# The effects of chrysin in an experimental model of acute pancreatitis

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**The effects of chrysin in an experimental model of acute pancreatitis**

PURPOSE: *This experimental study was conducted to evaluate the possible effects of orally administered chrysin on acute pancreatitis.*
MATERIAL AND METHOD: *Twenty four rats were procured. The animals were randomly divided into four groups. In Group I, only vehicle solution (5% dimethylsulfoksid) was administered, and in Group II, chrysin dissolved in the vehicle solution was administered for six days. In Group III and Group IV cerulein was administered to induce acute pancreatitis. In Group III, only vehicle solution was administered, and in Group IV, chrysin dissolved in the vehicle solution was administered orally for six days. Blood samples were analyzed and the pancreatic tissue specimens were evaluated for histopathological examination.*
RESULTS: *Group III and Group IV, exhibited markedly higher levels of serum WBC, amylase, and lipase, compared with Groups I and II. In the pancreatitis induced groups, CRP and TOS values were found to be significantly higher. In Group II and Group IV, TAS values were significantly higher. The highest calculated OSI values were observed in Group III. Group IV OSI values were significantly lower than those in Group III and even in Group I. Noticeable histopathological changes were identified in the pancreatitis induced Groups III and IV. Compared with Group III, the extent and severity of pancreatic injuries were markedly lower in Group IV.*
CONCLUSION: *Chrysin application reduced oxidative stress and histopathological parameters. The present study shows that chrysin can be used to treat pancreatic diseases.*

KEY WORDS: Acute pancreatitis, Cerulein, Chrysin

## Introduction

Acute pancreatitis is a severe clinical condition that can cause unintended consequences and may be life-threatening [1,2]. The most common causes of acute pancreatitis are cholelithiasis and excess alcohol consumption. Trauma, ductal obstructions, infections, metabolic disorders, and ischemia can also cause acute pancreatitis [3]. Although several treatment protocols have been suggested, their benefits are insufficient. In addition to standard medical treatments, new substances can be used for better results.

Flavonoids are the most common ingredients of the polyphenolic substances found in plants. Chrysin (5, 7-dihydroxyflavone) is a natural flavonoid that can be found in honey, bee propolis, and many plant extracts [4,5]. Many effects of chrysin have been identified with various experimental studies. These effects include anti-

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neoplastic, anti-inflammatory, anti-oxidant, and anti-diabetic effects [6,7]. Anti-inflammatory features are associated with regulation of the expression of pro-inflammatory genes [8,9]. Chrysin may have potential positive effects on the treatment of pancreatitis because of its anti-oxidant and anti-inflammatory properties, but there are no current studies on it. This experimental study was conducted to evaluate the possible effects of orally administered chrysin on acute pancreatitis.

We referenced Pushpavalli et al.'s study to determine the optimum dose of chrysin to be used in the present study. In this study, chrysin was given at different doses to different groups of animals. Among the different doses, the 25 mg/kg dose was found to be more effective in hepatotoxic rats [10]. Thus, we decided to give 25 mg/kg of chrysin to the animals in our study. Chrysin is a powder form plant extract; therefore, it must be dissolved in a vehicle solution for oral administration to the rats; 5% dimethylsulfoksid (DMSO) was used in this study as a vehicle solution for chrysin.

Cerulein, which is a cholecystokinin analogue, is used to induce acute pancreatitis. It causes premature proteolysis of zymogens and activates trypsin [11,12]. As a result of this, the active trypsin triggers the proteolytic enzymes that result in acute pancreatitis [13]. Cerulein-induced pancreatitis has been shown to be histologically similar to the early phase of acute pancreatitis. Cerulein can be administered intraperitoneally, intravenously, or subcutaneously in animal models [14,15]. Irrespective of the route of administration, many studies have demonstrated that cerulein leads to edematous pancreatitis. Furthermore, necrotizing pancreatitis may occur with administration of supramaximal doses of cerulein [16]. In Tani et al.'s study, it was suggested that cerulein at a concentration of 20 mcg/kg body weight was the minimal effective dose to cause the maximal degree of biochemical and histological alterations when given via four subcutaneous injections [17]. Therefore, in our study, we used cerulein at a 30 mcg/kg concentration administered as four subcutaneous doses (with one hour between each dose) to induce acute pancreatitis. Chrysin, cerulein, and DMSO were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

