

## The effects of 2-aminoethyl diphenylborinate on L-Arginine induced acute pancreatitis in the rats

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### Abstract

**Objective:** The aim of the study is to investigate the protective effect of 2-aminoethyl diphenylborinate on an acute pancreatitis model through an experimental study.

**Materials and Methods:** 30 Sprague-Dawley male rats were randomly divided into three groups: Sham, Pancreatitis and Pancreatitis + 2-APB. Pancreatitis was induced by L-arginine administration. The therapeutic agent 2-APB was injected i.v. at a dose of 2 mg/kg 10 min before pancreatitis induction. From blood samples, superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity, tumor necrosis factor alpha, interleukin-6, aspartate aminotransferase, alanine aminotransferase and creatinine levels were measured and the rats were sacrificed subsequently. Tissue samples were evaluated histopathologically. TUNEL staining method was used to visualize apoptotic cells.

**Results:** 2-APB significantly reduced serum MDA and creatinine levels in pancreatitis + 2-APB group. Unfortunately, SOD levels reduced significantly, too. Edema and hemorrhage in pancreatic tissue were lower, necrosis and fibrosis were higher in the 2-APB administered group. Additionally, in 2-APB given group, it was found that vacuolisation, epithelial desquamation, and congestion reduced in renal tubular epithelial. The number of apoptotic cells did not change in the pancreatic tissue in TUNEL staining.

**Conclusions:** 2-APB reduces renal damage caused by acute pancreatitis. However, protective effect has not been on pancreatic tissue with 2-APB administered group. Although 2-APB, which was shown to prevent the degradation of kidney functions due to pancreatitis, do not minimize the pancreas tissue damage, it can improve the prognosis of pancreatitis by reducing the damage of distant organs

**Key words:** 2-aminoethyl diphenylborinate, acute pancreatitis, kidney, oxidative stress, antioxidants

### Introduction

Acute pancreatitis is a nonbacterial inflammatory process which may affect pancreas, peripancreatic tissues and distant organs. Normally, it is self-limiting but it sometimes may lead to serious medical conditions such as systemic inflammatory response syndrome, sepsis, multiple organ failure and death (1). Some factors such as age, ischemia-necrosis and comorbid diseases affect the prognosis. Another factor affecting the prognosis is the increase of oxygen free radicals (ROS). ROS affects prognosis by causing damage to both pancreas and distant organs (2).

Intracellular and mitochondrial Ca<sup>2+</sup> concentrations, which increase in relation to ATP concentrations decreasing during ischemia, play an important role in ischemic cell damage and ROS

increase (3). Store-operated calcium channels (SOCs) are the members of ion channel family that enables the transition of Ca<sup>2+</sup> to the intracellular space and located in the membrane of many cells (3-6). 2-aminoethyl diphenylborinate (2-APB) inhibits the entry of calcium into the cell from the extracellular space by blockading SOCs (4). Additionally, it prevents ischemic cell damage by contributing to intracellular calcium hemostasis.

In some studies conducted earlier, it was mentioned that SOCs blockade of renal efferent arteriole myocytes increases blood flow by doing vasodilatation (6, 7). However, in this experimental study, the effect of 2-APB on acute pancreatitis was examined. Our hypothesis was that the 2-APB can

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provide vasodilation in pancreatic arterioles through SOCs channel blockade, which may be useful over the course of pancreatitis by increasing pancreatic blood flow. Thus, we expected to have an antioxidant effect. In our study, as a sign of the protective effect, the biochemical markers of antioxidant activity in serum samples were primarily measured. And then histopathological examination was conducted.

## Materials and Methods

### Chemicals

2-APB (Sigma-Aldrich), L- arginine (Sigma-Aldrich), ketamin (Ketalar-Pfizer) and xylazine (Alfazyne, EGE-VET)

### Animals and treatment protocol

The approval of Çanakkale onsekizmart University Ethical Committee of Animal experiments was granted before the study was conducted. Animals were allowed ad libitum access to food and drink up to the study. In addition, animals were treated humanely throughout the protocol according to national health institution guidelines and rules about the care and use of laboratory animals.

