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Exploring Promising Treatments for Acute Pancreatitis in Rats: Mesenchymal Stem Cells vs. Rosmarinic Acid - A Histological and Immunohistochemical Comparison

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ABSTRACT

Background: Acute pancreatitis (AP) remains a potentially fatal disease with limited effective therapies. Mesenchymal stem cells (MSCs) exhibit antioxidant and immunomodulatory properties, while Rosmarinic acid (RA) has anti-inflammatory and cytoprotective effects. **Objective:** This study aimed to evaluated their therapeutic potential in experimental AP. **Methods**: Forty-one male Sprague-Dawley rats were randomly assigned into four groups: Control (I), L-arginine–induced AP (II), L-arginine + RA (50 mg/kg, i.p., once) (III), and L-arginine + MSCs (1×10^6 cells/ml, i.v.) (IV). Assessments included serum amylase, TNF-α, and IL-10 levels, histopathology, and immunohistochemistry for proliferating cell nuclear antigen (PCNA) and inducible nitric oxide synthase (iNOS).

Results: Both RA and MSCs significantly reduced serum amylase and TNF-α and increased IL-10 compared to AP rats. MSCs produced the greatest improvements, though differences between groups III and IV were not statistically significant. Histologically, RA preserved acinar architecture with moderate protection, while MSCs restored near-normal pancreatic structure, reduced apoptosis and necrosis, enhanced PCNA expression, and markedly decreased iNOS reactivity.

Conclusions: RA and MSCs both demonstrated therapeutic benefits in arginine-induced AP, with MSCs showing superior efficacy in biochemical, histological, and immunohistochemical outcomes. MSCs appear more effective than RA in attenuating inflammation and promoting pancreatic repair, warranting further investigation into their mechanisms and potential clinical application.

Keywords: Acute pancreatitis, Reactive oxygen species, Mesenchymal stem cells, Rosmarinic acid.

INTRODUCTION

Acute pancreatitis (AP), one of the most common gastrointestinal disorders, which puts a severe emotional, physical, and financial strain on the sufferer. Worldwide, the yearly incidence of AP is estimated to be between 15 and 45/100,000, and 20% of patients have a progression of AP to severe acute pancreatitis (AP). Currently, analgesia, despam, fluid resuscitation, nutritional assistance, and other traditional clinical therapies for AP are available, but they are unable to halt the disease's course appreciably [1].

Pathologically, AP is caused by pancreatic dyshomeostasis, characterized by oxidative stress and the death of acinar cells. The first phases of AP, oxidative stress has a role in tissue damage. Pancreatic microcirculation may be disrupted by the overabundance of reactive oxygen species (ROS) generated by pancreatic acinar cells (PACs), which can exacerbate vasoconstriction and vascular injury. Conversely, a study stated that it stimulates nuclear factor kappa B (NF-κB). According to previous studies [1], the signaling pathway in PACs causes the inflammatory cascade and raises the synthesis of inflammatory cytokines. According to earlier research, pancreatitis inflammation may be decreased by blocking NF-κB. Reducing reactive oxygen species generation may also be beneficial clinically for treating AP [2]. Numerous plants contain Rosmarinic acid (RA), a caffeic acid ester with numerous medicinal uses, such as anti-inflammatory, anti-allergic, anticancer, and antioxidant actions. Research has indicated that RA may also block the NF-kB signaling pathway in some circumstances, such as cisplatin-induced nephrotoxicity and hepatotoxicity, and that it can reduce cell or tissue damage in certain inflammatory illnesses [3].

One form of adult stem cell that can develop into several different cell types is mesenchymal stem cells. Along with their differentiation capabilities, MSCs also exhibit immunological features. Because of these unique characteristics, MSCs are now being studied as a potential novel therapy method for AP. Research demonstrated that mesenchymal stem cells generated from bone marrow successfully repaired acute pancreatitis (AP) and facilitated the regeneration of damaged pancreatic tissue. This was achieved via the release of microRNA-9, a significant paracrine factor, and the inhibition of NF-κB signaling. Additional research has shown the magnitude of the effect of MSCs as a treatment for AP, which is linked to the suppression of inflammation, oxidative stress, and autophagy inhibition [4, 5]. Although these contributions provide insight into the therapeutic actions of MSCs, more research is required to investigate how MSCs specifically inhibit the first phases of inflammation in AP.

Prior studies have also shown that mesenchymal stem cells can restore pancreatic damage and exhibit anti-inflammatory and antioxidant properties in both moderate and severe acute pancreatitis. When administered alone, MSCs may not provide enough therapeutic benefits due to the restricted quantity of MSCs that may be transplanted, as well as the potential impact of ROS and

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inflammatory cytokines on MSC survival and differentiation post-transplantation[6].

Preconditioning MSCs with RA was discovered to have a cytoprotective effect. The present research aimed to examine the impact of a combination of Rosmarinic acid and MSCs on the treatment of an experimental rat model of AP.

MATERIALS AND METHODS

Establishment of AP model and experimental groups

Male SD rats, weighing 200–250 g and six weeks old, were acquired as wild-type specimens from the Holding Company for Biological Products & Vaccines (VACSERA) in Helwan, Egypt. Rats were kept in an environment with conventional laboratory water and food, and they were fed at 25 °C and 50% humidity with a 12-hour light/dark cycle. To generate the AP model, intraperitoneal injections (i.p.) of 99% L-arginine hydrochloride (bought from Sigma Aldrich®) at a dosage of 2.0g/kg, dissolved in 0.9% normal saline were given twice a day for three days, with a 1.5-hour interval between doses. Furthermore, 24 hours later, a single intravenous (i.v.) injection of 0.9% normal saline was given into the tail vein[7].

Forty-one male Sprague–Dawley rats with normal genetic characteristics were randomly assigned to four groups, with nine rats in each group. In the remaining five rats, mesenchymal stem cells were isolated from their bone marrow. Throughout the investigation, there were no instances of death documented.

Group I (Control):

Subgroup Ia: fed with normal pellet diet.

Subgroup Ib: given a single intravenous injection of 0.5 ml phosphate-buffered saline (PBS) through the caudal vein.

Subgroup Ic: administered intraperitoneally with 50 mg/kg of Rosmarinic acid [8].

Group II (Control group, AP model induced by L-Arginine administering): L-arginine hydrochloride solution was administered using the same method previously described).

Group III (Study group, L-Arginine + RA treated group): Before the development of acute pancreatitis, the rats were administered intraperitoneally with 50 mg/kg of Rosmarinic acid just once. Rosmarinic acid was obtained from Sigma Aldrich Chemie Gmbh, Munich, Germany in the form of water-soluble powder.

