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Pancreatic regeneration following an acute injury: Evidence from histological, haemato-biochemical and immunohistochemical analyses[#]

P. Shibla Nargees[1], K.S. Prasanna[1*], I.S. Sajitha[1], R. Anoopraj[1], C. Divya[1], Suresh N. Nair[2] and P. Krishna Veni[1]

[1]Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, [2]Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Pookode, Wayanad- 673 576, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad – 673 576, India

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Abstract

Acute pancreatitis is a major gastrointestinal disorder and current management is largely limited to supportive and symptomatic care. The disease manifests with oedema, necrosis, haemorrhage, and inflammatory changes in the exocrine pancreas, while its chronic form represents a fibroinflammatory condition with recurrent injury, fibrosis, and increased neoplasia risk. This study assessed hematobiochemical and histomorphological alterations in a Wistar rat model of L-arginine–induced pancreatitis. Induction was achieved using two intraperitoneal injections of L-arginine (2.5 g/kg, 1h apart) and the animals were sacrificed on days 8 and 15. Controls received saline. Blood samples were collected at baseline and on days 2, 8, and 15 for hematobiochemical assessment, while pancreatic tissue was subjected to biochemical, histopathological, and immunohistochemical analyses. Serum amylase and lipase sharply elevated within 24 h (amylase: 2922.93±294.25 vs. 476.11±37.75; lipase: 445.08±70.31 vs. 12.73±3.88), declining to near-normal by days 8 and 15. Glucose, total erythrocytes, haemoglobin, and packed cell volume remained unchanged, with only transient leukocytosis on day 2. Persistent glutathione depletion with elevated nitrite and myeloperoxidase indicated oxidative stress. Histopathology on day 8 showed necrosis, mononuclear infiltration, haemorrhage, and fatty changes, while fibroplasia, acinar-to-ductal metaplasia, and ductal hyperplasia were evident by day 15. Immunohistochemistry confirmed regeneration through PCNA expression, whereas IL-6 exhibited moderate localisation in acinar cells. This model replicates the progression from acute to chronic pancreatic damage.

Keywords: *Rats, pancreatitis, arginine, regeneration*

Pancreatitis is the most prevalent exocrine pancreatic disorder, typically characterised by severe abdominal pain and potential multi-organ dysfunction. Global incidence of pancreatitis is increasing, with severe cases linked to high morbidity and mortality (Iannuzzi *et al*., 2022). Current management remains largely supportive.

The pathogenesis of severe acute pancreatitis is highly complex and the autodigestion theory is the most widely recognised and accepted mechanism. A proper understanding of the patho- mechanisms of pancreatitis is essential for the development of effective therapeutic strategies. L-arginine induced pancreatitis is a well-established rat model for

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**Corresponding author: prasanna@kvasu.ac.in, Ph. 8589904780*

studying disease pathogenesis. Two doses administered one hour apart reliably induce acute pancreatitis without affecting other organs or the endocrine pancreas (Rehman *et al*., 2021). Its dose and time dependent response makes it suitable for investigating both early and late disease stages. Animal studies have suggested that the exocrine pancreas has an inherent ability to regenerate allowing it to recover rapidly and completely from disorders such as acute pancreatitis (Murtaugh and Keefe, 2015). The reparative process in the pancreas involves complex mechanisms. The present study aims to characterise injury and subsequent repair in L-arginine induced pancreatitis in Wistar rats. Comprehensive hematobiochemical, histomorphological, and immunohistochemical evaluations provide valuable insights into the regenerative potential of the pancreas contributing to a better understanding of its healing mechanisms and future therapeutic possibilities.

Materials and methods

The study was conducted on 12 Wistar albino rats of either sex weighing 120–150 g following approval from the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, as per the order number CVAS/MTY/IAEC /25/86 dated 17.02.2025. Pancreatitis was experimentally induced in six animals using two intraperitoneal doses of L-arginine (2.5 g/kg) administered at an interval of one hour on day 1. The animals were euthanized on the 8th and 15th days post-induction. Control animals received normal saline. All animals were maintained under standard management conditions.

