ABSTRACT

Background: Apigenin (AP) is a flavonoid substance, in nature abundance, which is described to have anti-inflammatory and anti-oxidative effects. We investigated the effect of AP on acute pancreatitis (ACP) by measuring the values of interleukin-6 (IL-6), interleukin-18 (IL-18) and resistin (R) in serum and CD45 as well as high mobility group box 1 protein (HMGB1) in intestinal tissue using a rat experimental model.

Methods: 126 male Wistar rats, (age 90-120 days, body weight 250-350 g), were used. The rats were divided in three groups: Sham (S), Control (C) and Apigenin (Ap). The S group comprised 20 rats, which underwent laparotomy and closure of the abdominal wall. The C group comprised 56 rats, which underwent ligation of the pancreatic duct to induce acute pancreatitis. The apigenin group comprised 50 rats, which, after induction of ACP, received AP. Each group was divided into 5 subgroups according to the time euthanasia was performed (i.e., 6, 12, 24, 48 and 72 hours after laparotomy). Blood and intestinal tissue were collected.

Results: Comparing the C with the Ap group we observed an improvement in IL-6 and IL-18 examined in serum and in HMGB1 as well as in CD45 expression in intestinal mucosa. Following AP administration IL-6 and IL-18 decreased at 12h in the Ap group. HMGB1 increased significantly in C group and decreased remarkably in Ap group over time. CD45 decreased at 24h after AP administration in the Ap group. In contrast to the previous results R remained at low levels in the Ap group.

Conclusions: AP administration in rats in a biliary-pancreatic duct ligation experimental model of ACP appears to have a protective effect on intestine and serum parameters reducing the severity of ACP. Therefore administration of that substance in humans could improve the damage of the organs affected in the early stages of ACP.
Within a few hours activated monocytes are attracted, further stimulating local and systemic hyperproduction of pro-inflammatory cytokines and interleukins such as IL-6 and IL-18 [3,4]. HMGB1 and CD45 play an important role in mediating inflammation and regulating immune responses, respectively. HMGB1 acts as pro-inflammatory cytokine when released extracellularly under stress conditions, like ACP [5,6]. CD45 is present on pancreatic acinar cells having various roles in activation-induced signaling in leukocytes, related to the inflammatory responses [7,8]. Moreover, according to recent studies, a new effective indicator that can predict the severity of ACP and is used for its assessment is R [9]. Studies in early stages of ACP have difficulties in being performed on humans, as the diagnosis is established when pancreatic injury has already been initiated. As a consequence there is missing information about the facts that occur during the initial stages of ACP. This gap can be filled through research on experimental animal models of ACP in order to enrich our knowledge [10]. The beneficial role of AP, a flavonoid substance, has been described in several studies [11]. It is proven that has anti-inflammatory, anti-oxidative, anti-carcinogenic, anti-mutant, anti-allergic and anti-osteoporotic effects [12]. The aim of this study is to determine whether AP has positive effect on experimental model of ACP. In order to accomplish it, we determined IL-6, IL-18 and R levels in serum and HMGB1 and CD45 expression in intestinal tissue of animal models with ACP after AP administration at different time points.

MATERIALS AND METHODS

Animals and Study Design

126 albino male wistar rats, 90-120 days old, with a median weight of 280g (IQR=220-350g), were used. The animals were maintained in groups of four, in polycarbonate cages, under controlled conditions of temperature (22°C – 25°C), humidity (55%-58%) and light (12/12 hours light/dark) with free access to rat food and tap water ad libitum.

All animals were supplied by Pasteur Institute, Athens, Greece. The experiments took place at the approved Experimental Research Center of ELPEN Pharmaceuticals, Athens, Greece, and the immunohistochemistry and ELISA assays took place at the laboratories of Medical School of Democritus University of Thrace. All experimental procedures conformed to the principles laid by the National Research Council Guide for the Care and Use of Laboratory Animals and the Directive 86/609 of the European Union (protocol number K/2284).

