of the significance, significant different was found between Group 1 and the Groups 2, 5, 6, and 7 ($P = 0.001$, $P = 0.003$, $P = 0.003$, and $P = 0.001$, respectively). No significant difference was found in Group 1 compared to the Groups 3 and 4 ($P = 1.000$, and $P = 0.268$, respectively). In comparisons of mean apoptotic values of the Group 2 with other groups, the mean apoptotic value in the Group 2 was significantly higher than in the Group 1, 3, 4, and 6 ($P = 0.001$, $P = 0.001$, $P = 0.001$, and $P = 0.007$, respectively). No significant difference was found in Group 2 comparison with Groups 5 and 7 ($P = 0.078$ and $P = 0.045$, respectively).

Histopathological scores

Histopathological presence of edema, hemorrhage, parenchymal necrosis, fat necrosis, leukocyte infiltration, and fibrosis in the pancreatic tissue of the Groups 1 to 7 was given in Figures 3A, 3B and 4. Figure 3. A. Distribution of histopathological scores for edema, hemorrhage and parenchymal necrosis in the pancreatic tissue among the groups. B. Distribution of histopathological scores for fat necrosis, leukocyte infiltration and fibrosis in the pancreatic tissue among the groups.

Figure 4. Group 6 (1), Group 5 (2), Group 7 (3) groups of hemorrhage and inflammation (A), edema (B), parenchymal necrosis (C-yellow arrows). (A: Hematoxylin & Eosin $\times 100$, Bar = 100 μm ; B, C: Hematoxylin & Eosin $\times 40$, Bar = 40 μm).

Distribution of mean edema scores of pancreatic tissue in the groups showed statistically significant difference ($P < 0.01$). When Mann-Whitney U test was applied after Kruskal-Wallis test in multiple comparisons to find source of the significance, significant differences were found between Group 1 and the Groups 2, 5, 6, and 7 ($P = 0.001$, $P = 0.001$, $P = 0.003$, and $P = 0.001$, respectively). There was no significant difference in Group 1 compared to other groups ($P > 0.05$). When the Group 2 was compared to other groups, edema in the groups 1, 3, and 4 was significantly lower than in the other groups ($P = 0.001$, $P = 0.002$, and $P = 0.001$, respectively). Edema was non-significantly lower in the Group 6 compare to Group 2 ($P = 0.215$). There was no difference for edema in Group 2 compared to Group 7 ($P = 0.661$). It was observed that edema was statistically significant higher in the Group 5 compared to Group 2 ($P = 0.049$) Edema scores were significantly lower in the diclofenac group when compared to the octreotide group ($P = 0.025$) (Figure 5).

Distribution of mean hemorrhagic scores of the pancreatic tissue in the groups showed statistically significant difference ($P < 0.01$). No hem-

orrhage was observed in the Groups 1, 3, and 4 whereas first and second degree of hemorrhage was observed in the Groups 2, 5, 6, and 7. When the Group 1 and other groups were compared in order to look at source of the significance, statistically significant differences were found compared to the Groups 2, 5, 6 and 7 ($P = 0.001$, $P = 0.001$, $P = 0.002$, and $P = 0.001$, respectively). No significant difference was found in Group 1 compared to Group 3 and Group 4 ($P > 0.05$). When the Group 2 was compared to other groups in terms of presence of hemorrhage, it was found that hemorrhage was significantly lower in the Groups 1, 3, 4, and 6 ($P = 0.001$, $P = 0.001$, $P = 0.002$, and $P = 0.052$, respectively). There was no significant difference in Group 2 compared to Group 5 and Group 7 for hemorrhage ($P > 0.05$).

Distribution of mean scores of parenchymal necrosis in pancreatic tissue among the groups showed statistically significant difference ($P = 0.001$). Parenchymal necrosis wasn't observed in the Groups 1, 3, and 4. Parenchymal necrosis was observed in the Groups 2, 5, 6, and 7. When the Group 1 and other groups were compared in order to look at source of the significance, statistically significant differences were found compared to the Groups 2, 5, 6 and 7 for parenchymal necrosis ($P = 0.001$, $P = 0.001$, $P = 0.002$, and $P = 0.001$, respectively). There was no significant difference in Group 1 compared to Group 3 and Group 4 ($P > 0.05$). When the Group 2 was compared to the other groups, parenchymal necrosis was significantly lower in the Groups 1, 3, 4, and 6 ($P = 0.001$, $P = 0.001$, $P = 0.001$, and $P = 0.002$, respectively). There was no significant difference in Group 2 when compared to Group 5 and Group 7 for parenchymal necrosis ($P > 0.05$).