Blood samples were collected on days 2, 8, and 15 of the experimental periods for the evaluation of haematological parameters including total leukocyte count, total erythrocyte count, packed cell volume and haemoglobin concentration, as well as biochemical parameters such as serum amylase, lipase, and glucose. After sacrifice, pancreatic tissue was collected in 10% neutral buffered formalin for histopathological and immunohistochemical analysis and in phosphate buffered saline for spectrophotometric analysis to estimate pancreatic glutathione, nitrite and myeloperoxidase levels.

Statistical analyses

Statistical analyses were performed to evaluate the blood parameters, oxidative stress markers and histopathological and immunohistochemical changes. Repeated measures ANOVA was used to assess changes in blood parameters over time. The Mann–Whitney U test was applied for the statistical analysis of histopathological parameters.

Results and discussion

The comparative findings of hematobiochemical, gross morphological, histopathological and immuno-histochemical evaluations at different time intervals of acute pancreatitis were as follows:

Serum amylase, lipase, and glucose

As depicted in table 1, serum amylase and lipase levels showed a significant rise in 24 hours after induction (amylase: 2922.93 ± 294.25 vs. 476.11 ± 37.75; lipase: 445.08 ± 70.31 vs. 12.73 ± 3.88 in controls), confirming the successful induction of pancreatitis as observed by Unni *et al*. (2023). Both enzyme levels were gradually returned towards normal by day 8 and 15, although they remained slightly higher than in the control group (Figs. 3.a and 3.b). According to Sidhu *et al*. (2011), who noted the similar findings in their study, this is because the kidneys clear more amylase through urine or due to severe pancreatic damage that stops amylase production. In contrast, serum glucose levels did not show any significant changes throughout the experimental period. This is likely due to the selective cytotoxic effect of L-arginine on acinar cells without affecting the pancreatic islets, as reported by Mizunuma *et al*. (1984).

Haematological parameters

A marked increase in total leukocyte count was observed on day 2 (7.10 ± 0.203 vs. 5.95 ± 0.178 in controls), reflecting an early inflammatory response. The return of leukocyte count to baseline values by days 8 and 15 suggests resolution of the acute inflammatory phase over time (Chen *et al*., 2022) (Fig. 3.c). A significant reduction in packed cell volume was recorded on day 2 (29.24 ± 0.43; vs. 32.99±0.24 in control), followed by gradual recovery by day 15, indicating a transient effect on erythropoiesis during the early stages of inflammation. Erythrocyte count and haemoglobin concentration, however, did not show significant changes throughout the experimental period, suggesting that overall red blood cell parameters remained largely stable despite the acute inflammatory response (Table 1). Consistent with this, Khan *et al*. (2021) also reported no significant alterations in erythrocyte count or haemoglobin concentration between control and diseased groups in their studies.

Spectrophotometric evaluation

Compared to the control group, pancreatic glutathione levels remained significantly reduced throughout the study period, with the decline being more pronounced on day 8 (5.16 ± 0.130 vs. control 7.53 ± 0.055) (Fig. 3. d) indicating enhanced oxidative stress at both local and systemic levels (Rau *et al*., 2000). Pancreatic nitrite was markedly elevated on comparison to the control group on day 8 (38.66±0.513 versus control 17.18±0.346; Fig. 3.e), indicative of heightened nitric oxide production. This increase in nitrite is consistent with upregulated inducible nitric oxide synthase (iNOS) activity during advanced acute pancreatitis, which promotes excessive nitric oxide

Table 1. Effect of L-arginine on the blood parameters on day 0, 2, 8 and 15 of the experiment (mean±SE)