Group IV (Study group, L-Arginine + MSCs-treated group): Before inducing acute pancreatitis, the rats were given a single systemic transplantation of MSCs (1×10^6 cells/ml) in 0.5 ml of PBS through the caudal vein [9].

Isolation, culture, and characterization of MSCs: Bone marrow cells were extracted from the marrow cavity by flushing it with low-glucose Dulbecco's modified Eagle's medium, which contained 100 U/ml of penicillin/streptomycin (PS) and 10% fetal bovine serum (FBS; Invitrogen-Gibco). The cells were kept at 37°C with 5% CO2. It is possible to identify MSCs with flow cytometry. For the therapy, MSCs from passages three through five were chosen. As previously stated, our previous study provided a comprehensive explanation of the procedure [9].

Immunophenotyping of BM-MSCs: The bone marrow mesenchymal stem cells were rinsed and suspended in phosphate-buffered saline. Monoclonal antibodies specific to CD34, CD90, CD105, and CD45 (obtained from Sigma, USA, SAB4501582 for CD34, CD90, CD105, and OX-1 84112004 for CD45) were introduced and incubated for 1 hour at a temperature of 4°C. Following that, the cells were exposed to a secondary antibody labeled by fluorescein, specifically immunoglobulin G (obtained from Millipore Corp., Temecula, CA), and this incubation was carried out for 45 minutes using rats as the experimental subjects. Following two rounds of washing, the cell suspensions were analyzed with a FACS Caliber flow cytometer [10].

Biochemical analysis: The serum amylase levels (mU/ml) were independently measured using a colorimetric test kit (Bio Vision, Milpitas, USA) in accordance with the manufacturer's instructions. Serum cytokines, including interleukin IL-10 and tumor necrosis factor (TNF)-α, were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA).

Histopathology: After three days, all rats were sacrified were sacrificed under ketamine anesthesia (80 mg/kg b.w., i.p.). The pancreas samples were preserved by immersing them in a 10% buffered formaldehyde solution and then embedded in paraffin. The slices, which were 5 μm thick, were stained with hematoxylin and eosin (H&E) for routine histological analysis. The sections were subjected to H&E staining by first staining them with hematoxylin for a duration of 3 minutes. After that, the sections were washed and stained with 0.5% eosin for 3 minutes. The slides were dehydrated in a series of ethanol solutions with concentrations of 70%, 96%, and 100%, followed by dehydration in xylene. To measure the extent of damage to acinar cells, 20 microscopic fields were selected randomly and evaluated using the established scoring method [11].

Immunohistochemistry protocol for assessment of PCNA and iNOS: The anti-proliferating cell nuclear antigen (PCNA) and anti-iNOS antibodies were acquired from Santa Cruz Biotechnology in Santa Cruz, CA, USA. These antibodies were used for immunohistochemistry. The pancreatic sections were treated to remove paraffin and restore hydration. The activity of the enzyme peroxidase was inhibited using a 3% hydrogen peroxide solution. The sections were then exposed to the anti-PCNA antibody (diluted 1:400) in PBS containing 5% bovine serum albumin. This incubation step was carried out overnight at a temperature of 4°C. The pancreatic sections underwent

deparaffinization, followed by rehydration in a series of alcohol washes, and were then washed in PBS. By treating the endogenous peroxidase with 3% hydrogen peroxide in ethanol, it became iRosmarinic acidative. Additionally, a 20% nonimmune serum solution in PBS was used to lessen any nonspecific antibody binding. The sections were exposed to the anti-iNOS antibody for an entire night at 4°C in a room with regulated humidity. As an alternative, they were treated with the other antibodies for an hour at room temperature. After a PBS wash, the sections were subjected to a biotinylated secondary antibody treatment. Vector Laboratories' goat anti-rabbit IgG was used to identify the anti-iNOS antibodies [12].

considerations: The Benha University Research Ethics Committee examined and approved the study protocol (approval code: RC 16-6-2025). All experimental methods were conducted in accordance with institutional policies for the care and use of laboratory animals in order to minimize pain and suffering.. Animals were closely observed during the trial, and humane endpoints were used. This work conforms to the ARRIVE criteria for reporting animal research and the tenets of the National Institutes of Health's (NIH) Guide for the care and use of laboratory Animals**.**

Statistical analysis:

The mean and standard deviation (±SD) were among the measurements that were recorded. "SPSS 22" was used to perform the statistical analysis (SPSS, Inc., Chicago, Illinois, USA). Post-hoc testing was performed after quantitative variables were compared using an analysis of variance (ANOVA). The significance level was set at a p-value ≤ 0.05.

RESULTS

The process of isolating, culturing, and expanding MSCs: Bone marrow cells were introduced to T-25 tissue culture flasks at a density of 1×10^6 cells per square centimeter. Within a span of 10 minutes, the cells settled down and took on a round shape, varying in size. The flasks containing the cells were placed in a cell culture incubator and incubated for 48 hours. During this incubation period, approximately 10% to 20% of the cells adhered to the surface of the flasks, and about 4% to 5% of these adhered cells displayed elongated and oval shapes, while the majority remained round-shaped. The non-adherent and floating cells were collected, and their viability was determined using trypan blue staining. It was found that around 70% of the collected cells were dead, whereas 30% were viable. The adherent cells were further cultured by replenishing the growth medium, and their growth and development were monitored until they reached a state where they covered a significant portion of the flask surface, referred to as a sub-confluent state. These sub-confluent cells were designated as passage 0 (P0) cells. Most of the P0 cells exhibited a spindle-shaped morphology, but a few retained an oval or round shape. The P0 cells were harvested, reseeded at a ratio of 1:3, and cultured further to obtain cells of passage 1, passage 2, and passage 3. Most of the cells in these subsequent passages displayed a spindle-shaped morphology with a visible nucleus and well-defined cell boundaries when observed under a light microscope using various staining techniques (Figure 1).

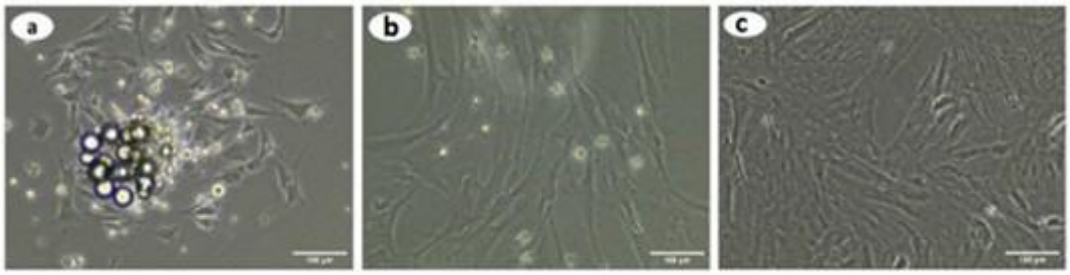

Figure (1): The morphological characteristics of bone marrow mesenchymal stem cells. a: isolated cells in culture in Passage 0; none adhesive rounded cells. b: MSCs in culture in first passage; adhesive fusiform cells. c: MSCs in culture in third passage; adhesive spindle shaped cells.

