Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 17s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

The Role of Rac-1 Inhibitor in Management of Acute Pancreatitis and Regulation of Chemokine in rat's model

Farman Naseh Othman[1], Nadir Mustafa Nanakali[2]

[1,2]College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq

[1]Al-Kitab University, Kirkuk, 36015, Iraq

***Corresponding Author:**

Farman Naseh Othman

Email: farmannasih2@gmail.com

Cite this paper as: Farman Naseh Othman, Nadir Mustafa Nanakali, (2025) The Role of Rac-1 Inhibitor in Management of Acute Pancreatitis and Regulation of Chemokine in rat's model. *Journal of Neonatal Surgery*, 14 (17s), 627-632.

ABSTRACT

Background: Acute pancreatitis is a common inflammatory disease of the exocrine pancreas, characterized by a mortality rate ranging from 1% to 5%. This condition has the potential to lead to organ dysfunction, pancreatic necrosis, and subsequent organ failure. Aim: Rac1, a G-protein with a molecular weight of approximately 21 kD, has been demonstrated to govern various platelet functions. We hypothesized that the inhibition of Rac-1 signaling could be implicated in severe acute pancreatitis (AP). Our study aimed to explore the impact of a Rac- 1 inhibitor on decrease of lipase enzyme and chemokine (IL-6 and CXCL1), and its potential in safeguarding against tissue damage associated with severe acute pancreatitis. Method: In this experimental study, male albino rats were employed, and the rats received pretreatment with a specific Rac1 inhibitor, NSC23766, at a dosage of 5 mg/kg. Subsequently, a midline laparotomy was performed on the anesthetized animals, followed by L-arginine injection intra-peritoneally. Blood Lipase levels were measured, and the concentration of serum chemokines (IL6 and CXCL1) were assessed using ELISA. Additionally, leukocyte counting, and histopathological examination were conducted to analyze morphological changes in the pancreas. Results: The blood lipase levels were notably elevated in rats with acute pancreatitis compared to the control rats. Our findings indicated that acute pancreatitis was induced in rats through L-arginine injection. Rac1 expression was upregulated in rats with acute pancreatitis, and pre-treatment with Rac-1 led to a non-significant decrease in lipase levels compared to the acute pancreatitis group. Furthermore, the levels of IL6 and CXCL1 were higher in acute pancreatitis group compared to the control group. Conclusion: Our investigation underscores the pivotal role of Rac-1 in regulating lipase activity during severe acute pancreatitis (AP). Additionally, it highlights the involvement of Rac-1 in platelet activation and the secretion of chemokines, namely IL6 and CXCL1, attributed to inflammation in organ tissues during acute pancreatitis. Targeting Rac1 emerges as a promising and innovative approach for the treatment of severe acute pancreatitis, offering potential control over inflammation and tissue damage.

Keywords: *Rac-1, Chemokines, acute pancreatitis, Inflammation, and leukocyte*

1. INTRODUCTION

Acute pancreatitis is an exocrine frequent inflammatory disease with a mortality rate between (1-5)%. Which can cause dysfunction of multiple organs, consequences pancreatic necrosis and the failure of the organs [1]. Two distinct phases have been identified in the progression of acute pancreatitis: an early and late phase. The severity of the condition is categorized as mild, moderate, or severe to diagnose acute pancreatitis. At least two of the specified symptoms must be evident: Enduring and intense pain in the upper central abdomen, often extending to the back, accompanied by a sudden onset, and elevated levels of serum lipase or amylase activity, surpassing at least three times the upper limit of normal [2]. Polymorphonuclear (PMN) leukocytes, also known as neutrophils are essential for the host's defense against fungal, bacterial, and viral infections. Additionally, neutrophils play a part in a number of chronic illnesses, including autoimmune disorders, cancer, atherosclerosis, and allergies [1], [3]. Neutrophils are predominantly produced in the bone marrow of the adult mammals and discharged into the bloodstream, A sequence of actions occurs to recruit circulating neutrophils into the tissue, including crawling, vessel wall rolling on, arrest, and transmigration. As a result of chemotactic clues, neutrophils migrate during transmigration from the lumen vessel toward the inflammatory tissue [4]. The onset of acute pancreatitis (AP) arises from the activation of trypsin within the pancreas, leading to self-digestion of pancreatic tissue, accompanied by edema, hemorrhages, necrosis, and inflammation. Currently, the complete understanding of the pathophysiology of acute pancreatitis