30 Sprague-Dawley male rats were randomly divided into three groups: Sham, Pancreatitis and Pancreatitis + 2-APB. Pancreatitis was induced by 2 doses of 1,5 g/kg of L-arginine which was administered intraperitoneally at a 1 hour interval. The therapeutic agent 2-APB was used intravenous at a dose of 2 mg/kg 10 min before pancreatitis induction. Anesthesia was performed with i.m. ketamine/xylazine (90/10 mg/kg) injection. All rats were sacrificed in 24 h after experimental procedure. After this, blood samples were collected through inferior vena cava, kidney-pancreas was excised, and the rats were sacrificed subsequently. Superoxide dismutase (SOD), malondialdehyde (MDA), total antioxidant capacity (TAS), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine levels were measured from blood samples, Renal and pancreatic tissue samples were evaluated histopathologically. Apoptotic cells visualization was evaluated by TUNEL staining method.

### Proinflammatory cytokines, Antioxidant enzymes, MDA, AST, ALT and creatinine measurement

Blood samples were kept for 2 h at room temperature to ensure proper clotting. The samples were then centrifuged at 2500 g at 4 °C for 15 min and stored at -20 °C until analysis.

Double sandwich Elisa kits (eBioscience USA) were used to measure serum concentrations of tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6). The samples were incubated with xanthine oxidase solution for 1 h at 37 °C to measure SOD

activity in serum. Absorbance was read at 490 nm to generate superoxide anions. SOD activity was determined as the inhibition of chromagen reduction. In the presence of SOD, superoxide anion concentration reduced by yielding less colorimetric signal. SOD activity was shown in %. Lipid peroxidation was determined using the procedure described by Yoshioka et al (8), in which MDA, an end product of fatty acid peroxidation, reacts with TBA to form a colored complex with a maximum absorbance at 532 nm. TAS of the serum was determined by using an automated measurement method with a commercial available kit developed by Rel. The antioxidative effect of the sample against the potent free radical reactions initiated by the reduced hydroxyl radical was measured by this method. The results were explained as mmol Trolox equiv/L. ELISA plates were measured using a microplate reader at 450 nm. Serum ALT (Archem, A2221, Istanbul, Turkey) and AST (Archem, A2212, Istanbul, Turkey) activities were measured by commercial available kits at a Biochemistry Auto Analyzer (Sinnowa D280, China). Likewise, serum creatinine (Archem, A2162, Istanbul, Turkey) levels were measured similarly by using commercial available kits at the same Auto Analyzer.

### Histological analysis

Kidney and pancreas tissue specimens were sliced transversely, fixed in formalin solution (10%), dehydrated in alcohol and embedded in paraffin. Sections at 5- $\mu$ m thick were taken using a microtome and stained with hematoxylen-eosin. A pathologist who was blind to the groups examined the specimens and investigated dispersion of kidney and pancreas.

### TUNEL staining for detection of apoptotic cells

Apoptotic cells in the pancreas sections were identified using TUNEL assay by an observer who was blind to the group assignments. TUNEL staining was performed using a TUNEL assay kit according to the manufacturer's instructions (ApopTaq Peroxidase In Situ Apoptosis Detection Kit; S7101-KIT, Millipore) decrease.

### Statistical Analysis

Data was presented as means  $\pm$  SD. All statistical analyses were performed on SPSS 20.0. The one-way ANOVA was used to test for differences among groups. Tukey's HSD test was used for multiple comparisons. P values < 0.05 were considered significant.