Characterization of MSCs (*Flow cytometry assay***)**

Our analysis using fluorescence-activated cell sorting (FACS) revealed that our MSCs did not exhibit lineage cell markers like CD34 and CD45. Still, they demonestated intense expression of common surface antigens, including CD105, and CD90 (Figure 2).

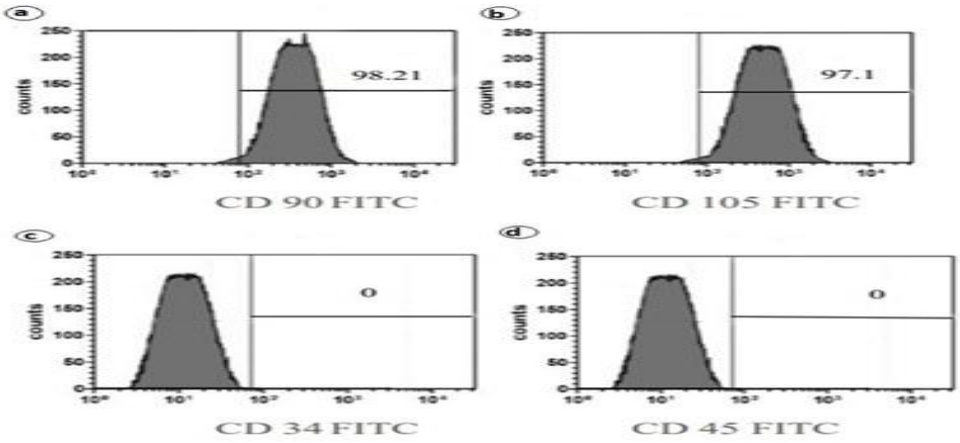

Figure (2): Analysis using fluorescence-activated cell sorting (FACS) was conducted to study bone marrow mesenchymal stem cells (BMMSCs) by employing specific markers. The proportion of CD90 + cells (a) and CD105 + cells (b) were strong expressd while MSCs were negative for hematopoietic markers CD34 (c) and CD45 (d).

Biochemical assay results: In comparison with group I, groups II, III, and IV showed significantly increase ($p<0.001$) of serum amylase and tumor necrosis factor-a (TNF-α) (Figure 3a). GIII and GIV showed significantly decreased amylase activity and serum levels of TNF-α compared to GII ($p<0.001$). Still, the enzyme activity and serum level of TNF-α were higher than those of G-I, but G-IV showed a non-significant decrease compared with G-III (Figure 3b). The serum levels of IL-10 showed significantly decreased in the G-II compared to G-I, G-III, and GIV ($p<0.001$). G-III and G-IV revealed significantly increased IL-10 levels compared to G-II ($p<0.001$), but its level was less than those of G-I. G-IV showed non-significant increases compared with G-III (Figure 3c).

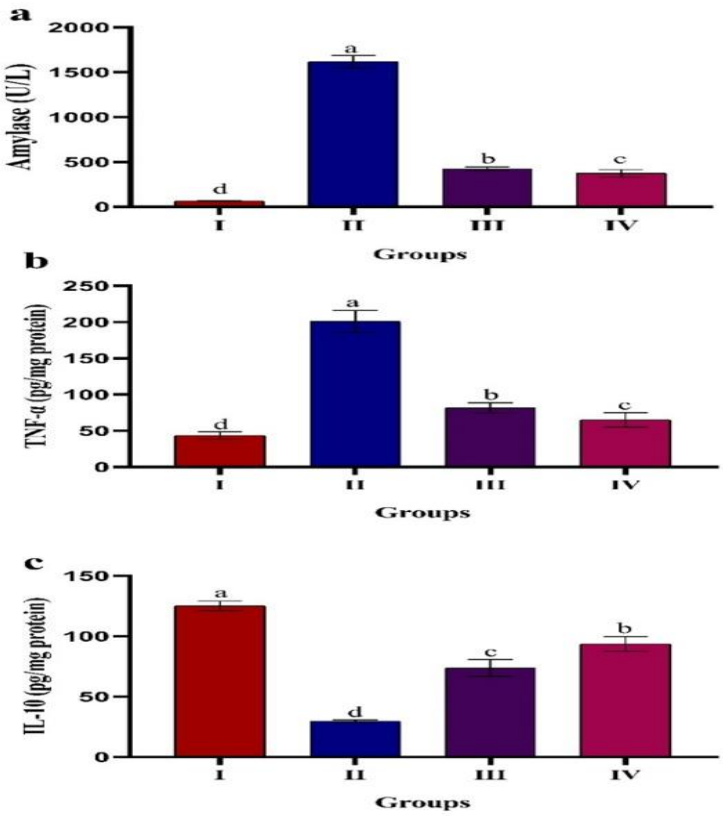

Figure (3): Comparison of a: amylase (U/L), b: TNF-α (pg/mg protein), and c: IL-10 (pg/mg protein) between different studied groups.

Histopathological changes in pancreas tissues

Hematoxylin and eosin (H&E) stain: Pancreatic samples from control group rats (Group I) were carefully studied. The examination revealed a typical pancreas structure consisting of closely arranged serous acini, with thin connective tissue separating them. The acinar cells displayed rounded nuclei at the base, stained blue with a basic dye. The cytoplasm of these cells had a reddish tinge when stained with an acidic dye. The islets of Langerhans, which are responsible for hormone production, appeared as light stains within the acinar tissue with regular outline. Additionally, the pancreatic duct and blood vessels were observed in the samples (Figure 4).