remains elusive. However, key concepts include self-digestion of the pancreas, elevated calcium levels in pancreatic cells, the presence of inflammatory mediators, apoptosis, and bacterial translocation from the intestine. Pancreatic acinar cells, rich in endoplasmic reticulum (ER), adjust to their physiological role of producing digestive enzymes. This involves the synthesis of various new digestive enzymes within the ER. The coordination of protein folding and post-translational modifications is facilitated by the collaborative action of ATP enzymatic activity, and the energy-consuming process of ATP hydrolysis. Furthermore, the Golgi apparatus aids in packaging, ultimately resulting in the secretion of zymogen granules extracellularly [5]. Rac1, a member of the Rho family, is a small GTPase that functions as a molecular switch, playing a role in the regulation of various essential cellular functions. [6].

The signal transducer Rac-1, expressed widely, oversees various activities associated with inflammatory responses, including cell adhesion, chemotaxis, and vascular permeability regulation [7]. Several research studies have emphasized the involvement of Rac1 in conditions like sepsis, hyperglycemia, lamellipodia formation, phospholipase activation, granule secretion, and platelet clot retraction. However, the potential impact of a Rac-1 inhibitor in mitigating neutrophil infiltration and tissue damage in acute pancreatitis remains unexplored. Given the aforementioned considerations, our hypothesis was that the inhibition of Rac-1 signaling could be significant in severe acute pancreatitis (AP). Therefore, the primary objective of this study was to explore the impact of a Rac-1 inhibitor on release of lipase and some chemokines, also focused on the protection against tissue damage caused by severe acute pancreatitis.

2. MATERIALS AND METHODS

A- Animals and housing

Male rats *Rattus norvegicus* were bred in the animal house of Biology Department - Education College -Salahaddin University-Erbil. During the entire period of experiments the rats were kept in special cages with a steel stainless wire mesh top to hold fed with standard rodent diet and water *ad libitum*. The room temperature was kept at about 22±4 ºC and the light dark cycle was kept in about 12/12 hours.

B- Design of the experiments

Twenty-four adult male rats weighting 240-300 gm and 12 weeks of age were divided equally into 3 groups each group eight rats were injected i.p. as the following:

Group 1 (control): Rats were given standard diet and tap water and injected with D.W i.p. twice one hour interval according to B.W.

Group 2 (L-arginine alone): Rats were given standard diet and tap water and L-arginine two dose of 250mg/100gm B.W one hour interval i.p.

Group 3:- Rac-1 +L-Arginine (pre-treatment): Rats were given standard diet and tap water and Rac-1inhibitor 5mg/kg B.W 15 minutes prior to L-arginine two dose of 250mg/100gm B.W 1 hour interval i.p.

C- Preparation of L-Arginine dose

The L-Arginine from sigma Aldrich, 500 mg/100g body weight (B.W) was prepared by dissolving it in distal water, each rat given specific volume according to exact B.W intra-pertonially (i.p.)

D- Induction of Pancreatitis in Rats

L-arginine hydrochloride was dissolved in normal saline to create a sterile solution. Non-fasting rats received an intraperitoneal injection of the L-arginine solution at a ratio of 250 mg/100 g body weight [5]. Animals were returned to the cages and allowed free access to feed and water. After one hour, animals were injected a second dose of L-arginine (250mg/100g) in saline. Control group was received saline alone. While the third group of rats received the same dose of L-arginine (250mg/100gm B.W.), after 15 minutes of Rac-1 injection (5mg/kg B.W.) intera-pertoneally. Animals were sacrificed after 72hours and a blood sample taken from the heart and tissue collected (pancreas). Tissue samples for histology were fixed in 10% formalin.