## Materials and Methods

### Study Design

Twenty-four Male Wistar albino rats were procured from the Selcuk University Experimental Medical Research and Practice Centre and were maintained in an air conditioned room (21°C) with a 12 hour light/12 hour dark cycle. Standard laboratory foods and drinking water were given to the rats ad libitum. This study was approved by the local ethics committee of the Selcuk University Experimental Medical Research and Application Center.

### Experimental Protocol

The animals were randomly divided into four groups consisting of six animals each. In order to determine whether the chrysin molecule and DMSO substance have any effects on normal pancreas tissue, Group I and Group II were created. In Group I, only DMSO was administered, and in Group II, chrysin dissolved in a DMSO vehicle solution was administered once a day for six days. This study aimed to determine whether the biochemical and pathological parameters of acute pancreatitis patients improved with chrysin administration; therefore, we induced acute pancreatitis in Group III and Group IV at the beginning of the experiment via subcutaneous cerulein injection. In Group III, only DMSO was administered, and in Group IV, chrysin dissolved in a DMSO solution was administered orally once a day for six days.

On the morning of the seventh day, the animals were sacrificed by cervical dislocation under general anesthesia, and their abdomens were opened under sterile conditions. Blood samples were collected from the abdominal aorta of each animal in clean, dry test tubes and tubes with ethylene diamine tetra acetic acid (EDTA). The samples in the dry tubes were centrifuged to separate the plasma. Pancreas tissues were removed and put into a 10% formaldehyde solution.

### Biochemical Evaluation

Blood samples were analyzed for the following biochemical parameters: white blood cell count (WBC) (K/uL), urea (mg/dL), creatinine (mg/dL), aspartate aminotransferase (AST) (U/L), alanine transaminase (ALT) (U/L), alkaline phosphatase (ALP) (U/L), lactate dehydrogenase (LDH) (U/L), amylase (U/L), lipase (U/L), albumin (g/dL), total protein (g/dL), calcium (Ca) (mg/dL), glucose (mg/dL), total antioxidant status (TAS) ( molTroloxEq/L), total oxidant status (TOS) ( mol H2 O2 Eq/L), and C-reactive protein (CRP) (mg/dL). In addition, the oxidative stress index (OSI) of each sample was calculated using the following formulation: (TOS/TAS) x 100.

### Histopathological Evaluation

The pancreatic tissue specimens from each rat were fixed in a 10% formaldehyde solution for 24 hours. The tissues were embedded in paraffin blocks and then stained with hematoxylin-eosin dye. All specimens were evaluated by a single experienced pathologist in a blinded fashion. Pancreatic edema, acinar necrosis, hemorrhage and fat necrosis, inflammation, and perivascular infiltrate were rated on a scale between 1 (no pathology) to 4 (most severe) for each parameter according to the criteria defined by Schmidt et al. (Table I).

TABLE I - *Histopathological scoring criteria according toSchmidt et al. Score Criteria*