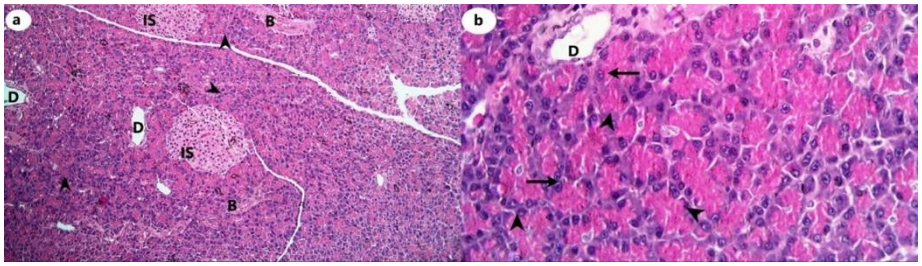

Figure (4): A photomicrograph of pancreatic section from group I (control group) showing: **a)** normal histological architecture, both exocrine and endocrine components. The exocrine pancreas is composed of pancreatic acini (head arrow). Blood vessels (B) and interlobular duct (D) are seen. Scattered throughout the exocrine tissue are the islets of Langerhans (IS), representing the endocrine portion. These appear as pale-staining, well-circumscribed clusters of polygonal cells. **b)** The pancreatic acini have cytoplasm with basal basophilia and apical acidophilia (head arrows). The round nuclei with prominent nucleoli and scattered chromatin are seen near the base (arrows). Notice interlobular duct (D) (H&E a x100 & b x400).

The sections of group II (L-arginine-treated group) indicated an irregular arrangement of acinar structures. Some acinar cells exhibited cell death characterized by dark, condensed nuclei indicating apoptosis. Other cells displayed the presence of empty spaces within the cytoplasm, suggestive of necrosis. Additionally, there was a significant presence of inflammatory cells and congested blood vessels in the surrounding tissue. The islets of Langerhans appeared minor and less distinct, with fewer cells than the control group (Figure 5).

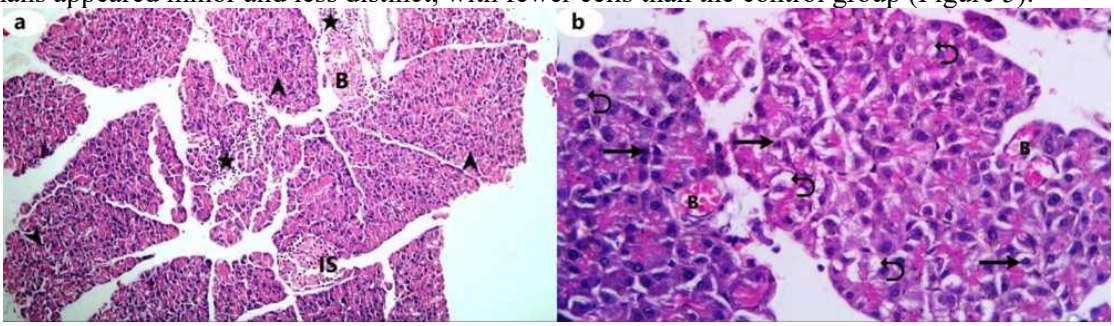

Figure (5): A photomicrograph of pancreatic section from group II showing: **a)** breakdown of the typical pancreatic structure (head arrow). Congested blood arteries (B) and infiltration of inflammatory cells (*) are observed. There are fewer islet cells and the islet of Langerhans (IS) has a smaller, less distinct border. **b)** the cells of acini appear with dark stained nuclei (arrow) and vacuolations (curved arrow). Notice congested blood vessels (B) (H&E a x100 & b x400).

Group III (L-arginine+ RA treated Group) showed some acini with apparently normal architecture, while others were distorted with vacuolated cells. Inflammatory cell infiltration was observed with congested blood vessels. Evidence of both apoptotic and necrotic cells was noted in the distorted areas (Figure 6).

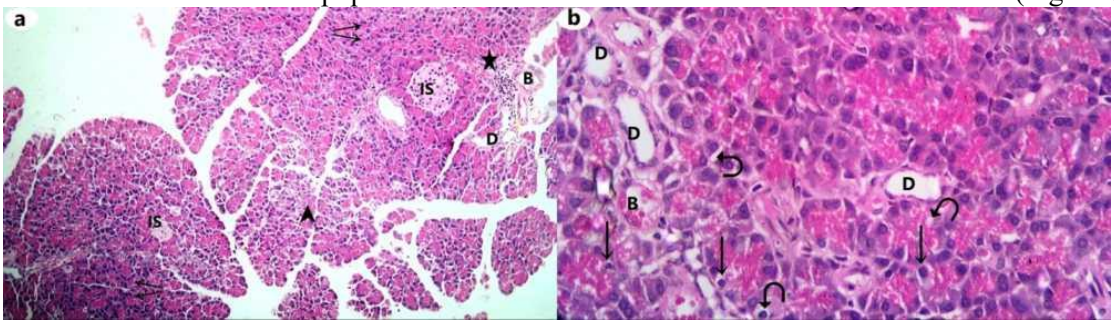

Figure (6): A photomicrograph of pancreatic section from group III showing: **a)** apparently normal architecture (double arrows). Some acini are distorted (head arrows). The congested blood vessel (B) and interlobular duct (D) are surrounded by inflammatory cell infiltration (*) in interlobular septa. The islet of Langerhans (IS) shows reduced dimension. **b)** some cells in acini appear with dark stained nuclei (arrows) while others show few cells appear vacuolated (curved arrow). Observe congested blood vessel (B) and interlobular duct (D). (H&E a x100 & b x400).

The pancreatic samples obtained from group IV, which underwent a combination treatment of MSCs and L-arginine, exhibited a pancreatic architecture that closely resembled that of the control group, specifically the normal islet. Congested blood vessels were noticed in this group (Figure 7).

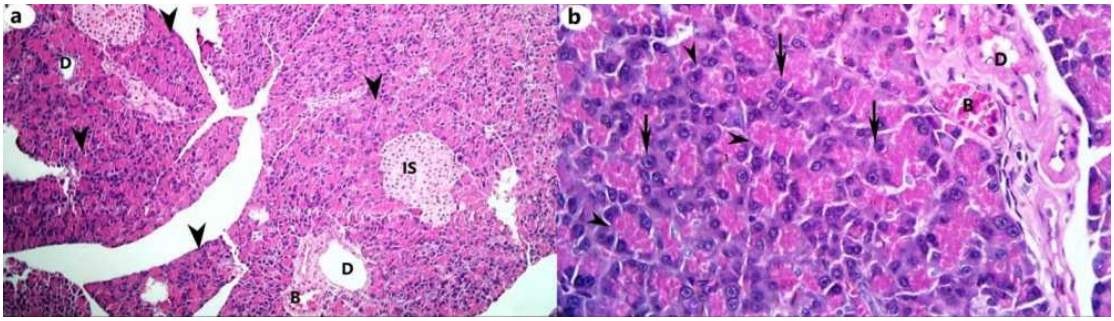

Figure (7): A photomicrograph of pancreatic section from group IV showing: **a)** apparently normal architecture of pancreatic acini (head arrows). Congested blood vessels (B) and interlobular duct (D) are seen. It contains normal structure of the islet of Langerhans (IS) with large number of islet cells. **b)** The pancreatic acini have cytoplasm with basal basophilia and apical acidophilia (head arrows). Round and basal nuclei are noticed with scattered chromatin and prominent nucleoli (arrows). Congested blood vessels (B) and interlobular duct (D) are noticed in interlobular connective tissue (H&E a x100 & b x400).