E-Rac-1 inhibitor

Rac1 inhibitor, NSC 23766 from Tocris (Bristol, United Kingdom) is a member of the Rho family, a small GTPase that functions as a molecular switch, playing a role in the regulation of various essential cellular functions [7]. The signal transducer, Rac-1 powder, dissolved in distill water Rac-1 is widely expressed and regulates a number of processes linked to inflammatory reactions, such as chemotaxis, cell adhesion, and vascular permeability[8].

F. Examination of the pancreas's morphology

The pancreatic tissue was fixed in a 10% formaldehyde phosphate buffer, followed by dehydration and embedding in paraffin. The embedded material was then cut into six micrometer slices, stained with hematoxylin and eosin, and observed under a light microscope. Employing the scoring system established earlier, which took into account edema, acinar cell

necrosis, bleeding, and neutrophil infiltration on a scale from absent to severe [9].

G. COUNTING THE LEUKOCYTES

Differential counts of systemic blood were conducted using blood taken from the heart and putted in the EDTA tube, fully automated haematology analyzer by sysmex (XP300 from india) was used to calculate the differential white blood cell count as well as neutrophil count.

H-ELISA

Lipase, CXCL1 and IL-6 levels in the serum were analyzed by use of double-antibody Quantitative enzyme linked immunosorbent assay kits (ELISA) (ELK Biotechnology from USA).

I-Anesthesia

The rats were anaesthetized by a combination of Ketamine (Rotexmedica and Tritta Germany) and xylazine (Xyla Ject Holland). Ketamine and xylazine were injected intraperitoneally in a dose of 90 mg/kg B.W and 10 mg/kg B.W, respectively.

J-Histological sectioning

A part of pancreas tissue was preserved in fixative solution (10% formalin) exposed to serial processes begin with dehydration, clearing and impregnation using a series of graded ethanol in ascending concentrations then immersed in xylene. Finally embedded in paraffin wax and cooled. Paraffin sections were cut by rotary microtome, and then stained with haematoxylin and eosin.

K-Statistical analysis

All data were expressed as Mean ± S.D, data analysis done by GraphPad Prism 10, comparison was made using one-way ANOVA, results compared by ANOVA and Duncan to determine significance among groups. Values were considered to be significantly different when $P<0.05$.

3. RESULTS

3.1. Rac-1 inhibitor regulates lipase activation in AP

To investigate the impact of a Rac-1 inhibitor on severe acute pancreatitis (AP) and its ability to regulate tissue damage, the study initially assessed blood lipase levels as a marker for tissue damage. Our findings revealed a 6- fold rise in lipase levels through retrograde L-arginine injection intra-pertoneally from **12.31 ± 0.53**ng/ml to **77.28 ± 3.81** ng/ml (Table 1, n = 8). Pretreatment with the Rac-1 inhibitor led to non- significant decrease in L-arginine-induced serum lipase levels, dropping from **77.28 ± 3.81**ng/ml to **42.07 ±2.79** ng/ml.

3.2. Rac-1 effects on leukocyte differential count in AP

Exposure to L-arginine resulted in an elevated count of circulating total leukocyte from **7.86 ±1.3 (cellsx10^3)** to **13.04±4.26 (cellsx10^3),** while pretreatment with Rac-1 prior to L-arginine injection 15 minutes led to non-significant decrease in leukocyte count to **12.11± 2.16 (cellsx10^3),** also exposure to L-arginine caused an elevate of special cells of leukocyte (lymphocyte cell) from **57.14 ± 2.59 % to 66.49 ± 8.3%**, indicating ongoing systemic activation. On the other hand treatment with Rac-1 (5mg/kg B.W.) makes a decrease in lymphocyte count **60.94 ± 8.62** % as shown in (Table 1). This is mean that injection of Rac-1, reversed alterations in leukocyte differential counts in the circulation, bringing them back to levels near control animals.