| | |
|---|---|
| **Edema** | |
| 0 | Absent |
| 0.5 | Focal expansion of interlobar septae |
| 1 | Diffuse expansion of interlobar septae |
| 1.5 | Same as 1 + focal expansion of interlobular septae |
| 2 | Same as 1 + diffuse expansion of interlobular septae |
| 2.5 | Same as 2 + focal expansion of interacinar septae |
| 3 | Same as 2 + diffuse expansion of interacinar septae |
| 3.5 | Same as 3 + focal expansion of intercellular spaces |
| 4 | Same as 3 + diffuse expansion of intercellular spaces |
| **Acinar necrosis** | |
| 0 | Absent |
| 0.5 | Focal occurrence of 1–4 necrotic cells/HPF |
| 1 | Diffuse occurrence of 1–4 necrotic cells/HPF |
| 1.5 | Same as 1+focal occurrence of 5–10 necrotic cells/HPF |
| 2 | Diffuse occurrence of 5–10 necrotic cells/HPF |
| 2.5 | Same as 2+focal occurrence of 11–16 necrotic cells/HPF |
| 3 | Diffuse occurrence of 1 1–16 necrotic cells/HPF (foci of confluent necrosis) |
| 3.5 | Same as 3+focal occurrence of >16 necrotic cells/HPF |
| 4 | >16 necrotic cells/HPF (extensive confluent necrosis) |
| **Hemorrhage and fat necrosis** | |
| 0 | Absent |
| 0.5 | 1 focus |
| 1 | 2 focus |
| 1.5 | 3 focus |
| 2 | 4 focus |
| 2.5 | 5 focus |
| 3 | 6 focus |
| 3.5 | 7 focus |
| 4 | >7 focus |
| **Inflammation and perivascular infiltrate** | |
| 0 | 0-1 intralobuler or perivascular leukocytes/HPF |
| 0.5 | 2–5 intralobuler or perivascular leukocytes/HPF |
| 1 | 6–10 intralobuler or perivascular leukocytes/HPF |
| 1.5 | 11–15 intralobuler or perivascular leukocytes/HPF |
| 2 | 16–20 intralobuler or perivascular leukocytes/HPF |
| 2.5 | 21–25 intralobuler or perivascular leukocytes/HPF |
| 3 | 26–30 intralobuler or perivascular leukocytes/HPF |
| 3.5 | >30 leukocytes/HPF or focal micro abscesses |
| 4 | >35 leukocytes/HPF or confluent micro abscesses |

HPF: high-power field

### Statistical Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences software package (SPSS; version 15.0; SPSS Inc., Chicago, Illinois, USA). For continuous data, arithmetical mean, standard deviation (SD), and ranges were calculated. For discrete data, frequency and percentage were calculated. The Kruskal-Wallis test was used in comparisons of continuous and ordinal data between more than two groups, and a significance level of $p<0.05$ was chosen. To determine significance is from which group, the Mann-Whitney U test was performed to compare the groups in pairs, and with a Bonferroni correction, the p value was adjusted to <0.008.

## Results

The biochemical values of the groups are summarized as mean and standard deviation in Table II. There were no statistically significant differences in AST, ALT, ALP, LDH, total protein, albumin, Ca, or glucose levels among the groups. On the other hand, analyses of urea, creatinine, WBC, amylase, lipase, CRP, TAS, TOS, and OSI differed significantly between groups (Table III).

In Groups I and II, serum WBC, amylase, and lipase values remained within normal ranges. Compared with Groups I and II, the acute pancreatitis-induced groups (Groups III and IV) exhibited markedly higher levels of serum WBC, amylase, and lipase. However, these parameters were significantly lower in Group IV than in Group III.

Although urea and creatinine values differed significantly among groups, they were still within normal ranges.

In Group III and Group IV, CRP and TOS values were found to be significantly higher. In Group II and Group IV (the chrysin administered groups), TAS values were significantly higher. The highest calculated OSI values were observed in Group III. Group IV OSI values were significantly lower than those in Group III and even in Group I. The lowest OSI values were found in Group II.

The histopathological analyses revealed statistically significant differences among groups (Table IV ). There were no apparent histopathological changes identified in the pancreases of rats from Group I and Group II. Noticeable necrosis, pancreatic edema, and interstitial leukocyte and erythrocyte infiltration were observed in the pancreatitis induced Groups III and IV. Pancreatic histological scores were highest in Group III. Compared with Group III, the extent and severity of pancreatic injuries were markedly lower in Group IV (Table V). Representative histological sections are presented in Fig. I.