Distribution of mean scores of fat necrosis in the pancreatic tissue among the groups showed statistically significant difference ($P = 0.001$). Fat necrosis wasn't observed in the Groups 1, 3, and 4. Fat necrosis was observed in the Groups 2, 5, 6, and 7. When the Group 1 and other groups were compared in order to look at source of the significance, statistically significant differences were found compared to the Groups 2, 5, 6 and 7 ($P = 0.006$, $P = 0.005$, $P = 0.002$, and $P = 0.017$, respectively). There was no significant difference in Group 1 compared to the Group 3 and Group 4 for fat necrosis ($P >$

0.05). When the Group 2 was compared to the other groups, statistically significant differences were found compared to the Groups 1, 3, and 4 ($P = 0.006$, $P = 0.006$, and $P = 0.006$, respectively). There was no difference in Group 2 compared to Group 5, Group 6 and Group 7 for fat necrosis ($P > 0.05$).

Distribution of mean scores of leukocyte infiltration in the pancreatic tissue among the groups showed statistically significant difference ($P = 0.002$). Leukocyte infiltration in the Groups 1, 3, and 4 were not observed or first degree of leukocyte infiltration was observed. Second and third degree of leukocyte infiltrations were observed in the Groups 2, 5, 6, and 7. When the Group 1 and other groups were compared in order to look at source of the significance, statistically significant differences were found compared to the Groups 2, 5, 6 and 7 ($P = 0.003$, $P = 0.007$, $P = 0.004$, and $P = 0.002$, respectively). There was no difference compared to the other groups ($P > 0.05$). When the Group 2 was compared to the other groups, statistically significant differences were found compared to the Groups 1, 3, and 4 ($P = 0.003$, $P = 0.003$, and $P = 0.003$, respectively). There was no difference in Group 2 compared to Group 5, 6 and 7 for leukocyte infiltration ($P > 0.05$).

Distribution of mean fibrosis scores in the pancreatic tissue among the groups showed statistically significant difference ($P = 0.002$). No fibrosis was observed in the Groups 1, 3, and 4, although first degree of fibrosis was observed in the Groups 2, 5, 6, and 7. When the Group 1 and other groups were compared in order to look at source of the significance, statistically significant differences were found compared to the Groups 2, 5, 6 and 7 ($P = 0.005$, $P = 0.011$, $P = 0.020$, and $P = 0.017$, respectively). No difference was found compared to the other groups ($P > 0.05$). When the Group 2 was compared to the other groups, statistically significant differences were found compared to the Groups 1, 3, and 4 ($P = 0.005$, $P = 0.005$, and $P = 0.005$, respectively). There was no difference in Group 2 compared to Group 5, 6 and 7 for fibrosis ($P > 0.05$).

Discussion

There is no specific treatment for acute pancreatitis, suggesting a complex pathophysiology

involving many factors. Alternative treatment strategies involving one or more than one therapeutic agents alone or in combination targeting multiple stages in pathogenesis of the condition should be investigated. Caerulein-induced acute pancreatitis is a widely used model in exploring and developing such therapeutic strategies [23]. Octreotide is a long-acting somatostatin analogue. It has been studied in many studies on AP. Logic for using octreotide in treatment of acute pancreatitis is based on several observations. Based on these observations, it was observed that its effects originated from its ability to decrease focal pancreatic tissue necrosis and reduce the leukocyte infiltration in acute pancreatitis and its inhibitory effect on exocrine pancreatic secretion [7, 24]. Octreotide has been used in treatment and prevention of post-ERCP pancreatitis (PEP) due to its ability to reduce tonus of Oddi's sphincter and reducing reflux of duodenal content into the pancreatic canal [25]. In regard to therapeutic agents in acute pancreatitis, effects of octreotide on exocrine pancreatic secretion have been studied in many studies on man and animals [7-9, 26-31]. It was shown in previous studies that octreotide improved acute pancreatitis through inhibition of baseline and stimulated exocrine pancreatic secretion [32]. In experimentally induced acute pancreatitis, octreotide has been shown to improve acute pancreatitis by keeping the pancreas in rest, reducing the degree of leukocyte infiltration, and limiting the focal pancreatic tissue necrosis [7, 33]. Publications on effects of somatostatin and octreotide on exocrine pancreatic secretion, however, are conflicted and the studies have emphasized complexity of the system and use of concentrations with different species and that timing of starting the treatment affected the outcomes of treatment [7, 8, 10-14].

In the current study, we observed that the octreotide + acute pancreatitis group showed decreased level of amylase compared to the acute pancreatitis group but the difference wasn't statistically significant. This failure of octreotide to decrease plasma enzyme levels has been shown in previous studies using similar models [10, 11, 28].

Involved mechanisms, however, are not clear. A possible mechanism has been suggested by

Jenkins et al. [34]. The authors suggested that the enzyme level resulted in "escape phenomenon" although inhibition of all fluid secretions continued for 4 to 5 hours during continuous use of the agent following initial inhibition of the pancreatic enzymes and all other fluids in acute pancreatitis. They said that this "escape phenomenon" might reflect desensitization of the somatostatin receptors involving regulation of pancreatic amylase secretion. Thus, they advocated that caerulein-induced endogenous release of somatostatin occupied the somatostatin receptors regulating the acinar cellular secretion and thus, as a consequence of preventing binding of octreotide, no change would be observed with octreotide treatment in enzymatic elevation induced by acute pancreatitis [34]. In another mechanism described by Garvin et al., the authors suggested that this might have originated from the fact that octreotide failed to reduce cholecystokinin-induced amylase secretion [35].