Table1. Blood lipase levels, white blood cells and Lymphocyte cells were measured in control rats and exposed L-arginine rats, and group three receiving pretreatment with a Rac-1 inhibitor (5mg/kg). Blood samples were obtained 72 hours following the onset of pancreatitis.

Groups / Parameter	Control	L-Arginine alone	Rac-1+L-Arginine
S. Lipase (ng/ml)	**12.31 ±5.34^a**	**77.28 ± 3.81^b**	**42.07 ± 2.79^{ab}**
WBC (cellx**10^3**)	**7.86 ±1.3^a**	**13.04 ±4.26^a**	**12.11± 2.16^a**

Lymphocyte %	**57.14 ±2.59[a]**	**66.49 ± 8.3[a]**	**60.94 ± 8.62[a]**

3.3. Histopathology

The tissue of the control pancreas had typical pancreatic anatomy, with lobules divided by thin interlobular septa. The acini, ducts, and islets of Langerhans make up the pancreatic lobules. Figure 1a and b showed well-formed ducts bordered by cuboidal epithelium. Significant fluid collection, cell swelling, and alteration of histoarchitecture were observed in the L-arginine-administered group (Fig. 1 C). In response to an infusion of L-arginine, acinar cell vacuolization and cytoplasmic expansion were observed in pancreatic tissue (Fig. 1d).

Furthermore, as seen in (Fig.1e), the injection of high dosages of L-arginine results in the broad necrosis of acinar cells. After rats were injected with 500 mg/100g body weight of L-arginine, (fig. 1-f) demonstrated the infiltration of many immune cells in response to tissue inflammation, whereas Langerhans cells remained unaffected. Finally, when Rac-1 was injected 15 minutes before to the injection of L-arginine, (fig. 1g) displayed minimal immune cell infiltration and minimal cell degradation.