Blood parameters	Group	Day 0	Day 2	Day 8	Day 15	F- value (p- value)
Serum amylase (U/L)	Control	594±31.87	476.11^{a}±37.75	448.32^{a}±23.53	424.49^{a}±16.34	1.174^{ns} (0.339)
	L-arginine	603.35^{A}±40.67	2922.93^{Bb}±294.25	786.17^{bC}±26.27	613.16^{bD}±45.65	14.963** (<0.00 1)
Serum lipase (U/L)	Control	5.51^{a}±0.61	12.73^{b}±3.88	9.05^{b}±2.05	8.53^{a}±0.94	0.227^{ns} (0.877)
	L-arginine	12.82^{aA}±2.58	445.08^{aB}±70.31	35.41^{aC}±5.59	11.43^{aD}±3.22	26.000** (<0.001)
Serum glucose (mg/dL)	Control	106.10±3.33	104.00±1.17	101.43±0.80	98.08±2.13	0.313^{ns} (0.816)
	L-arginine	101.60±7.15	96.56±5.62	97.70±7.35	98.64±10.08	0.056^{ns} (0.982)
Total leukocyte count (10^3/µL)	Control	5.79±0.242	5.95^{b}±0.178	6.02±0.202	6.00±0.10	0.343^{ns} (0.795)
	L-arginine	5.79^{A}±0.281	7.10^{aB}±0.203	6.29^{A}±0.179	7.43^{C}±0.20	10.492** (<0.001)
Total erythrocyte count (10^6 /µL)	Control	5.43^{A}±0.176	5.16±0.229	5.13^{bB}±0.185	5.39±0.282	2.001^{ns} (0.138)
	L-arginine	5.46±0.219	5.63±0.219	5.88^{aA}±0.179	5.00^{B}±0.158	2.072^{ns} (0.128)
Packed red cell volume (%)	Control	32.93±0.244	32.99^{a}±0.244	32.69±0.266	33.36±0.270	0.140^{ns} (0.935)
	L-arginine	31.90^{A}±0.431	29.24^{bB}±0.431	32.49^{A}±1.03	33.88^{A}±0.751	13.725** (<0.001)
Hemoglob in (g/dL)	Control	11.39±0.243	11.28±0.276	11.11±0.260	10.81±0.238	0.657^{ns} (0.586)
	L-arginine	11.82±0.281	11.38±0.253	11.70±0.254	11.32±0.503	0.474^{ns} (0.703)

generation, contributing to oxidative stress and pancreatic tissue injury (Que *et al*., 2010). However, a reduction in these values was observed on day 15. Myeloperoxidase (MPO) exhibited a similar trend, with levels peaking on day 8 (1.81 ± 0.031 versus control 0.992 ± 0.017; Fig. 3.f). Comparable observations were reported by Sahota *et al*. (2021), who reported significantly elevated serum MPO levels in acute pancreatitis patients compared to healthy controls, suggesting its potential as a prognostic marker (Table 2).

Gross morphology

On day 8, gross examination of the pancreas revealed necrosis of the surrounding peripancreatic fat tissues in some animals, a finding supported by Patel *et al*. (2015) who reported that pancreatic injury triggers lipase release, promoting visceral fat lipolysis and contributing to peripancreatic fat necrosis. In addition, a small cyst was observed within the pancreatic tissue, along with diffuse congestion of the gland. By day 15, these alterations had subsided, with only minimal changes evident in the gross morphology of the pancreas (Fig. 1a -1d).

Histopathological evaluation

Pancreatic tissues were subjected to histopathological examination at different time intervals. On day 8, microscopic evaluation revealed extensive pathological alterations. Multifocal areas of necrosis were

Table 2. Effect of L-arginine on pancreatic glutathione, nitrite and myeloperoxidase levels on days 0, 2, 8 and 15 of the experiment (mean±SE)