The experimental animals were randomly assigned into 3 groups: 1) S group (S; n=20), in which animals underwent midline laparotomy without induction of ACP or AP administration; 2) C group (C; n=56), in which animals underwent midline laparotomy with induction of ACP via ligation of the common bilio-pancreatic duct; and 3) Ap group (Ap; n=50), in which animals underwent midline laparotomy with injection of ACP following postoperative per os administration of AP. The animals of each group were further subdivided into five subgroups according to the euthanasia time, i.e., 6h (C6, Ap6), 12h (C12, Ap12), 24h (C24, Ap24), 48h (C48, Ap48) and 72h (C72, Ap72). In the Ap group, the substance was administered as follows: i) Ap1 (n=10): 1 dose of 4mL, ii) Ap2 (n=10): 1 dose of 4mL, iii) Ap3 (n=10): 1 dose of 4mL, iv) Ap4 (n=10): 2 doses of 4mL with a 24 h interval, v) Ap5 (n=10): 3 doses of 4mL with 24 h intervals, followed by euthanasia. The overall study design was based on a previous publication by members of our research group [13].

Protocol of Experimentally Induced ACP in Rats

Induction in anaesthesia was performed by placing each experimental animal in a special glass box connected to isoflurane supply for about 2 to 3 min, followed by subcutaneously injected 0.25mL of Butorphenol 10 mg/mL (Dolorex;Intervet/Schering/Plough Animal Health, Boxmeer, Holland). Endotracheal intubation was performed under direct laryngoscopy by trained staff using a 16-G venous catheter connected to an Ugo-Basile ventilator for rodents (Harvard Apparatus, Holliston, MA), which was adjusted at tidal volume of 3 mL and respiratory rate of 70 breaths/min. Chest expansion and lung auscultation was indicating a proper intubation.

With the experimental animal placed in a supine position, a midline laparotomy of about 3cm was performed under sterile conditions. After the entry in the abdominal cavity the bilio-pancreatic duct was identified and ligated close to the duodenum with a non-absorbable 4/0 silk suture for the induction of ACP [14]. 1 mL of NaCl 0.9% was instilled in the abdominal cavity before closing the linea alba with vicryl 3/0 suture and the skin with nylon 3/0 suture. Postoperatively the animal was fed ad libitum while analgesia (2mL of butorphenol, Dolorex) was administered subcutaneously every 4 hours, and then, according to the animal’s needs based on their clinical picture. In S group, operation was followed by identification and manual mobilization of the pancreas without ligation of the bilio-pancreatic duct or further action before the abdominal closure. In Ap group, after the surgical ligation of the duct, once the animal was becoming conscious, 4 mL of prepared AP solution was administered orally.

Nine rats died before scheduled euthanasia, two during the surgical experiment due to intubation complications from the C group and seven post-surgery, one from the Ap group and six from the C group. These animals were not included in the statistical analysis.

AP Solution Preparation

AP was supplied by Sigma-Aldrich, Taufkirchen, Germany in vials with >99% purity by thin layer chromatography, as yellow powder with tan cast. AP was dissolved as follows: 660 mg AP was dissolved in 500mL corn oil, 2mL dimethyl sulfoxide and 10mL Tween 80. The final concentration was 5 mg AP in 4mL solution.

was set at \( p < 0.05 \). Data are presented as median values.

Figure 1. Changes in IL-6 in three groups over the 72h period. (A) indicates statistical significance between Sham and Apigenin groups at 12 (\( p = 0.007 \)) and 24 hours (\( p = 0.005 \)). (D) indicates statistical significance between 6-72 hours (\( p = 0.001 \)) at the Apigenin group. (E) indicates statistical significance between 12-72 hours (\( p = 0.001 \)) at the Apigenin group. (F) indicates statistical significance between 6-48 hours (\( p = 0.001 \)) at the Apigenin group. Significance was set at \( p < 0.05 \). Data are presented as median values.

**RESULTS**

**AP Reduces IL-6 in Rats with Experimental ACP at 12h**

IL-6 concentrations were lower in S group compared to C and Ap groups, at every time point (Figure 1). More specifically, in S group, the initial value was the highest (mean: 61.10; range: 30.80 – 64.70) and diminished gradually for every time point that followed (Figure 1). In C group IL-6 reached its highest value at 12h (mean: 129.30; range: 63.70 – 263.50) due to the inflammation caused by ligation of the bilo-pancreatic duct. At this time point of 12h, values in C and Ap groups almost coincided and presented their highest levels (mean: 162.50; range: 110.90 – 311.60). It is shown that after 12h, in the Ap group the level of IL6 is reduced, leading to a steady decline, whereas in the C group, fluctuations of the level of IL6 are seen. Probably, the beginning of the anti-inflammatory effect of the substance starts at 12h, result which also reaches statistical significance from the pairwise comparisons between S and Ap groups at 12h (\( p = 0.007 \)) and at 24h (\( p = 0.005 \)) (Table 1). From comparisons between the 5 time points for each group, in the Ap group, every time point combination presented statistical significant results (\( p < 0.001 \)), underlying the effect of AP administration (Table 2).