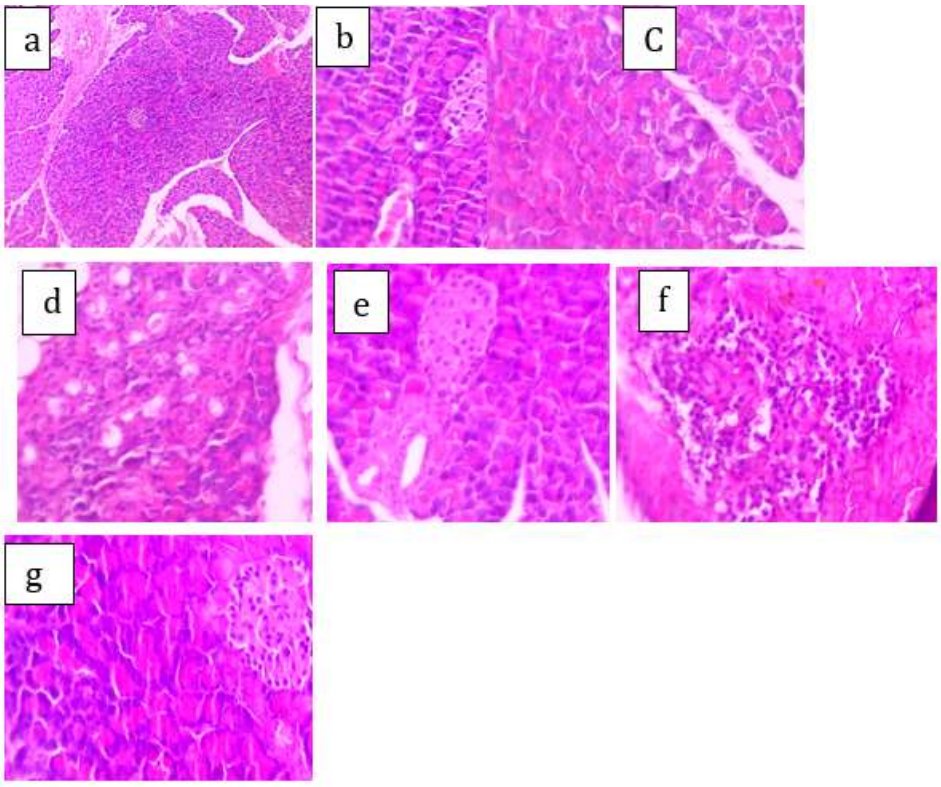

Figure1:- -a: normal section of pancreas 100x b: normal section of pancreas 400x . c- pancreatic cells 100x shows swelling of pancreatic acini cells d- section of pancreatic cells 400x show formation of vacule (vaculization). e-Pancreatic cell 400x show necrosis of acini cells. f- section of pancrease cell show infilitration of immune cells. g- pancrease tissue of Rac-1 group showed little cell infiltration and little degeneration in comparision with L-arginine treated group.

3.4. Rac-1 controls serum IL6 levels in acute pancreatitis

Acute pancreatitis increased plasma levels of IL6 from **1.32 ±0.11** pg/ml in control rats up to **6.01± 0.53** pg/ml, corresponding to about 5 -fold increase. We found the induction of acute pancreatitis by L-arginine suggesting that acute pancreatitis induces IL6 secretion, Notably Rac-1 inhibitor, significantly reduced chemokine release brought by acute pancreatitis. In acute pancreatitis pretreated with Rac-1 inhibitor reduced blood levels of IL6 from **6.01±0.53** pg/ml to **3.76 ±0.13** pg/ml as shown in (Table 2).

3.5. Rac-1 controls the levels of CXCL1 in acute pancreatitis

Levels of CXCL1 in the serum were assessed in both control animals and rats exposed to L-arginine, with prior treatment

using Rac1 inhibitor (5mg/kg). Blood specimens were gathered 72 hours after the initiation of pancreatitis, as found in the (Table 2) exposure to L-arginine significantly increased CXCL1 levels in the serum, escalating from **6.13±0.28** to **8.93±0.33** pg/ml. On the contrary, pre administration of the Rac-1 inhibitor significantly decreased CXCL1 levels from **8.93 ±0.33 to 6.93 ± 0.4** pg/ml.

Groups / parameter	Control	L-Arginine alone	Rac-1+L-Arginine
IL-6(pg/ml)	**1.32 ±0.11[a]**	**6.01± 0.53[c]**	**3.76 ±0.13[b]**
CXCL1(pg/ml)	**6.13 ± 0.28[a]**	**8.93 ± 0.33[c]**	**6.93 ± 0.4[b]**

Table1. Serum IL-6 levels and CXCL1 were measured in control rats and exposed L-arginine rats, and group three receiving pretreatment with a Rac-1 inhibitor (5mg/kg). Blood samples were obtained 72 hours following the onset of pancreatitis.