PCNA-immuno-stained pancreatic sections: The study observed different levels of PCNA reactions in the pancreatic acinar cells across four groups. Group I had few number PCNA reactions, as indicated by brown nuclei. Group II exhibited very few number of PCNA reactions, while Group III displayed a moderate number of brown nuclei in the pancreatic acinar cells. In contrast, Group IV had a higher number of brown nuclei in the pancreatic acinar cells, as depicted in figure (8).

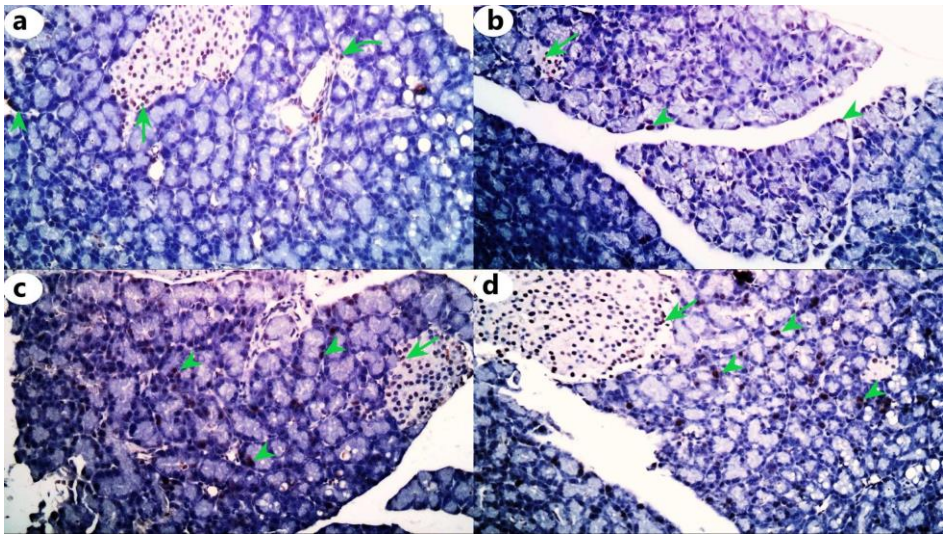

Figure (8): Photomicrographs of sections in the pancreatic acini of rats showing nuclear immunoreactivity: (a) few in acinar (head arrows), duct(curved arrows), and islet cells (arrows) of group I; (b) very few nuclei in acinar (head arrows) and islet cells (arrows) of group II; (c) present in some islet (arrows) and occasional acinar cells (head arrows) in group III; (d) abundant in most islet cells (head arrows) and some acinar cells (arrows) in group IV. (Anti-PCNA Immunostaining x200).

Anti-iNOS stained pancreatic sections: In the study, different groups were observed for their immune responses. Group I showed basal levels of iNOS in immune reaction. In contrast, Group II exhibited a robust immune response characterized by distinct brown granules in the cytoplasm of acini and islet cells. Group III displayed a moderate immune response with cytoplasmic reactivity observed in acini and immune reactivity in islet cells. Finally, group IV demonstrated a weak cytoplasmic reactivity in acinar cells, along with observable immune reactivity in islet cells (Figure 9).

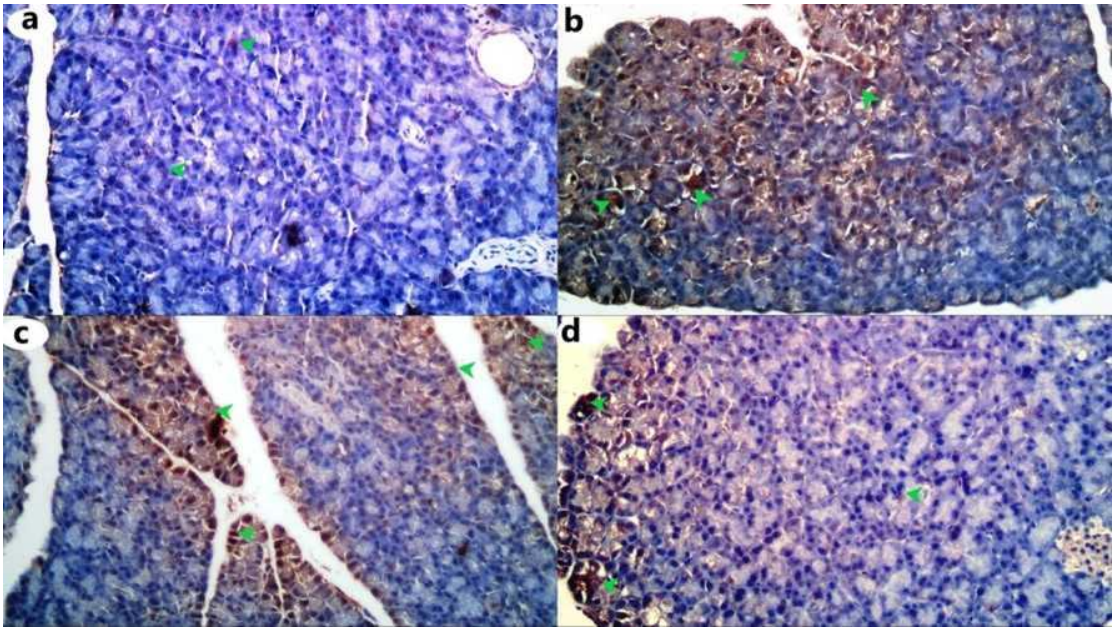

Figure (9): Photomicrographs of sections in the pancreatic acini of rats showing: **a)** Acinar cells in group I show negative cytoplasmic immunoreactivity (head arrows). **(b)** Dense cytoplasmic immunoreactivity is observed in acinar cells of group II (head arrows). **(c)** In group III, some acinar cells exhibit moderate cytoplasmic immunoreactivity (head arrows). **(d)** Group IV demonstrates mild cytoplasmic immunoreactivity in acinar cells (head arrows). (Anti-iNOS Immunostaining x200).