**AP Reduces IL-18 in Rats with Experimental ACP at 6 h**

IL-18 remained at low levels in the S group, with a peak at 24 h time point (Figure 2). In the C group, it reached the highest value at 6 h time point, diminished until 12 h and then augmented until 48 h, where it started to fall again. In the Ap group, the onset value was the highest of all 3
The effect of AP started at 12h, followed by a huge drop in value of IL-18 until 24h, when it rose again. Another drop appeared at 48h with the lowest value at 72h. The only statistically significant result was at the onset point (p=0.028), in which Ap group showed the highest and the S group, one of the lowest values (Table 1).

### Table 1. Between group differences over the 5 time points.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time points</th>
<th>SHAM</th>
<th>CONTROL</th>
<th>APIGENIN</th>
<th>p-value for between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukine 18</td>
<td>6H</td>
<td>0.09 (0.01-0.45)</td>
<td>1.56 (0.01-3.33)</td>
<td>2.03 (0.22-3.68)</td>
<td>0.028&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12H</td>
<td>0.40 (0.3-5.7)</td>
<td>2.15 (1.18-2.94)</td>
<td>0.662</td>
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<tr>
<td></td>
<td>24H</td>
<td>0.85 (0.23-1.80)</td>
<td>0.57 (0.2-0.9)</td>
<td>0.038</td>
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</tr>
<tr>
<td></td>
<td>48H</td>
<td>0.95 (0.5-0.3)</td>
<td>0.97 (0.2-2.6)</td>
<td>0.796</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72H</td>
<td>0.03 (0-0.52)</td>
<td>0.18 (0.2-2.4)</td>
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<tr>
<td>Resistin</td>
<td>6H</td>
<td>0.15 (0.1-0.26)</td>
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<tr>
<td></td>
<td>12H</td>
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<td>0.07 (0.04-0.14)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24H</td>
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<td>0.14 (0.08-0.25)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>48H</td>
<td>0.44 (0.14-0.72)</td>
<td>0.19 (0.11-0.36)</td>
<td>0.028</td>
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</tr>
<tr>
<td></td>
<td>72H</td>
<td>0.21 (0.04-0.26)</td>
<td>0.25 (0.08-0.51)</td>
<td>0.17 (0.13-0.39)</td>
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<tr>
<td>CD45</td>
<td>6H</td>
<td>1 (1-1)</td>
<td>2 (1-2)</td>
<td>2 (1-2)</td>
<td>0.049&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12H</td>
<td>1 (1-1)</td>
<td>1.5 (1-2)</td>
<td>2 (1-2)</td>
<td>0.127</td>
</tr>
<tr>
<td></td>
<td>24H</td>
<td>1 (1-1)</td>
<td>2 (1-3)</td>
<td>1 (1-2)</td>
<td>0.043&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>48H</td>
<td>1 (1-1)</td>
<td>2 (2-3)</td>
<td>2 (1-3)</td>
<td>0.002&lt;sup&gt;2,6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>72H</td>
<td>1 (1-1)</td>
<td>3 (2-3)</td>
<td>1 (0-1)</td>
<td>&lt;0.001&lt;sup&gt;2,6&lt;/sup&gt;</td>
</tr>
<tr>
<td>HMGB1</td>
<td>6H</td>
<td>0.50 (0-1)</td>
<td>2 (2-3)</td>
<td>3 (2-3)</td>
<td>0.002&lt;sup&gt;2,6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>12H</td>
<td>0.50 (0-1)</td>
<td>2 (2-3)</td>
<td>2 (1-2)</td>
<td>0.002&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>24H</td>
<td>1 (0-1)</td>
<td>3 (2-3)</td>
<td>1 (1-2)</td>
<td>&lt;0.001&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>48H</td>
<td>1 (1-1)</td>
<td>3 (2-3)</td>
<td>1 (0-1)</td>
<td>&lt;0.001&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>72H</td>
<td>1 (1-1)</td>
<td>3 (3-3)</td>
<td>0.50 (0-1)</td>
<td>&lt;0.001&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