4. DISCUSSION

The present study indicates that Rac-1-mediated neutrophil infiltration and tissue damage are crucial factors in severe acute pancreatitis. These results imply a pivotal role for neutrophils in the development of acute pancreatitis. The Rac-1 inhibitor was employed to elucidate the involvement of Rac-1 in severe acute pancreatitis. The digesting enzyme lipase is mostly secreted by the pancreas. Lipase can rise quickly in acute pancreatitis can spike three to six hours after symptoms appear stay high for up to 5 days [10]. Earlier research has emphasized the crucial involvement of lipase in the initiation of acute pancreatitis. However, in our present study, we noted a significant rise in blood lipase levels after the injection of L-arginine into the rat's intrapertoneally in comparison to the control group. Consistent with our findings, prior studies have also shown an increase in lipase levels following severe acute pancreatitis [11], [12], [13]. However, our investigation revealed that pretreatment with the Rac-1 inhibitor (5 mg·kg−1) led to non- significant reduction in blood lipase (Table 1). This underscores the involvement of both lipase and Rac1 in acute pancreatitis. Additionally, histological examination demonstrated substantial pathological changes in the pancreatic tissue of L-arginine-exposed rats, indicative of significant tissue damage. In our study, administration of the Rac-1 inhibitor notably mitigated the extent of tissue damage (Figure 1). Previous studies have also highlighted the role of Rac-1 in the limitation of tissue damage in acute pancreatitis [14], [15], [16], [17]. Our results indicated that the Rac-1 inhibitor reversed alterations in leukocyte counts (Table 1). Yang et al. had previously noted the significance of leukocyte especially lymphocyte and neutrophil infiltration in severe acute pancreatitis [18]. Our findings align with this pattern, as demonstrated in (Table 1). The present results indicate the significance of Rac1-mediated platelet activation and the secretion of IL6 and CXCL1 derived from platelets in the context of acute pancreatitis. The role of Rac1 in acute pancreatitis was explored using the Rac1 inhibitor NSC23766. Serum samples were collected from rats in control, L-arginine-exposed, and Rac1 inhibitor pre-treated groups. It was observed that platelet reactivity is heightened in the presence of L-arginine induction. In agreement with this finding, better Rac-1 inhibitor has been linked to reduced platelet reactivity [19]. We discovered that rat's exposure to L-arginine, increased platelet activity and chemokine secretion (IL6 and CXCL1), the level of CXCL1 in serum from acute pancreatitis rats were higher than in control rats (Table 2). Remarkably, The Rac1 inhibitor (NSC23766) demonstrated a substantial decrease in the release of chemokines induced by L-arginine. The initiation of acute pancreatitis through L-arginine was effectively counteracted through the administration of the Rac1 inhibitor, resulting in a reduction of severity of pancreatitis. Additionally, other research included the control of CXCL1, a platelet-derived chemokine, is involved in abdominal sepsis [20]. Interleukin-6 is a good marker for early diagnosis of the severe acute pancreatitis, in this research, we showed that acute pancreatitis markedly increased IL6 by 5-fold higher than control rats, moreover, we discovered that the Rac1 inhibition strongly decreased IL6 levels in rats induced with acute pancreatitis, (Table 2). Rac1 inhibitor may thus be a therapeutic agent for controlling acute pancreatitis by inhibiting chemokine, a signaling molecule in exaggerated inflammation.

5. CONCLUSION

In this investigation, we illustrated the regulatory role of Rac1 in severe acute pancreatitis, particularly in modulating lipase activity. Additionally, we observed overexpression of the chemokines IL6 and CXCL1 in platelet activation among acute pancreatitis rats. The inhibitory effect of Rac-1 on leukocyte count especially lymphocyte and neutrophil infiltration was also highlighted. Furthermore, the morphological changes associated with acute pancreatitis in rats were attributed to platelet

activation. These findings suggest that Rac1 inhibition may serve as a therapeutic approach in regulating acute pancreatitis by mitigating the production of chemokines, key signaling molecules in inflammation, and subsequently reducing organ damage caused by activated platelets in acute pancreatitis.

REFERENCES

[1] K. Ley et al., "Neutrophils: New insights and open questions," Sci. Immunol., vol. 3, no. 30, p. eaat4579, 2018.

[2] J. Walkowska, N. Zielinska, R. S. Tubbs, M. Podgorski, J. Dłubek-Ruxer, and Ł. Olewnik, "Diagnosis and treatment of acute pancreatitis," Diagnostics, vol. 12, no. 8, p. 1974, 2022.

[3] N. Reusch et al., "Neutrophils in COVID-19," Front. Immunol., vol. 12, p. 652470, 2021.

[4] K. Ley, C. Laudanna, M. I. Cybulsky, and S. Nourshargh, "Getting to the site of inflammation: the leukocyte adhesion cascade updated," Nat. Rev. Immunol., vol. 7, no. 9, pp. 678–689, 2007.