## Discussion

Acute pancreatitis is a disease that poses challenges to physicians during treatment and follow-up, and in cases of progression, the currently available treatment options are not very promising. Therefore, for many years, there have been ongoing studies to understand the etiology and pathophysiology of acute pancreatitis and to improve treatment options. The primary approach to acute pancreatitis treatment is often medical and designed to reduce the patient's pain, reduce the exocrine function of the patient's pancreas, and restore the patient's metabolic homeostasis. The idea that restriction of enteral nutrition is helpful in treating acute pancreatitis has been increasingly weakened by recent studies. With enteral nutrition, rates of infectious complications, multi-organ dysfunction syndrome, and mortality decrease, and length of hospital stay decreases as well [18,19].

A rat model of acute pancreatitis was established in this

TABLE II - *Biochemical parameters of the groups*

| | Group 1 | Group2 | Group 3 | Group 4 | p |
|---|---|---|---|---|---|
| WBC | 7653.16±1405.55 | 7615±1064.49 | 16339.50±1463.89 | 13683±676.59 | p<0.001* |
| Urea | 45,91±1,50 | 40,88±3,95 | 39,28±2,84 | 33,78±2,93 | p:0.001* |
| Creatinine | 0,51±0,04 | 0,51±0,01 | 0,47±0,03 | 0,42±0,05 | p: 0.009* |
| AST | 116,66±18,18 | 113,16±19,99 | 127±29,70 | 130,16±27,49 | p:0,892 |
| ALT | 58±11,15 | 60,50±17,98 | 64±17,26 | 75±25,21 | p:0,611 |
| ALP | 126,50±35,35 | 190±39,84 | 179,33±41,62 | 170,50±46,94 | p:0,063 |
| LDH | 1740,83±531,82 | 1504,83±323,92 | 1998±428,51 | 1598,50±405,95 | p:0,186 |
| Amylase | 1047,66±147,79 | 1066,50±131,96 | 2541±443,68 | 1537±169,82 | p<0.001* |
| Lipase | 13±5,2 | 17,33±6,37 | 153±49,28 | 71±11,93 | p<0.001* |
| Total Protein | 6,26±0,31 | 6,15±0,21 | 6,35±0,10 | 6,41±0,16 | p:0,179 |
| Albumin | 3,03±0,16 | 2,95±0,20 | 3,05±0,05 | 3,01±0,14 | p:0,827 |
| Ca | 10,41±0,24 | 10±0,25 | 9,98±0,11 | 9,88±0,40 | p:0,027 |
| Glucose | 145,50±30,23 | 165,33±37,53 | 201,66±50,59 | 193,33±33,91 | p:0,123 |
| CRP | 22,16±8,47 | 19,33±5,12 | 158,66±35,68 | 158,66±65,17 | p:0,001* |
| TAS | 2,33±0,08 | 4,96±0,47 | 2,31±0,11 | 5,05±0,22 | p<0.001* |
| TOS | 23,95±3,35 | 23,41±2,94 | 46,75±5,32 | 39,58±3,29 | p<0.001* |
| OSI | 1025,43±128,33 | 470,67±21,19 | 2026,34±287,79 | 783,68±53,69 | p<0.001* |

*Kruskal-Wallis test wasused in comparisonsand results were given as the mean ± standard deviation (SD)
*significance level was chosen as p <0.05

TABLE III - *Biochemical compare of the groups in pairs*

| | Group 1/2 | Group 1/3 | Group 1/4 | Group 2/3 | Group 2/4 | Group 3/4 |
|---|---|---|---|---|---|---|
| WBC | p:0.873 | p:0.004* | p:0.004* | p:0.004* | p:0.004* | p:0.004* |
| Urea | p:0,16 | p:0,004* | p:0,004* | p:0,337 | p:0,013 | p:0,010 |
| Creatinine | p:0,627 | p:0,053 | p:0,025 | p:0,040 | p:0,004* | p:0,199 |
| Amylase | p:1 | p:0,004* | p:0,004* | p:0,004* | p:0,004* | p:0,004* |
| Lipase | p:0,200 | p:0,004* | p:0,004* | p:0,004* | p:0,004* | p:0,004* |
| CRP | p:0,470 | p:0,004* | p:0,004* | p:0,004* | p:0,004* | p:0,575 |
| TAS | p:0,004* | p:0,676 | p:0,004* | p:0,004* | p:0,373 | p:0,004* |
| TOS | p:0,688 | p:0,004* | p:0,004* | p:0,004* | p:0,004* | p:0,020 |
| OSI | p:0,004* | p:0,004* | p:0,006* | p:0,004* | p:0,004* | p:0,004* |