Parameters	Group	Day 8	Day 15	F- value (P-value)
Pancreatic glutathione	Control	7.53^{a}±0.055	7.61^{a}±0.038	0.079^{ns} (0.780)
	L-arginine	5.16^{b}±0.130	5.55^{b}±0.173	2.046^{ns} (0.158)
Pancreatic nitrite	Control	17.18^{a}±0.346	17.02^{a}±0.328	0.032^{ns} (0.859)
	L-arginine	38.66^{bA}±0.513	36.79^{bB}±0.531	4.133* (0.047)
Pancreatic myeloperoxidase	Control	0.992^{b}±0.017	1.04^{b}±0.044	1.185^{ns} (0.281)
	L-arginine	1.81^{aA}±0.031	1.70^{aB}±0.011	5.024* (0.029)

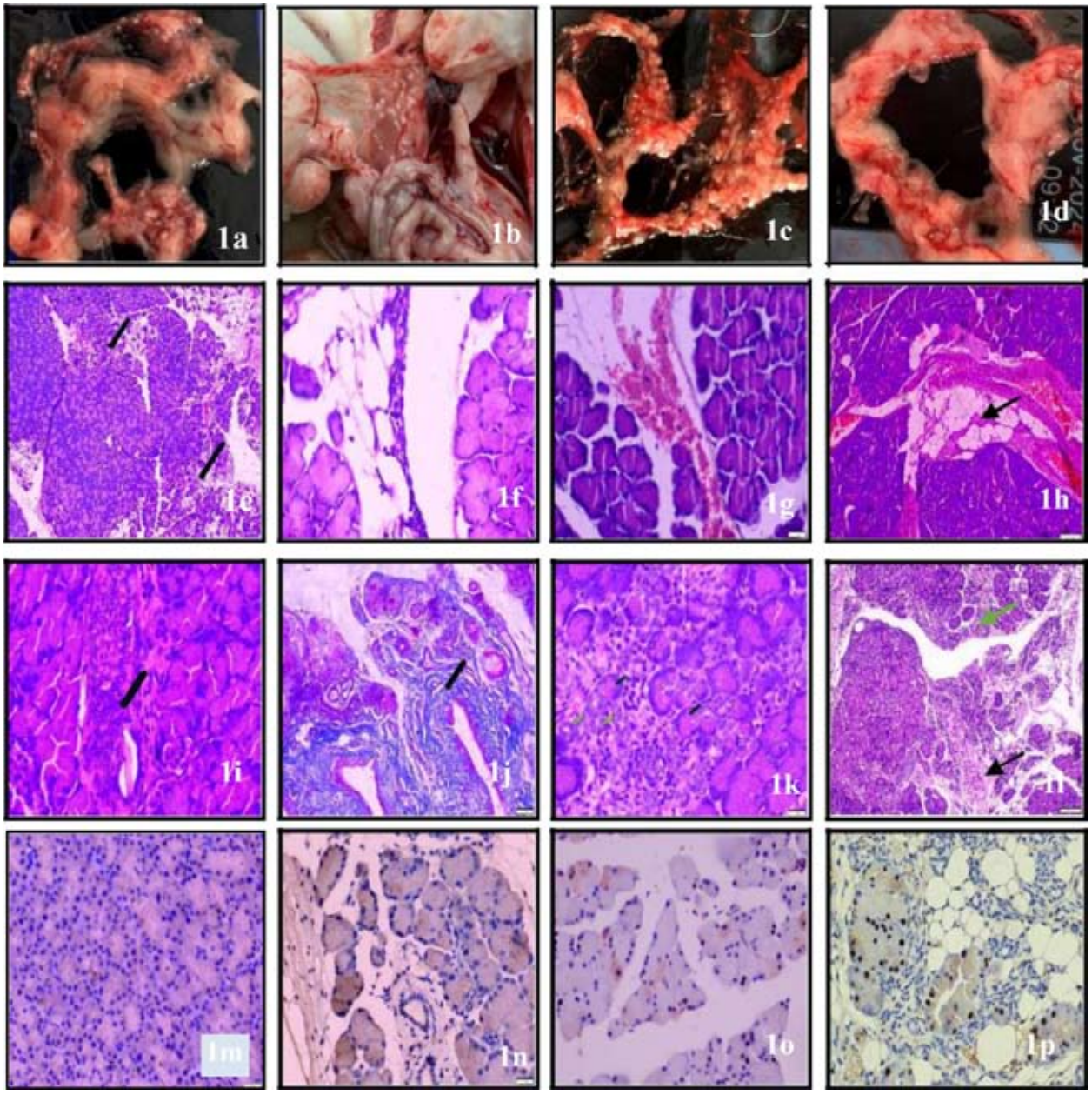

Fig. 1. a. Rat- Pancreas-Disease control b. Rat- Disease control- Pancreas-Day 8-Peripancreatic fat necrosis and diffuse congestion c. Rat- Disease control- Pancreas-Day 8-diffuse congestion d. Rat- Disease control- Pancreas-Day 15-mild congestion and oedema e. Rat- Disease control- Pancreas-Day 8- Multifocal areas of necrosis (black arrow) (H&E-x100) f. Rat- Disease Control-Pancreas-Day 8- Infiltration of inflammatory cells (H&E-x400) g. Rat- Disease control- Pancreas- Day 8- Severe haemorrhage(H&E-x400) h. Rat- Disease control- Pancreas- Day 8- Fatty infiltration (H&E-x400) i. Rat- Disease control- Pancreas-Day 15-Moderate ductal cell hyperplasia (Black arrow) (H&E-x400) j. Rat- Disease control- Pancreas-Day 15-Periductal fibrosis (Black arrow) (Masson's trichrome-x400) k. Rat- Disease control- Pancreas-Day 15-Acinar cells are transformed in to duct like cells(yellow arrow).Surviving acini(black arrow) l. Rat- Disease control- Pancreas-Day 15-Acinar ductal metaplasia (Green arrow), fibrosis (Black arrow) (H&E x100) m. Rat-Control- pancreas-IL-6-No immunoreactivity (IHC, X100) n. Rat- Disease control- pancreas-Day8-IL-6- Moderate immunoreactivity (IHC, X400) o. Rat- Disease control- pancreas-Day 15- IL-6-Weak immunoreactivity (IHC, X400) p. Rat- Disease control- pancreas-Day 15- PCNA-Strong immunoreactivity (IHC, X400)