Table 1: The mean levels of serum samples and statistical results in all experimental groups

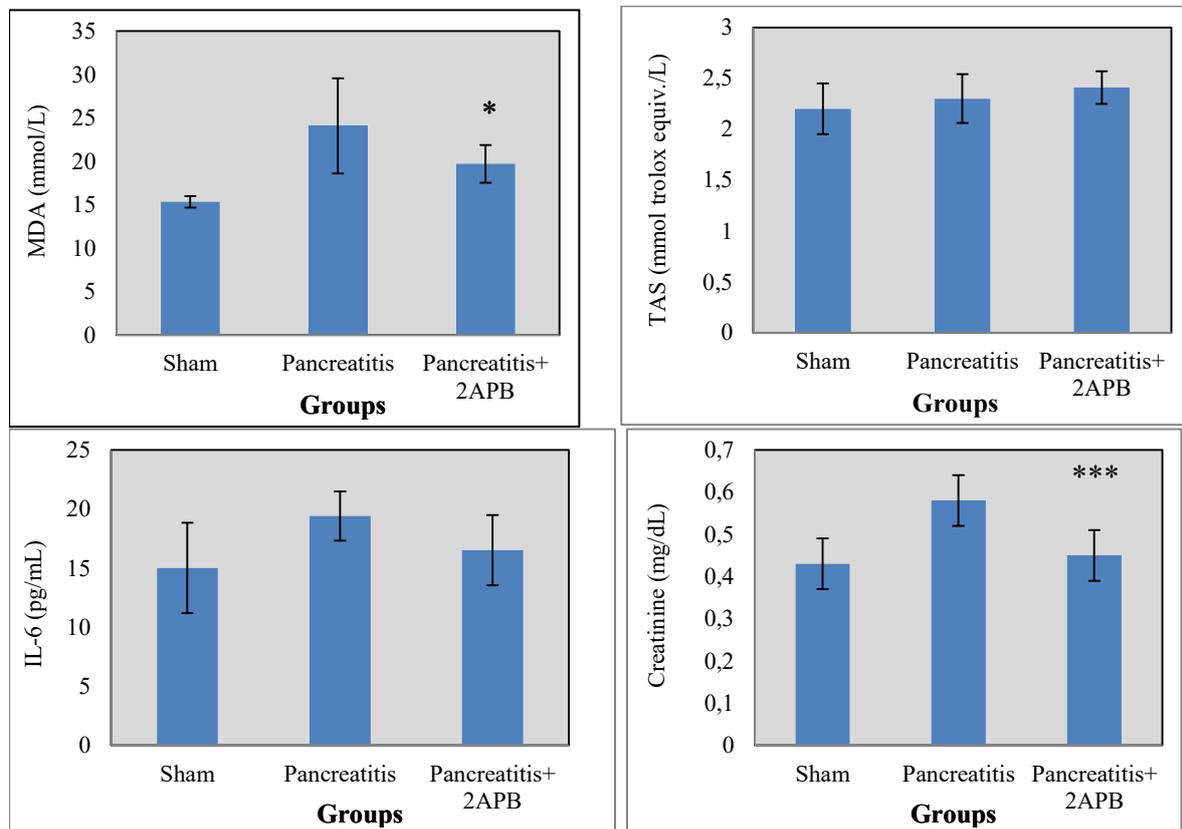
Groups	SOD (%)	MDA (mmol/L)	TAS (mmol trolox equiv./L)	TNF- $\alpha$ (pg/mL)	IL-6 (pg/mL)
Sham	64,75 $\pm$ 1,32	15,34 $\pm$ 0,67	2,20 $\pm$ 0,25	31,04 $\pm$ 6,80	15,01 $\pm$ 3,81
Pancreatitis	66,68 $\pm$ 0,80*	24,08 $\pm$ 5,49*	2,30 $\pm$ 0,24	23,79 $\pm$ 1,47*	19,39 $\pm$ 2,08*
Pancreatitis+2 APB	61,60 $\pm$ 2,29*†	19,70 $\pm$ 2,17*†	2,41 $\pm$ 0,16	22,46 $\pm$ 2,60*	16,51 $\pm$ 2,98

Results are expressed as the mean  $\pm$  standard deviation. \* P < 0.05 compared with the sham group.  
† P < 0.05 compared with the pancreatitis group.