Morphometric results: Quantitative morphometric analysis of immunohistochemical staining for PCNA and iNOS revealed significant differences among the studied groups **(Table 1)**. The mean PCNA percent area was lowest in the control group (0.34 ± 0.06%) and showed a slight, non-significant increase in the AP group (0.81 ± 0.13%). Treatment with Rosmarinic acid markedly elevated PCNA expression (1.94 ± 0.32%, $p<0.001$ vs. groups I and II), while MSCs induced the highest proliferative response (4.90 ± 0.82%, $p<0.001$ vs. all groups). Conversely, iNOS expression was significantly upregulated in the AP group (25.17 ± 4.19%) compared to the control (0.32 ± 0.06%, $p<0.001$). Both RA (9.33 ± 1.22%) and MSCs (3.78 ± 0.21%) significantly reduced iNOS immunoreactivity compared to the AP group ($p<0.001$), with MSCs achieving a greater reduction ($p<0.001$ vs. RA). These findings confirmed the superior effect of MSCs in enhancing cellular proliferation and attenuating oxidative stress markers in arginine-induced acute pancreatitis **(Table 1)**.

Table (1): Comparison of area percent of positive reaction of PCNA and iNOS (%) between different studied groups (One way ANOVA followed by post-hoc tukey)

	Group I	Group II	Group III	Group IV	P value
PCNA percent area(%)	**0.34±0.06**	**0.81±0.13**	**1.94±0.32**	**4.90±0.82**	**<0.001***
Post-hoc		**P1=0.1**	**P1=<0.001* P2= <0.001***	**P1=<0.001* P2=<0.001* P3=<0.001***	
iNOS percent area(%)	**0.32±0.06**	**25.17±4.19**	**9.33±1.22**	**3.78±0.21**	**<0.001***
Post-hoc		**P1=<0.001***	**P1=<0.001* P2= <0.001***	**P1=0.006* P2=<0.001* P3=<0.001***	

Data expressed as mean±SD, SD: standard deviation, P:Probability *:significance <0.05, P1: significance vs group I, P2: significance vs group II, P3: significance vs group III.

DISCUSSION

The prognosis for AP is still dismal, and it ranks as the fourteenth most common cause of mortality from digestive system disease. For AP, there are still no specific treatments. The treatment is largely supportive of symptoms. Antioxidant and anti-inflammatory treatments are currently exclusive emphases [13].

Based on our laboratory findings, the group treated with arginine showed a significantly higher serum amylase concentration compared to the control group 72 hours after inducing acute pancreatitis (AP). These results are consistent with similar study [14]. These findings support the diagnosis of acute pancreatitis as proposed by **Stojanović *et al.*** [15], who stated that elevated levels of pancreatic enzymes are key diagnostic markers for AP. As suggested by a prior study [16], this could be attributed to the breakdown of zymogen granule membranes due to increased free radicals, resulting in the release of amylase and lipase into the interstitium. Conversely, the group treated with arginine-MSCs and arginine-RA demonstrated a significant reduction in serum amylase levels compared to the arginine-treated group, which aligns with previous researchs [10, 17].

The present investigation found that TNF-α levels in the bloodstream increased significantly after 72 hours of inducing acute pancreatitis (AP) in the group administered with arginine, compared to the control group. This discovery supports the findings of a prior study conducted by researchers cited as [18], which documented elevated levels of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6, as well as other inflammatory mediators like iNOS in severe acute pancreatitis (AP). Furthermore, this study demonstrated a significant decrease in serum IL-10 levels in the arginine-treated group after 72 hours of AP induction compared to the control group. **Lin *et al.*** [19] , observed a decline in serum IL-10 in rats with AP and reported that management with IL-10 reduced the severity of inflammation in AP models.

In this study, we discovered that the group treated with both of arginine and Rosmarinic acid exhibited significantly reduced levels of TNF-α and significantly increased levels of IL-10 compared to the group treated only with arginine. These levels were also significantly different from the control group. Furthermore, the levels of IL-10 in this combination group were significantly different from the results observed in the arginine MSCs-treated group. These findings support a previous study that had similar outcomes. A study found that MSCs was observed to delay the activation of NF-κβ in a condition called induced AP [20]. Consequently, this led to the inhibition of TNF-α production, leading to a significant decrease in the infiltration of white blood cells and swelling in the pancreas. A researcher proposed that Rosmarinic acid significantly reduced TNF-α levels and exhibited anti-inflammatory properties when used to treat AP [21]. These findings indicate that Rosmarinic acid could benefit AP treating, as observed through microscopic analysis.

In the current study, laboratory investigations of (the arginine-MSCs-treated group) showed a significantly reduction level of TNF-α (an inflammatory cytokine) and a significantly higher IL-10 (an anti-inflammatory mediator) than that of the arginine-treated group. These findings are consistent with other research, where **Ma *et al.*** [22] demonstrated that the anti-inflammatory properties of MSCs made them potentially effective for treating pancreatitis. Also, our study noted that the results of the serum level of TNF-α in (arginine-MSCs-treated group) had no significant difference compared to the control group. These outcomes concurred with **Li *et al.*** [23], who stated that MSCs could control the immune response in AP rat models through the reduction of inflammatory factor expression and the upregulation of anti-inflammatory mediators.

Moreira *et al.* [24] noted that significant histological changes occurred approximately 72 hours after inducing AP, and the timing of scarification was chosen as the focus of this investigation. In this study, histopathological examination of pancreatic sections from rats treated with arginine revealed extensive damage to the exocrine pancreas. The histological analysis confirmed significant necrosis of acinar cells, along with congested blood vessels and increased cellular infiltration. According to **Li *et al.*** [25], the presence of acinar cell necrosis was quantified by measuring the percentage of necrotic areas relative to the total pancreatic tissue area, which was increased significantly in the arginine-treated group comparing with the control.

The inflammatory response in the arginine-treated group was further elucidated by quantifying the number of inflammatory cells per unit area of pancreatic tissue. This approach corroborates the proposed mechanism of local inflammatory infiltration due to excessive nitric oxide generation by L-arginine, leading to vasodilation and subsequent influx of inflammatory cells, as discussed by **Yang *et al***. [26]. Moreover, the cytoplasmic vacuolations observed in the arginine-treated rats were quantified by counting the number of vacuoles per acinar cell. **Mareninova *et al.*** [27] suggested that such vacuolations in acute pancreatitis could result from disrupted autophagy.

In contrast, the arginine-MSC co-treatment group exhibited significant structural improvement in the pancreas. The histopathological analysis revealed a bsence in necrotic areas, and inflammatory cell infiltration compared to the arginine-only group. The observed amelioration in pancreatic architecture and function is consistent with **Zhou *et al.*** [28] who documented MSCs' potential to migrate to injured pancreatic tissues and substantially reduce necrosis and inflammation.