### Table 2. Within group differences over the time points.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>6H</th>
<th>12H</th>
<th>24H</th>
<th>48H</th>
<th>72H</th>
<th>p-value for between time points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukine 18</td>
<td>SHAM</td>
<td>0.09 (0.01-0.45)</td>
<td>0.03 (0-0.52)</td>
<td>0.686 &lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>CONTROL</td>
<td>1.56 (0.01-3.33)</td>
<td>1.50 (1-2)</td>
<td>0.007 A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>APIGENIN</td>
<td>2.03 (0.22-3.68)</td>
<td>2.15 (1.18-2.94)</td>
<td>0.054 (0.0-1.26)</td>
<td>0.032</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistin</td>
<td>SHAM</td>
<td>0.15 (0.1-0.26)</td>
<td>0.21 (0.04-0.26)</td>
<td>0.086</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONTROL</td>
<td>0.19 (0.05-0.31)</td>
<td>0.31 (0.02-0.75)</td>
<td>0.001&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>APIGENIN</td>
<td>0.12 (0.01-0.23)</td>
<td>0.07 (0.04-0.14)</td>
<td>0.011&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45</td>
<td>SHAM</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td>0.002&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONTROL</td>
<td>2 (1-2)</td>
<td>2 (1-2)</td>
<td>2 (1-2)</td>
<td>2 (1-2)</td>
<td>0.011&lt;sup&gt;2,6&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>APIGENIN</td>
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<td>2 (1-2)</td>
<td>2 (1-2)</td>
<td>2 (1-2)</td>
<td>0.281</td>
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<tr>
<td>HMGB1</td>
<td>SHAM</td>
<td>0.50 (0-1)</td>
<td>1 (0-1)</td>
<td>1 (1-1)</td>
<td>1 (1-1)</td>
<td>0.001&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CONTROL</td>
<td>2 (2-3)</td>
<td>2 (2-3)</td>
<td>3 (2-3)</td>
<td>3 (2-3)</td>
<td>0.001&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>APIGENIN</td>
<td>3 (2-3)</td>
<td>2 (1-2)</td>
<td>1 (1-2)</td>
<td>1 (0-1)</td>
<td>&lt;0.001&lt;sup&gt;2,6&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>6</sup>HVs 72H; <sup>6</sup>HVs 72H; <sup>6</sup>HVs 48H; <sup>6</sup>HVs 48H

AP Reduces HMGB1 Expression

HMGB1 expressions among the 3 groups reached statistically significant differences almost at all-time points (Table 1). HMGB1 presented low expressions in the S group, whereas in the C group, it increased after 12h and reached its highest value at 24h (Table 2, Figure 3). The beneficial role of AP started at 48h (p=0.02), while
HMGB1 gained its lowest value in the Ap group at 72 h time point ($p=0.001$) (**Table 1**), with statistical significance in pairwise comparison of 6h vs. 72 h ($p<0.001$) (**Table 2**).

**AP Presents Anti-Inflammatory Effect, as CD45 Expression is Decreased**

In the S group, CD45 remained low and stable at every time point (**Figure 2**). In the C group, it increased reaching its highest value at 72 h. On the contrary, in the Ap group, the expressions of CD45 coincided with those of the S group after 24h. Statistical significance for pairwise comparisons between the S and C group was found for every time point after 24h (24h: $p=0.043$; 48h: $p=0.002$; 72h: $p<0.001$) and between C and Ap at 48h ($p=0.002$) and 72 h ($p=0.001$) (**Table 1**). From the comparisons between the 5 time points for each group, both C and Ap groups presented statistical significant results for 6h vs. 72h ($p=0.001$) and 12h vs. 72h ($p=0.011$), underlying for the C group the upward trend of values and for the Ap group the downward trend of values (**Table 2, Figure 4**).

**AP Improves Inflammatory Effect, as Levels of RR Remain at Low Points**

In both S and C groups, R ranged from low to high values without any specific pattern (**Figure 5**). On the contrary, the Ap groups presented the lowest values compared to C.