[5] Y. Chen et al., "Melatonin induces anti-inflammatory effects to play a protective role via endoplasmic reticulum stress in acute pancreatitis," Cell. Physiol. Biochem., vol. 40, no. 5, pp. 1094–1104, 2016.

[6] F. Mouawad, H. Tsui, and T. Takano, "Role of Rho-GTPases and their regulatory proteins in glomerular podocyte function," Can. J. Physiol. Pharmacol., vol. 91, no. 10, pp. 773–782, 2013.

[7] R. Hwaiz et al., "Rac1 signaling regulates sepsis-induced pathologic inflammation in the lung via attenuation of Mac-1 expression and CXC chemokine formation," J. Surg. Res., vol. 183, no. 2, pp. 798–807, 2013.

[8] L. A. Martinez, "Effects of Regulating Rac1 in a Mouse Model of Fragile X Syndrome," 2016.

[9] Y. Hong et al., "Effects of castanospermine on inflammatory response in a rat model of experimental severe acute pancreatitis," Arch. Med. Res., vol. 47, no. 6, pp. 436–445, 2016.

[10] O. Z. Ismail and V. Bhayana, "Lipase or amylase for the diagnosis of acute pancreatitis?," Clin. Biochem., vol. 50, no. 18, pp. 1275–1280, 2017.

[11] X. Wu et al., "Protective effect of tetrandrine on sodium taurocholateinduced severe acute pancreatitis," Evid. Based Complement. Alternat. Med., vol. 2015, 2015.

[12] T. Plusczyk, S. Westermann, D. Rathgeb, and G. Feifel, "Acute pancreatitis in rats: effects of sodium taurocholate, CCK-8, and Sec on pancreatic microcirculation," Am. J. Physiol.-Gastrointest. Liver Physiol., vol. 272, no. 2, pp. G310–G320, 1997.

[13] P. Xu et al., "Pioglitazone attenuates the severity of sodium taurocholateinduced severe acute pancreatitis," World J. Gastroenterol. WJG, vol. 13, no. 13, p. 1983, 2007.

[14] M. E. Soliman, M. A. Kefafy, M. A. Mansour, A. F. Ali, and W. A. I. I. Esa, "Histological study on the possible protective effect of pentoxifylline on pancreatic acini of l-arginine-induced acute pancreatitis in adult male albino rats," Menoufia Med. J., vol. 27, no. 4, p. 801, 2014.

[15] X.-X. Hong et al., "Systemic injury caused by taurocholate-induced severe acute pancreatitis in rats," Exp. Ther. Med., vol. 24, no. 1, pp. 1– 12, 2022.

[16] Y. Wang, Y. Li, S. Gao, X. Yu, Y. Chen, and Y. Lin, "Tetrahedral framework nucleic acids can alleviate taurocholate-induced severe acute pancreatitis and its subsequent multiorgan injury in mice," Nano Lett., vol. 22, no. 4, pp. 1759–1768, 2022.

[17] E. Seyhun et al., "Tauroursodeoxycholic acid reduces endoplasmic reticulum stress, acinar cell damage, and systemic inflammation in acute pancreatitis," Am. J. Physiol.-Gastrointest. Liver Physiol., vol. 301, no. 5, pp. G773–G782, 2011.

[18] Z. Yang, X. Meng, and P. Xu, "Central role of neutrophil in the pathogenesis of severe acute pancreatitis," J. Cell. Mol. Med., vol. 19, no. 11, pp. 2513–2520, 2015.

[19] G. G. Schiattarella et al., "Rac1 modulates endothelial function and platelet aggregation in diabetes mellitus," J. Am. Heart Assoc., vol. 7, no. 8, p. e007322, 2018.

[20] Z. Hasan et al., "Rho-kinase signaling regulates pulmonary infiltration of neutrophils in abdominal sepsis via attenuation of CXC chemokine formation and Mac-1 expression on neutrophils," Shock, vol. 37, no. 3, pp. 282–288, 2012.