evident within the pancreatic parenchyma, accompanied by marked interstitial haemorrhage. Prominent fatty infiltration was observed, which replaced and disrupted the normal acinar cell architecture. In addition, mild infiltration of mononuclear inflammatory cells was noted in the interstitial spaces, suggesting the initiation of an inflammatory response (Fig. 1e-1h). Similar observations were reported by Sreelekshmi (2024) who observed enlargement of interlobular spaces with oedema, widespread acinar cell necrosis, cytoplasmic vacuolation, and marked disorganization of acinar architecture within 24 hours of induction in L-arginine induced pancreatitis models. By day 15, these acute pathological changes had subsided, and the histological picture revealed features of tissue regeneration. Pancreatic acinar cells appeared shrunken and had undergone transformation into duct-

like cells, a process known as acinar-to-ductal metaplasia (ADM). Several surviving acini were observed interspersed within the tissue indicating preservation of some functional units. New acinar cells are believed to originate from the proliferation of these surviving acini (Fig. 1k and 1l). The transformation of acinar cells into ductal cells plays a pivotal role in the reparative and regenerative process of the pancreas (Murtaugh and Keefe, 2015). Marked fibrosis was evident on day 15 further supporting the ongoing reparative process. Fibrotic changes were highlighted and confirmed using Masson's trichrome stain (Fig. 1j), which clearly demonstrated collagen deposition within the interstitium. This is in-consistent with the findings of Sidhu *et al*. (2011) who explained that pancreatic stellate cells drive regeneration by proliferating, adopting a myofibroblastic phenotype, and regulating extracellular matrix deposition and degradation. In addition, ductal cell hyperplasia was prominently observed, signifying active regenerative changes within the pancreatic tissue (Fig. 1i). Collectively, these findings indicate that pancreatic acinar cells possess an inherent ability to undergo spontaneous