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**Figure 2.** Changes in IL-18 in three groups over the 72h period. (A): indicates statistical significance between Sham and Apigenin groups at 12 hours ($p=0.028$). Significance was set at $p<0.05$. Data are presented as median values.

**Figure 3.** Changes in HMGB1 expression in three groups over the 72h period. (A): indicates statistical significance between Sham and Apigenin groups at 6 ($p=0.002$) and 24 hours ($p=0.001$). (B): indicates statistical significance between Sham and Control groups at 6 ($p=0.002$), 12 ($p=0.002$), 24 ($p=0.001$) and 48 hours ($p=0.002$). (C): indicates statistical significance between Control and Apigenin groups at 48 ($p=0.001$) and 72 hours ($p=0.001$). (D): indicates statistical significance between 6-72 hours at the Control ($p=0.001$) and Apigenin group ($p=0.001$). (F): indicates statistical significance between 6-48 hours at the Control group ($p=0.001$). Significance was set at $p<0.05$. Data are presented as median values.

**Figure 4.** Changes in CD45 expression in three groups over the 72h period. (A): indicates statistical significance between Sham and Apigenin groups at 6 ($p=0.049$). (B): indicates statistical significance between Sham and Control groups at 24 ($p=0.043$), 48 ($p=0.002$) and 72 hours ($p<0.001$). (C): indicates statistical significance between Control and Apigenin groups at 48 ($p=0.002$) and 72 hours ($p<0.001$). (D): indicates statistical significance between 6-72 hours at the Control ($p=0.001$) and Apigenin group ($p=0.011$). (E): indicates statistical significance between 12-72 hours at the Control ($p=0.001$) and Apigenin group ($p=0.011$). Significance was set at $p<0.05$. Data are presented as median values.
DISCUSSION

ACP is an inflammatory process of the pancreas with adverse outcome. It is characterized by damage of peri-pancreatic tissues and/or distant organs, and it seems to rather comprise a variety of diseases than a sole one [15]. Pathophysiological mechanisms involved in the cause of ACP, especially these of the early stages of the disease, are under study. All mechanisms lead to release of cytokines and free radicals of oxygen, increasing the inflammatory process, the apoptosis and the oxidative stress [16].

Due to the continuous research interest on pancreatitis, several experimental models of pancreatitis have been developed. In our study, we used the experimental model of ligation of the biliary-pancreatic duct [14] which mimics acute obstruction similar to gallstone pancreatitis in humans.

Our study refers to the effect of a flavonoid, AP, on known inflammation indices such as IL-6 [3], IL-18 [4], HMGB1 [17], CD45 [18] and R [9] in rat serum and intestinal tissue. Flavonoids are phenolic phytochemicals, naturally existing in plants and abundant in particular plant species, vegetables and fruits. They are considered important constituents of the human diet, although their daily intake varies with dietary habits. The beneficial role of flavonoids is well known in the traditional chinese medicine (TCM). TCM is one of the most popular complementary and alternative medicine modalities worldwide for the treatment of ACP, and one of its most powerful medicines is scutellarin. In the last decade, further studies enhance scutellarin’s beneficial effect on inflammation, as a family member of flavonoids [19,20]. Several properties have been ascribed to flavonoids including AP. It seems that AP down-regulated Tumor Necrosis Factor–α in an experimental model of ACP, so it is expected, to also down-regulate other inflammatory cytokines [21]. AP is characterized by anti-cancerous, anti-oxidative, anti-inflammatory and other actions [12].

In our study, AP showed its anti-inflammatory effect, mainly at 12 h reducing IL-6 serum levels. Concerning IL-6, it acts via gp130 protein, leading the activation of Janus kinase/signal transducer and activator (JAK/STAT) signaling pathway [22,23]. In vivo studies proved that patients suffering from pancreatitis developed high serum values of IL-6 compared to healthy individuals [52]. A recent study of Mrazek et al which is in accordance with our findings showed that AP reduces IL-6 levels [11].