Rosmarinic acid reduced the severity of acute pancreatitis induced by L-arginine. This was observed

through a decrease in degeneration of acinar cells, and infiltration of inflammatory cells, as analyzed under a light microscope using H&E-stained sections of the pancreas. Previous research has demonstrated that Rosmarinic acid inhibits proinflammatory cytokines, has antioxidant and cytoprotective properties, and improves microcirculation in pancreatitis. Numerous studies have revealed that adding Rosmarinic acid to other treatment plans enhances AP results [8, 29]. As an illustration, Rosmarinic acid therapy reduced paroid damage and safeguarded the delicate acinar cell structure [30].

In contrast, while beneficial, the Rosmarinic acid treatment was less effective than BM-MSCs. Rosmarinic acid mitigates oxidative stress by neutralizing ROS and enhancing antioxidant enzyme activities. However, Rosmarinic acid's impact on inflammation is comparatively limited as it primarily addresses oxidative damage rather than directly modulating the immune response [31]. This difference in action mechanisms might explain why Rosmarinic acid alone did not achieve the same level of improvement as BM-MSCs. Rosmarinic acid's role in reducing oxidative stress and inflammation is significant but does not encompass the broader spectrum of immunomodulatory effects exhibited by BM-MSCs.

In this study, we used histomorphometry measures to quantitatively evaluate the level of PCNA reaction, which serves as a marker for tissue proliferation. The main objective was to assess the effectiveness of different treatment approaches by analyzing this response. According to our findings, the rat group treated with arginine exhibited a rise in acinar proliferation after acinar damage induced by L-arginine comparing with the control. **Zhang *et al.*** [32] demonstrated a causal relationship between increased DNA damage and acinar damage, leading to enhanced proliferation. The most pronounced increase in acinar cell proliferation occurred 72 hours after the cerulean administration, which was confirmed by labeling with the proliferating nuclear antigen (PCNA) marker.

In this research, the therapeutic use of MSCs or Rosmarinic acid demonstrated favorable prognostic markers, specifically a notably elevated level of PCNA, compared to the AP group. However, the MSCs treated group exhibited a significantly higher PCNA level than the Rosmarinic acid-treated group. This observation aligns with previous studies [33]. Moreover, our findings support the conclusions of another study [34], which indicated that the regenerative effects of MSCs result from either a localized reduction in inflammation or their paracrine activity.

This work used immunohistochemistry analysis to examine the expression of iNOS (inducible nitric oxide synthase). Rats given arginine showed a markedly higher expression of iNOS than the control group. This result is consistent with a prior investigation [34], which demonstrated a significant expression of iNOS in mice 72 hours after l-arginine-induced pancreatitis. The researchers concluded that arginine therapy raises tissue nitric oxide levels via increasing iNOS activity. The pancreas, in particular the acinar cells, are directly harmed by this. One of the reactive nitrogen species connected to oxidative stress, nitric oxide which is the primary cause of AP.

The research demonstrated that the utilization of stem cells derived from bone marrow (referred to as MSCs) as a therapeutic intervention had a notable decrease in iNOS reaction compared to a group of rats treated with arginine. This outcome is consistent with prior investigations conducted by researchers [34]. Additionally, our findings unveiled a significant reduction in pancreatic iNOS expression within the group treated with arginine and Rosmarinic acidcompared to the results obtained from the arginine-treated rat group. However, the iNOS expression in this group remained significantly higher than that of the arginine-MSCs-treated group, suggesting a potentially superior role of MSCs compared to Rosmarinic acid.this is in agreement with previous study, which showed that daily administration of RA improved oxidative stress in brain tissue induced by doxorubicin and showed no expression of iNOS as seen in normal rats [35]. Overall, our quantitative data substantiated the qualitative observations and underscore the therapeutic potential of MSCs in ameliorating arginine-induced pancreatic damage. Future studies should consider employing additional quantitative methodologies, such as biochemical assays and advanced imaging techniques, to further elucidate the mechanisms underlying MSC-mediated protection and repair.

LIMITATIONS

The comparatively limited number of rats employed in the study is one of its shortcomings, potentially limiting the applicability of the results to larger populations or to humans.Second, only short-term outcomes were assessed at a single time point (72 hours), without evaluation of long-term pancreatic recovery or recurrence. Third, while biochemical, histological, and immunohistochemical markers were examined, molecular mechanisms underlying the observed effects, such as specific signaling pathways or gene expression changes, were not explored. Finally, the study compared Rosmarinic acid and MSCs separately but did not investigate their potential synergistic effects when combined, which could provide further insight into therapeutic strategies for acute pancreatitis.

CONCLUSIONS

This study demonstrated that arginine, Rosmarinic acid (RA), and bone marrow-derived stem cells (MSCs) had potential effects in the application of AP. The arginine-MSC-treated group showed significant improvement in pancreatic structure and decreased inflammation compared to the arginine-treated group. Applied with RA also reduced the

severity of pancreatitis and preserved the acinar cell structure. Furthermore, MSCs were more effective in reducing pro-inflammatory cytokine expression than RA. These findings suggest that arginine, RA, and MSCs hold promise as potential interventions for AP by attenuating inflammation, improving tissue regeneration, and reducing oxidative stress.