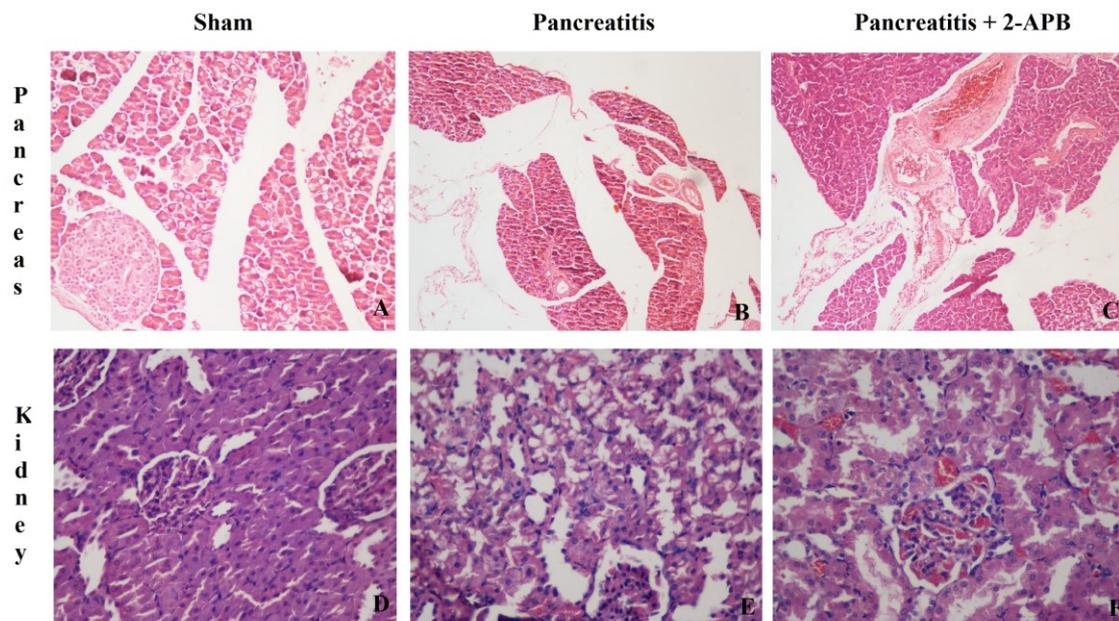
Table 2: The mean levels of serum samples and statistical results in all experimental groups

Groups	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)
Sham	224,50 $\pm$ 36,73	83,80 $\pm$ 43,17	0,43 $\pm$ 0,06
Pancreatitis	122,10 $\pm$ 44,32*	48,30 $\pm$ 11,70*	0,58 $\pm$ 0,06*
Pancreatitis + 2 APB	79,00 $\pm$ 9,51*†	48,50 $\pm$ 4,95*	0,45 $\pm$ 0,06*†