To determine significance source, Mann-Whitney U test was performed to compare the groups in pairs and with Bonferroni correction p value was adjusted
*significance level wasdetermined as p<0.008.



study using cerulein-induced pancreatitis-which is one of the main experimental models for the study of the disease-to determine the flavonoid derivate chrysin's effects. Histological examination of the pancreas tissues indicates that the experimental model resembles major features of severe acute pancreatitis in patients [20]. In the present study, pancreatic tissue sections of the acute pancreatitis-induced groups exhibited noticeable histopathological differentiation characterized by acinar cell necrosis, inflammatory cell infiltration, and edema. These results confirm that the experimental model employed in our study induced severe acute pancreatitis.

Flavonoids have been widely and traditionally used to treat diseases associated with oxidative injury and acute inflammation [21-23]. Previous studies [24,25] have reported that luteolin (3, 4', 5', 7-tetrahydroxyflavone), which is a natural flavonoid, is a protective agent and a potential drug for the treatment of acute pancreatitis [25]. Chrysin, which is in the same group as luteolin, is likely to have similar effects on pancreatitis. This is why this study was designed.

Elevated WBC is an expected condition in inflammatory and infectious processes. Thus, elevated WBC is often seen in the patients with acute pancreatitis. Inadequate reduction of WBC despite treatment gives clinicians important information about the course of the acute pancreatitis during treatment and follow-up. The degree to which the patient's WBC is elevated is also associated

TABLE IV - *Histopathological Scores*

| | | N(%) | | | | |
|---|---|---|---|---|---|---|
| | **score** | **Group 1** | **Group 2** | **Group 3** | **Group 4** | **P** |
| Edema | 0 | 6 (%100) | 6 (%100) | 0 (%0) | 1 (%16.7) | p<0.001* |
| | 0.5 | 0 (%0) | 0 (%0) | 0 (%0) | 1 (%16.7) | |
| | 1 | 0 (%0) | 0 (%0) | 0 (%0) | 3 (%50) | |
| | 1.5 | 0 (%0) | 0 (%0) | 0 (%0) | 1 (%16.7) | |
| | 2 | 0 (%0) | 0 (%0) | 1 (%16.7) | 0 (%0) | |
| | 2.5 | 0 (%0) | 0 (%0) | 1 (%16.7) | 0 (%0) | |
| | 3 | 0 (%0) | 0 (%0) | 2 (%33.3) | 0 (%0) | |
| | 3.5 | 0 (%0) | 0 (%0) | 1 (%16.7) | 0 (%0) | |
| | 4 | 0 (%0) | 0 (%0) | 1 (%16.7) | 0 (%0) | |
| Acinar necrosis | 0 | 6(100%) | 6(100%) | 0(%0) | 1(%16,7) | p<0.001* |
| | 0.5 | 0(%0) | 0(%0) | 0(%0) | 2(%33,3) | |
| | 1 | 0(%0) | 0(%0) | 0(%0) | 3(%50) | |
| | 1.5 | 0(%0) | 0(%0) | 0(%0) | 0(%0) | |
| | 2 | 0(%0) | 0(%0) | 3(%50) | 0(%0) | |
| | 2.5 | 0(%0) | 0(%0) | 0(%0) | 0(%0) | |
| | 3 | 0(%0) | 0(%0) | 2(%33,3) | 0(%0) | |
| | 3.5 | 0(%0) | 0(%0) | 1(%16,7) | 0(%0) | |
| | 4 | 0(%0) | 0(%0) | 0(%0) | 0(%0) | |
| Hemorrhage andfat necrosis | 0 | 6(100%) | 6(100%) | 0(%0) | 0(%0) | p<0.001* |
| | 0.5 | 0(%0) | 0(%0) | 0(%0) | 1(%16,7) | |
| | 1 | 0(%0) | 0(%0) | 0(%0) | 4(%66,7) | |
| | 1.5 | 0(%0) | 0(%0) | 1(%16,7) | 1(%16,7) | |
| | 2 | 0(%0) | 0(%0) | 1(%16,7) | 0(%0) | |
| | 2.5 | 0(%0) | 0(%0) | 1(%16,7) | 0(%0) | |
| | 3 | 0(%0) | 0(%0) | 1(%16,7) | 0(%0) | |
| | 3.5 | 0(%0) | 0(%0) | 0(%0) | 0(%0) | |
| | 4 | 0(%0) | 0(%0) | 2(%33,3) | 0(%0) | |
| Inflammation and perivascular infiltration | 0 | 6(100%) | 6(100%) | 0(%0) | 0(%0) | p<0.001* |
| | 0.5 | 0(%0) | 0(%0) | 0(%0) | 3(%50) | |
| | 1 | 0(%0) | 0(%0) | 0(%0) | 2(%33,3) | |
| | 1.5 | 0(%0) | 0(%0) | 0(%0) | 1(%16,7) | |
| | 2 | 0(%0) | 0(%0) | 2(%33,3) | 0(%0) | |
| | 2.5 | 0(%0) | 0(%0) | 1(%16,7) | 0(%0) | |
| | 3 | 0(%0) | 0(%0) | 2(%33,3) | 0(%0) | |
| | 3.5 | 0(%0) | 0(%0) | 0(%0) | 0(%0) | |
| | 4 | 0(%0) | 0(%0) | 1(%16,7) | 0(%0) | |