Octreotide has been observed to reduce pancreatic enzyme elevation in acute pancreatitis model created by biliary tract ligation [8]. It has also been shown to reduce biochemical serum parameters compared to the group of acute necrotizing pancreatitis in experimental studies using sodium taurocholate injection [11, 36, 37]. In another study, it was shown to have minimal impact on biochemical and morphometric values [29]. No useful effect of octreotide was observed in decreasing pancreatic enzymes and reducing edema in the pancreatic tissue in sodium taurocholate-induced acute pancreatitis [38]. Octreotide has been shown to reduce lipase levels significantly in experimental acute pancreatitis induced by ischemia-reperfusion [39, 40].

In a study, it was suggested that octreotide induced apoptosis in the pancreatic acinar cells and thus, improved acute pancreatitis by reducing inflammatory reaction. In the same study, no difference was found with acute pancreatitis group in decreasing plasma amylase level [41, 42]. In our study, no significant difference was found for it in decreasing pancreatic amylase and lipase levels compared to pre-treatment levels in the acute pancreatitis group. Furthermore, apoptotic values were observed to be lower than in octreotide + acute pancreatitis group than in the acute pancreati-

tis group. But no significant difference was observed between two groups.

Studies conducted so far have shown that edema is minimally less in the octreotide group than in the acute pancreatitis group [41, 43], and minimal decrease occurs in plasma levels of amylase [44]. Another study showed that octreotide had no effect on plasma levels of amylase and lipase but had positive effects on pancreatic myeloperoxidase activity and pancreatic histopathology [23]. In the current study, we observed that edema was more in the octreotide + acute pancreatitis group compared to the acute pancreatitis group but didn't find significant difference between two groups ($P = 0.049$).

Non-steroidal anti-inflammatory drugs (NSAIDs) are inhibitor of cyclooxygenase (COX) enzyme catalyzing production of the prostaglandins and thromboxanes from arachidonic acid. Studies on effects of the SAIDs on experimental acute pancreatitis are conflicted and no effect of the NSAIDs on mortality has been demonstrated [45-48]. In a study looking at therapeutic effect of NSAIDs on acute pancreatitis, no difference was found in serum levels of amylase and calcium between the indomethacin and placebo groups and it was shown that analgesic need was less in the indomethacin group [49].

In regard to effects of diclofenac sodium and H₂S-releasing diclofenac sodium on lung damage associated with caerulein-induced acute pancreatitis, it has been shown that both drugs had no effect on pancreatic damage, H₂S-releasing diclofenac sodium reduced lung inflammation significantly but had no effect on pancreatic damage [50]. In many studies conducted so far, diclofenac sodium has been shown to reduce post-ERCP pancreatitis [15-19], but no effect of oral diclofenac sodium has been demonstrated on PEP [20].

In the current study, effects of diclofenac sodium in reducing apoptosis, improving histopathological findings of hemorrhage and parenchymal necrosis was statistically significant compared to the acute pancreatitis group. However, no significant effect on mean apoptotic values was found in the octreotide and combination groups compared to the acute pancreatitis group. No significant difference was found wh-

en effects of diclofenac sodium in decreasing serum levels of amylase and lipase and reducing pancreatic MPO activity was compared to the octreotide and combination groups. Compared to the acute pancreatitis group, plasma level of amylase was found to be lower in the octreotide group and plasma level of lipase in the diclofenac sodium group. But the difference was not significant. Mean values of pancreatic MPO activity was lower in the octreotide, diclofenac sodium, and combination treatment groups than in the acute pancreatitis group, suggesting that all three therapeutic options decreased pancreatic neutrophil infiltration. The difference, however, was not significant. Non-significance might mean lack of homogenization in values of pancreatic MPO activity in the acute pancreatitis group. Compared to the acute pancreatitis group, no statistically significant difference was found in the octreotide and combination groups in terms of histopathological healing (edema, hemorrhage, pancreatic parenchymal necrosis, fat necrosis, leukocyte infiltration, and fibrosis).

Reduced apoptosis and parenchymal necrosis in the Group 6 compared to Group 2 may indicate that diclofenac sodium might provide a therapeutic benefit through reduction of cellular damage during acute pancreatitis process. However, further studies are warranted to have firm evidence supporting such an effect. The lack of favorable effects in the combination group may be due to an interaction between the two agents.

Conclusions

In conclusion, diclofenac sodium, a cost-effective agent with a favorable side-effect profile, may represent a novel therapeutic agent for the treatment of acute pancreatitis. Our observations suggest a better efficacy for diclofenac sodium monotherapy as compared to octreotide alone or octreotide/diclofenac combination in terms of reduced apoptosis and improved histopathology. However, it should be noted that no effect on acute pancreatitis itself have been found for the other treatments including the combination.

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Disclosure of conflict of interest

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