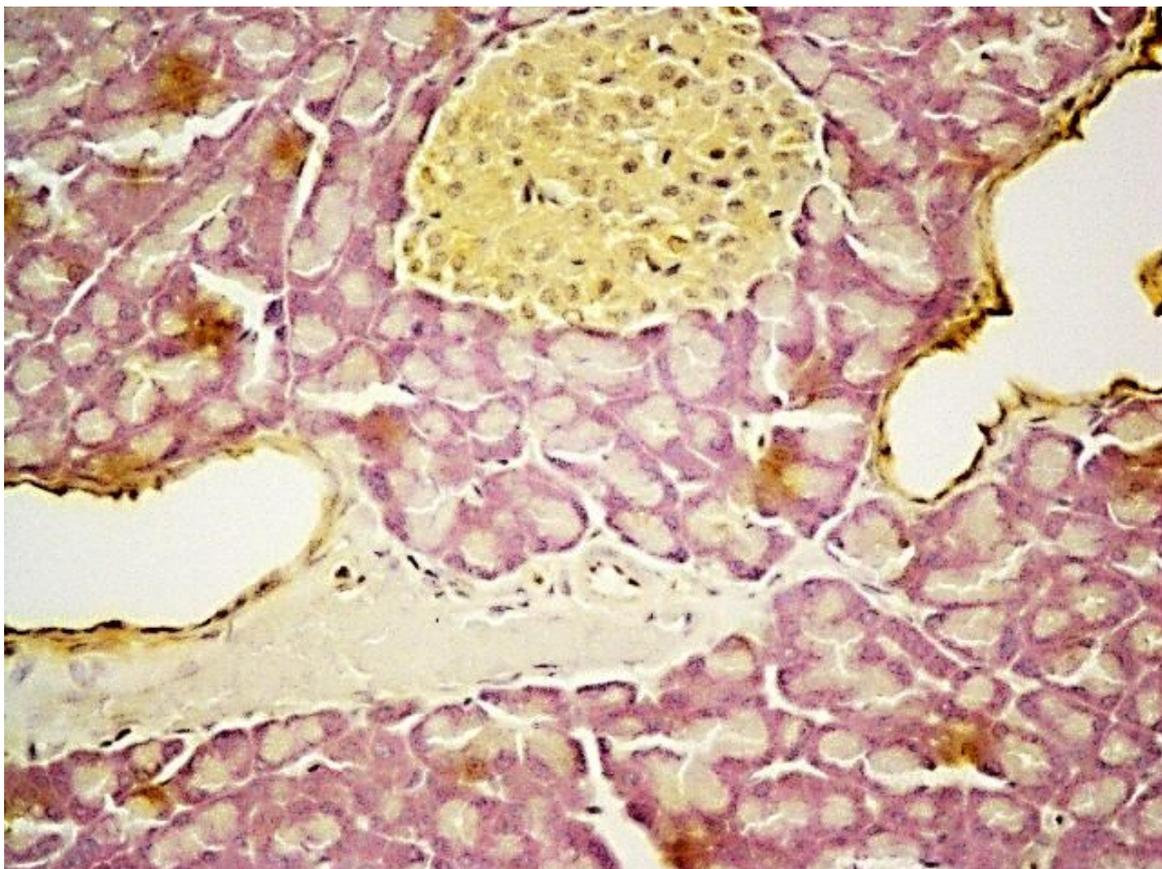
Results are expressed as the mean  $\pm$  standard deviation. \* P < 0.05 compared with the sham group.  
† P < 0.05 compared with the pancreatitis group.



**Figure 1.** Mean serum MDA, TAS, IL-6, and Creatinine levels in all experimental groups. Data are expressed as the mean  $\pm$  standard deviation. \*P < 0.05 compared with the Pancreatitis group, \*\*\*P < 0.001 compared with the Pancreatitis group.



**Figure 2.** Histopathologic examination with hematoxylin-eosin of pancreatic tissue shown in images (A), (B) and (C) (magnification, X200, X100 and X100 respectively ). Kidney tissue shown in images (D),(E) and (F) (magnification, X400).



**Figure 3.** TUNEL analysis for apoptotic cells in Pancreatitis group. TUNEL-positive cells were not encountered (magnification, X400).

## Results

### The effect of 2-APB on TNF- $\alpha$ and IL-6 levels

Mean values of TNF- $\alpha$  in the serum were significantly lower in the pancreatitis group than in the sham group ( $p=0.002$ ). Mean values of IL-6 in the serum were significantly higher in the pancreatitis group than in the sham group ( $p=0.009$ ). The pancreatitis induced increase in IL-6 and it was attenuated in the 2-APB administrated group ( $p=0.105$ ). However, decrease in TNF- $\alpha$  was relatively induced (Table 1 and Figure 1).

### The effect of 2-APB on SOD, TAS and MDA levels

Mean levels of SOD and TAS in the serum were higher in the pancreatitis group than in the sham group ( $p=0.030$  and  $p=0.557$ , respectively). Pancreatitis induced increase in TAS induced in the 2-APB administrated group ( $p=0.542$ ). However, SOD levels decreased ( $p<0.001$ ). Mean levels of MDA in the serum were higher in the pancreatitis group than in the sham group ( $p<0.001$ ). The pancreatitis that induced increase in this marker was significantly attenuated in the 2-APB administrated group ( $p=0.021$ ). The results are shown in Table 1 and Figure 1.