*Kruskal-Wallis test wasused in comparisonsand results were given as frequency and percentage*significance level was chosen as p<0.05

TABLE V - *Histopathologicalcompare of the groups in pairs*

| | **Group 1/2** | **Group 1/3** | **Group 1/4** | **Group 2/3** | **Group 2/4** | **Group 3/4** |
|---|---|---|---|---|---|---|
| Edema | p:1 | p:0,002* | p:0,007* | p:0,002* | p:0,007* | p:0,004* |
| Acinar Necrosis | p:1 | p:0,002* | p:0,007* | p:0,002* | p:0,007* | p:0,003* |
| Hemorrhage and fat necrosis | p:1 | p:0,002* | p:0,002* | p:0,002* | p:0,002* | p:0,004* |
| Inflammation and perivascular infiltration | p:1 | p:0,002* | p:0,002* | p:0,002* | p:0,002* | p:0,004* |

To determine significance source, Mann-Whitney U test was performed to compare the groups in pairs and with Bonferroni correction p value was adjusted
*significance level was determined as p<0.008.

with the severity of acute pancreatitis [26]. In our study, in accordance with previous studies, WBCs were significantly higher in pancreatitis induced groups than in other group. In Group IV, despite being higher than normal, WBCs were found to be significantly lower than in Group III. This suggests that chrysin leads to a statistically significant reduction in WBC and in the severity of acute pancreatitis; however, because the acute pan-