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REFERENCES

1. **Lee P, Papachristou G (2020)**: Management of severe acute pancreatitis. Curr Treat Options Gastroenterol., 18:670–81.
2. **Checa J, Aran J (2020)**: Reactive oxygen species: drivers of physiological and pathological processes. Journal of Inflammation research, 13: 1057–73.
3. **Xiang Y, Ji M, Wu L *et al.* (2022):** Rosmarinic acid prevents cisplatin-induced liver and kidney injury by inhibiting inflammatory responses and enhancing total antioxidant capacity, thereby activating the Nrf2 signaling pathway. Molecules, 27(22): 7815.
4. **Shi Y, Wang S, Liu D *et al.* (2024)**: Exosomal miR-4645-5p from hypoxic bone marrow mesenchymal stem cells facilitates diabetic wound healing by restoring keratinocyte autophagy. Burns & Trauma, 12:tkad058.
5. **AbdRabou M, Atwa A, Massoud D *et al.* (2024)**: Therapeutic effect of mesenchymal stem cells and olive leaf extract against cytotoxicity induced by streptozotocin in gestational diabetes rats. Cellular Physiology and Biochemistry, 58:702–21.
6. **Mukkala A, Jerkic M, Khan Z *et al.* (2023)**: Therapeutic effects of mesenchymal stromal cells require mitochondrial transfer and quality control. Int J Mol Sci., 24:15788.
7. **Kaur J, Sidhu S, Chopra K *et al.* (2016):** Calendula officinalis ameliorates l-arginine-induced acute necrotizing pancreatitis in rats. Pharmaceutical Biology, 54(12): 2951-2959..
8. **Fan Y T, Yin G J, Xiao W Q *et al.* (2015):** Rosmarinic acid attenuates sodium taurocholate-induced acute pancreatitis in rats by inhibiting nuclear factor-κB activation. The American Journal of Chinese Medicine, 43(06):1117-1135.
9. **Gong J, Meng H, Hua J *et al.* (2014)**: The SDF-1/CXCR4 axis regulates migration of transplanted bone marrow mesenchymal stem cells towards the pancreas in rats with acute pancreatitis. Mol Med Rep., 9:1575–82.
10. **Spencer L (2018):** Microtomy for paraffin and frozen sections. Bancroft's Theory and Practice of Histological Techniques, 8th ed.; Suvarna, SK, Layton, C., Bancroft, JD, Eds, Pp: 84-95.
11. **Van Laethem J, Marchant A, Delvaux A *et al.* (1995)**: Interleukin 10 prevents necrosis in murine experimental acute pancreatitis. Gastroenterology, 108:1917–22.
12. **Hsu SM, Raine L, Fanger HX. (1981):** Application of the avidin–biotin–peroxidase complex (ABC) method in immunoperoxidase staining: comparative evaluation with the peroxidase–antiperoxidase (PAP) technique. *J Histochem Cytochem*., 29(4):577-80.
13. **Pagliari D, Brizi M, Saviano A *et al.* (2019)**: Clinical assessment and management of severe acute pancreatitis: a multi-disciplinary approach in the XXI century. European Review for Medical and Pharmacological Sciences, 23:771–87.
14. **Kui B, Balla Z, Vasas B *et al.* (2015)**: New insights into the methodology of L-arginine-induced acute pancreatitis. PLoS One, 10:e0117588.
15. **Stojanović N, Mitić K, Randjelović P *et al.* (2021)**: Thymol regulates the functions of immune cells in the rat peritoneal cavity after L-arginine-induced pancreatitis. Life Sci., 280:119704.
16. **Ye Y, Zhang H, Deng J *et al.* (2022)**: L-Arginine inhibits the activity of α-amylase: rapid kinetics, interaction and functional implications. Food Chem., 380:131836.
17. **Rencber M, Oguz A, Yıldızhan E (2022**): Protective Efficacy of Rosmarinic Acid on Acute Pancreatitis in Rats. J Cukurova Anesth Surg., 5(2):250-258
18. **Atwa A, Latif A, Moustafa M *et al.* (2024)**: Integrating nanosensors into stem cells technologies and regenerative medicine. In: Handbook of Nanosensors: Materials and Technological Applications, Pp:1113–47.
19. **Lin R, Chen F, Wen S *et al.* (2018)**: Interleukin-10 attenuates impairment of the blood-brain barrier in a severe acute pancreatitis rat model. Journal of Inflammation, 15:4.
20. **Abdelhafez D, Aboelkomsan E, El Sadik A *et al.* (2021)**: The role of mesenchymal stem cells with ascorbic acid and N-acetylcysteine on TNF-α, IL-1β, and NF-κβ expressions in acute pancreatitis in albino rats. J Diabetes Res., 2021:6229460.
21. **Ailing Z, Ken C, Naiyu Q *et al.* (2023):** Effect of rosmarinic acid on oxidative damage in rats with acute pancreatitis based on the Nrf2/HO-1 signaling pathway. Journal of Guangxi Medical University, 40(9): 1494-1500.
22. **Ma Z, Song G, Zhao D *et al.* (2019)**: Bone marrow-derived mesenchymal stromal cells ameliorate severe acute pancreatitis in rats via hemeoxygenase-1-mediated antioxidant and anti-inflammatory effects. Cytotherapy, 21:162–74.
23. **Li S, Li H, Zhangdi H *et al.* (2022)**: Hair follicle-MSC-derived small extracellular vesicles as a novel remedy for acute pancreatitis. J Control Release, 352:1104–15.
24. **Moreira M, Matias J, Souza C *et al.* (2011)**: Action of tacrolimus in arginine induced experimental acute pancreatitis. Rev Col Bras Cir., 38:260–5.
25. **Li Y, Zhu Y, Li S *et al.* (2022)**: Deoxyarbutin attenuates severe acute pancreatitis via the HtrA2/PGC-1α pathway. Free Radic Res., 56:651–65.
26. **Yang Y, Huang Z, Li L (2021)**: Advanced nitric oxide donors: chemical structure of NO drugs, NO nanomedicines and biomedical applications. Nanoscale, 13:444–59.
27. **Mareninova O, Jia W, Gretler S *et al.* (2020)**: Transgenic expression of GFP-LC3 perturbs autophagy in exocrine pancreas and acute pancreatitis responses in mice. Autophagy, 16:2084–97.
28. **Zhou Z, Zhu X, Huang H *et al.* (2022)**: Recent progress of research regarding the applications of stem cells for treating diabetes mellitus. Stem Cells Dev., 31:102–10.
29. **Zhu A, Chen K, Qin N*et al.* (2023):** Effect of rosmarinic acid on oxidative damage in rats with acute pancreatitis based on the Nrf2/HO-1 signaling pathway. Journal of Guangxi Medical University, 40(9): 1494-1500.

30. **Zhang T, Liu C, Ma S *et al.* (2020):** Protective effect and mechanism of action of rosmarinic acid on radiation-induced parotid gland injury in rats. Dose-Response,18(1): 1559325820907782.
31. **Sadeghi A, Bastin A R, Ghahremani H *et al.* (2020):** The effects of rosmarinic acid on oxidative stress parameters and inflammatory cytokines in lipopolysaccharide-induced peripheral blood mononuclear cells. Molecular biology reports, 47(5): 3557-3566.
32. **Zhang D, Li J, Zhao L *et al.* (2025):** Mitochondrial DNA Leakage Promotes Persistent Pancreatic Acinar Cell Injury in Acute Pancreatitis via the cGAS-STING-NF-κB Pathway. Inflammation, 48(3): 1420-1437.
33. **Fareed S , Yousef E , Abd El-Moneam S *et al.* (2023):** Assessment of effects of rosemary essential oil on the kidney pathology of diabetic adult male albino rats. Cureus, 15(3): e35736.
34. **Aboulkhair A , Nasr S , Radwan D et al. (2019):** Comparative Histological Study on The Effect of Bone Marrow-Derived Mesenchymal Stem Cells Versus Wheat Germ Oil on Acute Pancreatitis in A Rat Model. Egyptian Journal of Histology, 42(3): 540-553
35. **Rizk H , Masoud M , Maher O (2017):** Prophylactic effects of ellagic acid and rosmarinic acid on doxorubicin-induced neurotoxicity in rats. Journal of biochemical and molecular toxicology, 31(12): e21977.