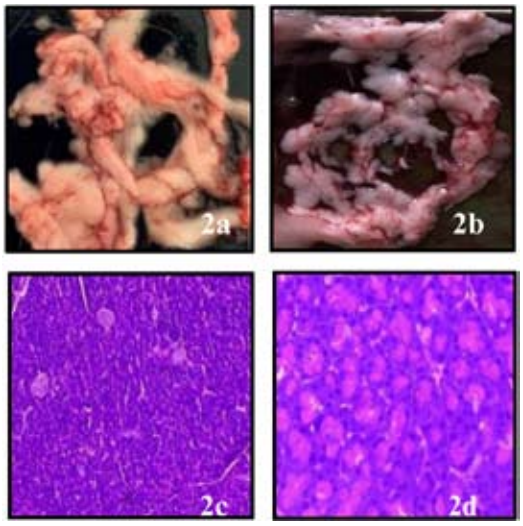

Fig. 2. **a.** Rat- Control-Pancreas-Day 8-No gross changes noticed **b.** Rat- Control-Pancreas-Day 15-No gross changes noticed **c.** Rat- Control- Pancreas –Healthy pancreas with normal acinar cells, ducts and islets (H&E-x100) **d.** Rat- Control- Pancreas –Acinar pattern of cells with basal nuclei (H&E-x400)