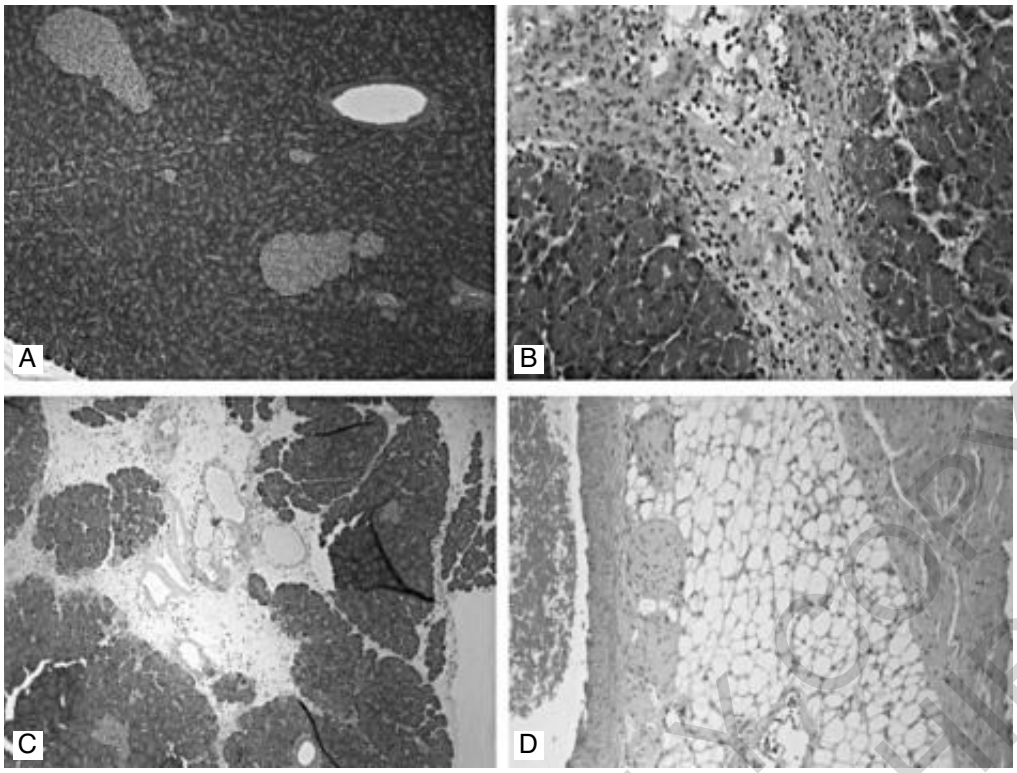


Fig. 1: Histopathological examination of the pancreas (hematoxylin and eosin staining). A- normal pancreatic tissue in group I rats, B- nflammation in Group IV rats, C- Edema and inflammation in Group III rats, D- Fat necrosis in Group III rats.

creatitis cases in this study did not heal completely, WBC values did not fall entirely to the normal ranges.

Amylase and lipase have historically been utilized more frequently than other laboratory tests in the evaluation of acute pancreatitis and a multitude of studies have examined their utility, often with conflicting conclusions. The sensitivities and specificities of amylase and lipase vary considerably among studies examining their diagnostic utility in acute pancreatitis [27]. Therefore, amylase and lipase evaluation alone are not currently considered reliable methods for the diagnosis of acute pancreatitis. However, these values are known to be elevated in pancreatitis. In our study, compared with Groups I and II, groups in which acute pancreatitis was induced exhibited significantly higher serum concentrations of amylase and lipase serum. However, in Group IV, chrysin administration appeared to lead to significantly lower amylase and lipase levels compared to Group III.

In acute pancreatitis, as in all acute events, acute phase reactants are elevated. CRP is an acute-stage protein and a nonspecific inflammation marker [26]. It is usually used because it is simple and cheap [28]. Higher serum CRP concentrations are found in severe pancreatitis [26]. In our study, in Groups III and IV, CRP values were significantly higher than those in other groups due to acute pancreatitis. However, CRP values were not significantly lower in the chrysin administered pancreatitis group.

In the normal physiology of living organisms, there is a delicate balance between pro-oxidants and anti-oxidants. The disturbance of this balance in favor of pro-oxidants causes oxidative damage (oxidative stress) [29]. Oxidative stress is known to increase throughout the course of pancreatitis [30]. Moreover, supplementation with an anti-oxidant mixture containing N-acetyl cysteine, selenium, and vitamin C has been shown to reduce pancreatic injury in rats [31]. There are many different markers and methods to determine the state of oxidative stress. However, the measurement of these markers separately is both time-consuming and expensive [29,32]. Therefore, the measurement of TOS and TAS values has recently been adopted, and OSI is calculated as the TOS/TAS ratio for use as an indicator of oxidative stress [33-35]. In our study, TAS levels in chrysin administered groups and TOS levels in acute pancreatitis groups were found to be significantly elevated. We thus conclude that chrysin increases TAS values but does not have any effect on TOS values. In acute pancreatitis, TOS values were significantly elevated, and calculated OSI levels were significantly elevated as well. The highest calculated OSI values were observed in Group III. In Group IV, both TOS and TAS values were elevated. The elevation in TAS levels were higher than the TOS levels; therefore, the calculated OSI values were lower in Group IV than in Group III, and the OSI values in Group IV were more significantly lower than in Group I. The lowest OSI levels were observed in Group II, in which chrysin was administered without inducing pancreatitis. As a result, we observed that OSI values increased with the severity of acute pancreatitis but decreased with chrysin's anti-oxidant effects.