### The effects of 2-APB on AST, ALT, creatinine levels and histopathological results

AST and ALT levels in the serum were lower in the Pancreatitis group than in the sham group ( $p<0.001$  and  $p=0.013$ , respectively). AST levels were significantly lower in the group administrated 2-APB compared to the pancreatitis group ( $p=0.021$ ). However, levels of ALT did not change ( $p=1.000$ ), (Table 2). Mean serum level of creatinine was significantly elevated in the pancreatitis group compared to the sham group ( $p<0.001$ ). Serum levels of creatinine significantly decreased in 2-APB administrated group compared to the pancreatitis group ( $p<0.001$ ), (Table 2 and Figure 1).

Examination of pancreatic tissue sections stained with hematoxylen and eosin in the pancreatitis group revealed edema, hemorrhage, leukocyte infiltration. Edema and hemorrhage in pancreatic tissue were lower, while necrosis and fibrosis were higher in the 2-APB administered group. Examination of kidney tissue sections stained with hematoxylen and eosin in the pancreatitis group revealed vacuolization and desquamation of tubular epithelial cell and bleeding around the intertubular region and blood vessels. Severity of kidney tissue damage in the group administered 2-APB was lower than in the pancreatitis group (Figure 2).

### Effects of 2-APB on TUNEL staining

TUNEL-positive cells were not encountered in the pancreas from pancreatitis group (Figure 3).

## Discussion

2-APB reduces renal damage caused by acute pancreatitis. However, protective effect has not been on pancreatic tissue. Results of the present study have demonstrated that 2-APB can be used as an effective agent for reducing distant organ injury on pancreatitis as 2-APB significantly reduced creatinine levels in this study.

Up to now, several experimental pancreatitis models have been proposed and various drugs have been tested in order to reduce morbidity and mortality in acute pancreatitis. One of these models is formed with L-Arginine (9). Rakonczay Z et al. explained 300 mg/100 g as an appropriate dose for L-arginine in the induction of severe acute pancreatitis (10). Thus, 300 mg/100 g dose L-arginine was used to form acute pancreatitis in our study.

The pathogenesis of acute pancreatitis is multifactorial. It is stated in the studies that excessive production of oxygen free radicals and increase in cytokine levels are effective in the pathogenesis (2). Excessive production of ROS may damage cells by causing protease activation and lipid peroxidation. In this study, it was found that MDA level, which is an indicator of lipid peroxidation, decreased by means of 2-APB treatment (11).

Endogenous antioxidants such as SOD and endogenous antioxidant systems that involve these components protect cells from ROS damage. When ROS production increases, levels of antioxidant systems decrease (12). In our study, TAS levels increased once 2-APB was given.

In the studies conducted, it has been demonstrated that ROS causes the release of proinflammatory cytokines by stimulating macrophage, and these cytokines induce the inflammatory response which increases tissue damage. Proinflammatory cytokines such as TNF- $\alpha$  and IL-6 have an important role in the damage occurring in both tissue and distant organs by inducing polymorphomonuclear leukocytes activation and infiltration (13). In our study, only IL-6 levels decreased with 2-APB treatment.

Excessive ROS, proinflammatory cytokine release, or hypovolemia can cause kidney damage in acute pancreatitis (14). In our study, it was discovered that vacuolisation in renal tubular epithelial, desquamation and congestion in intertubular regions increased after pancreatitis. However, it was observed that the complaints decreased with 2-APB treatment. Additionally, after pancreatitis, it was seen that renal functions impaired probably due to increased systemic renal tubular injury, which caused an increase in serum creatinine levels. However, with 2-APB treatment, significant reduction of creatinine levels was observed.

## Conclusions

It was revealed in this study that the presence of 2-APB showed antioxidant activity in acute pancreatitis. However, the protective effect of this activity on pancreas was not shown in this study. Although 2-APB, whose protective effect on the degradation of kidney functions caused by pancreatitis was demonstrated, cannot minimize damage in pancreas tissue, it can improve the prognosis of pancreatitis by reducing the damage distant organs.

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**Conflict of interest statement:** Authors declare that there is no financial support or relationships that may pose potential conflict of interest.

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