IL-18, is a member of IL-1 family cytokines. There are many studies that prove the presence of IL-18 in the blood of patients suffering from ACP. Moreover its values increase during mild and severe forms of the disease compared to healthy people [4]. In experimental models of ACP, high levels of IL-18 have also been described [24]. In our study, AP shows its anti-inflammatory action between 12h and 24h, as we observe an important reduction of IL-18 value in AP group. In general, IL-18 seems to be present at the early stages of ACP acting as an immunomodulator of the inflammatory response especially in severe forms of the disease, however, without knowing the exact mechanisms of pathogenesis [24].

HMGB1 is a nuclear protein which regulates transcriptional process and genetic expression, while it plays an important role in replication and repair of DNA. Loss of HMGB1 leads to structural and functional anomalies of the nucleus. Under stress conditions it is moved from nucleus to cytoplasm, where it mediates autophagy. Beyond its intracellular action, it can act as extracellular protein as well, and more specifically as damage associated molecular pattern (DAMP) molecule. These molecules act as cytokines which through HMGB1 receptors or toll-like receptors (TLRs), activate specific signaling pathways mediating the inflammatory and immune response. According to experimental studies, oxidative stress is responsible for the translocation, release and action of HMGB1 [25,26].

Studies try to explain the relationship between HMGB1 and ACP. Most of them showed linear correlation of HMGB1 and other inflammatory parameters [25,27], while in one study, a reduction of HMGB1 levels was described through the action of siRNA that inhibited NF-kB, decreasing the inflammation in severe ACP [28]. In general, HMGB1 expression in patients with ACP, seems to increase. Two are the possible mechanisms responsible for this. The first one is “HMGB1 circulation” [29], which is a phenomenon under study. According to this, reduction of HMGB1 through administration of anti-HMGB1 antibody causes reduction of the severity of ACP and vice versa. The second mechanisms based to the fact that in ACP, HMGB1 is released from the injured pancreas as well as from...
lungs and intestine that are also affected [30]. The results of our study are in total agreement with the majority of the studies, as in the C group we observed statistically significant increase of HMGB1 expression with time while in the Ap group we detected the positive effect of AP after 48 hours of the experiment. Moreover, at every time of the experiment there was statistically significant difference between two of the groups, undoubtedly pointing at the beneficial role of the substance’s administration on the intestinal mucosa [31].

CD45 is another antigen related with inflammation. As it was expected, in S group, CD45 remained low and stable for every time point. On the other hand, in both groups with ACP it increased, coming in line with the existing bibliography [32,33]. The AP started its action at 24 hours, by down-regulating the expression of CD45. To the rest of our knowledge, this is the first study that has noticed a great and undoubtable impact of AP on CD45 of the intestine mucosa.

Concerning R, the lower levels of its concentration may correlate with the early action of AP. Generally, R is involved in the early stages of inflammation. Daniel et al. observed that R serum concentration in patients with ACP was significantly increased on the first, third and fifth day of hospitalization compared to the control group [34] while Xue et al. indicated in their study that R may be associated with the occurrence and development of AP and as a result the protein may be a valuable indicator for assessing the severity of ACP [35]. In our study, the beneficial role of AP on R was also obvious.

As it is well known, ACP is also associated with acute lung and kidney injury. Recent studies have proven that the administration of anti-inflammatory substances, such as aseugenol and AP, can protect kidneys and lung injury in an experimental model of ACP in rats [36,37]. In our study, we observed the beneficial role of AP not only in serum parameters, but also in the intestinal mucosa. It seems that the therapeutic target of ACP is not just the pancreas but also other organs – targets of the disease, such as the intestine, the kidneys, the lungs. The prevention of multiple organ dysfunction can be useful in diminishing the morbidity and mortality of the disease.

In the present study, 4 mL of AP solution were administered every 24 hours containing 5 mg of AP, as described previously. This dose proved to be capable to satisfy our hypothesis that AP is a potent anti-inflammatory agent [13], after ACP. The selection of the dosage was based on previous studies investigating daily dietary exposure of AP to humans [38]. Dosages of 100-200 mg/kg or more induce cytotoxicity and produce phenoxyl radicals and reactive oxygen species [39,40].

CONCLUSION

In conclusion, AP administration in a rat experimental model appears to be beneficial on the examined parameters as it reduces inflammation, caused in that experimental model of ACP. AP administration seems to be a promising therapeutic non-toxic agent which should be further tested in clinical use.

REFERENCES