In acute pancreatitis, varying degrees of pathological changes-including hemorrhagic necrosis, pancreatic ede-

ma, and interstitial leukocyte and erythrocyte infiltration-are seen [36]. In the present study, there were no evident histopathological changes identified in the pancreases of rats in Group I and Group II, because acute pancreatitis was not induced in these groups. Pancreatic tissue injury in rats with acute pancreatitis was distinct, and pathological scores were higher in acute pancreatitis groups than in other groups. Treatment with chrysin ameliorated pancreatic injury and resulted in significantly lower pathological scores. These findings demonstrate that chrysin has positive effects on histopathological parameters in acute pancreatitis.

## Conclusion

In the present study, the levels of amylase, lipase, WBC, and OSI were significantly elevated with acute pancreatitis induction and were significantly lower in the chrysin-treated group. This was in accordance with the results of the histopathological examination, which also showed less pancreatic edema, acinar necrosis, hemorrhage and fat necrosis, inflammation, and perivascular infiltration in pancreatic tissue in the chrysin-treated group. Chrysin application reduced oxidative stress and histopathological parameters. The present study shows that chrysin can be used to treat pancreatic diseases due to its antioxidant and pancreatoprotective features.


## Riassunto

Si tratta di uno studio sperimentale finalizzato alla valutazione dei possibili effetti della somministrazione orale di Chrysina sulla pancreatite acuta. Sono stati utilizzati 24 ratti divisi a random in quattro gruppi.
Nel primo gruppo è stata somministrata agli animali soltanto la soluzione eccipiente (5% dimethylsulfoksid - DMSO). Nel Gruppo II è stata somministrata per 6 giorni la Chrysina disciolta nella stessa soluzione veicolo. Nel Gruppo III e nel Gruppo IV è stata somministrata Ceruleina per indurre una pancreatite acuta, Nel III Gruppo è stata somministrata la sola soluzione veicolo, mentre nel IV Gruppo è stata somministrata per 6 giorni la Chrysina disciolta nella soluzione veicolante. Sono stati analizzati campioni di sangue e sono stati prelevati campioni di tessuto pancreatico per esame istopatologico.
Nei Gruppi III e IV si sono riscontrati più elevati livelli di leucocitosi, di amilasi e lipasi in paragone con i Gruppi I e II. Nei gruppi in cui è stata indotta la pancreatite acuta sono stati riscontrati valori significativamente più alti di Proteina C Reattiva (CRP) e un più elevato stato totale di ossidanti (TOS). Nei Gruppi II e IV i valori di stato totale di antiossidanti (TAS) sono stati rilevati significativamente più elevati. I più alti valori calcolati di Stress ossidative Index (OSI) sono stati riscontrati nel Gruppo III. Nel IV Gi valori di OSI sono risultati significativamente inferiori rispetto a quelli dei Gruppi III ed anche I.
Notevoli alterazioni istopatologiche sono state osservate nei gruppi III e IV di pancreatite acuta indotta.
A confronto con il Gruppo III la diffusione e la gravità dei danni pancreatici sono stati rilevati significativamente minori nel Gruppo IV.
L'utilizzo della Chrysina riduca dunque lo stress ossidativo e i parametri istopatologici, e dunque questo studio indica che la Chrysina può essere usata per il trattamento delle patologie del pancreas.


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