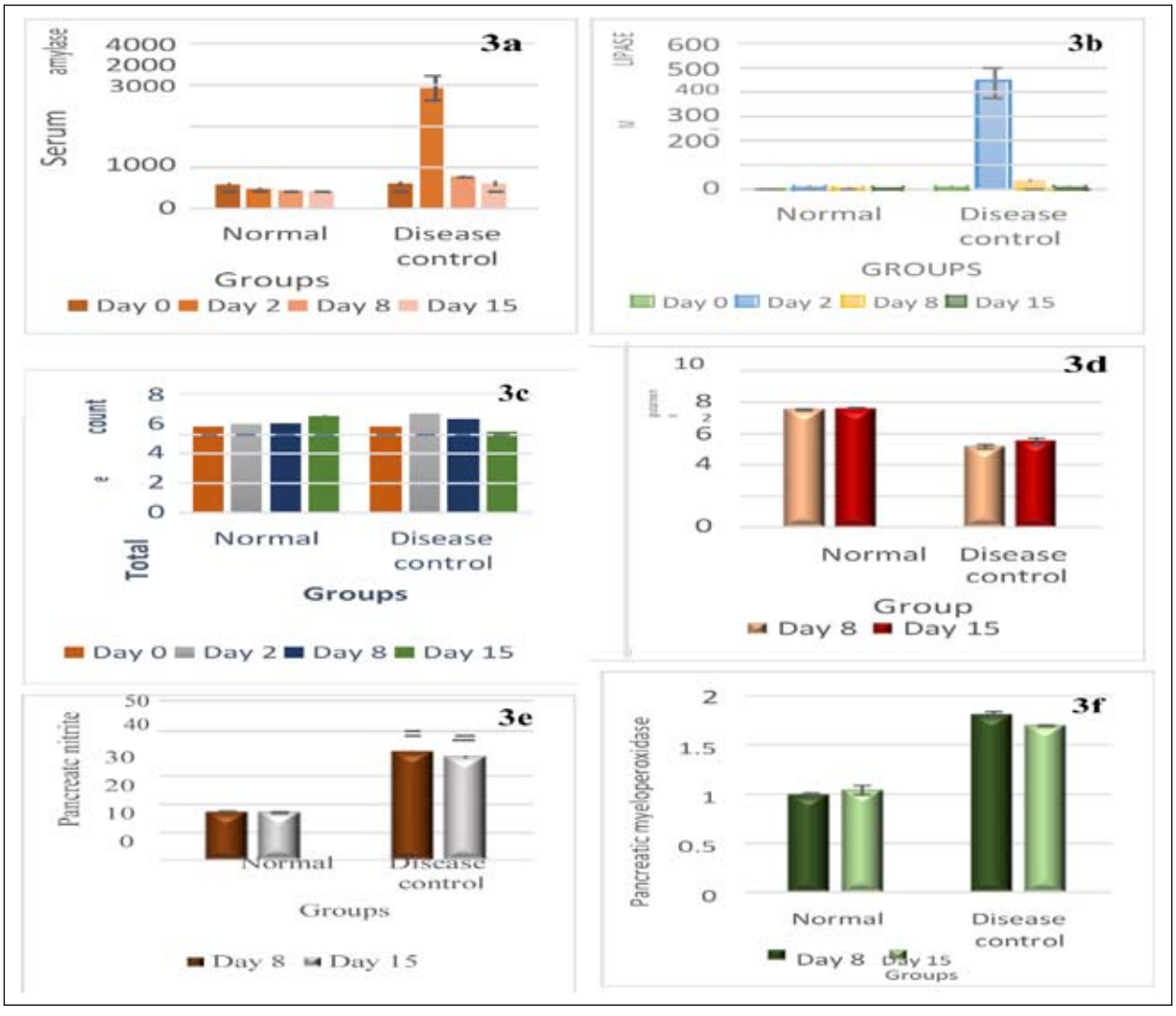

Fig. 3. a. Serum amylase b. Serum lipase c. Total leukocyte count d. Reduced pancreatic glutathione e. Pancreatic nitrite f. Pancreatic myeloperoxidase

recovery following the onset of pancreatitis. The combined processes of acinar-to-ductal metaplasia, fibrosis, and ductal cell hyperplasia reflect the dynamic regenerative potential of the pancreas during the later stages of injury.

Immunohistochemistry

Pancreatic tissue sections were subjected to immunohistochemical evaluation for interleukin-6 (IL-6) and proliferating cell nuclear antigen (PCNA). IL-6 is a proinflammatory cytokine that plays a key role in regulating inflammatory responses. In the present study, IL-6 was primarily localized in the cytoplasm of acinar cells near the nucleus, with no expression detected in the control group (Fig. 1m). A study by Jablonowska *et al*. (2008) in patients with chronic pancreatitis also reported strong cytoplasmic IL-6 expression in both acinar and ductal pancreatic cells when compared to control group. On day 8, moderate cytoplasmic localization of IL-6 was observed in acinar cells indicating an enhanced inflammatory response (Fig. 1n). By day 15, only mild localization of IL-6 was evident suggesting a reduction in proinflammatory cytokines and a corresponding decrease in pancreatic inflammation (Fig. 1o).

PCNA is a nuclear protein that plays a critical role in DNA replication and repair, and it is widely used as a marker of cell proliferation. Immunohistochemical analysis of PCNA expression was carried out to evaluate the regenerative activity in the pancreas. Strong nuclear expression of PCNA was observed in acinar cells undergoing metaplastic changes on day 15 (Fig. 1p). This indicates a high degree of proliferative activity and reflects an active reparative process in the pancreatic tissue at this stage (Elsasser *et al*., 1995).

Taken together, the decreasing expression of IL-6 and the strong nuclear localization of PCNA suggest a transition from an inflammatory to a regenerative phase highlighting the capacity of pancreatic tissue to undergo spontaneous recovery following pancreatitis.

Conclusion

Based on our study, L-arginine–induced pancreatitis in Wistar rats successfully replicates key features of acute pancreatic injury and its subsequent reparative processes. The model demonstrates transient enzymatic, haematological and oxidative stress changes, followed by histological and immunohistochemical evidence of pancreatic regeneration, highlighting its utility for studying the mechanisms of pancreatic injury and repair.

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Conflict of interest

The authors declare that they have no conflict of interest.

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