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Impact of high protein diet on the formation and healing of L-arginine induced acute pancreatitis in male wistar rats

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Abstract

The condition known as acute pancreatitis is defined as the inflammation of the pancreas, commonly caused by gallstones and alcohol ingestion. The paucity of information on its management in combination with other factors such as adverse effects resulting from treatment has redirected the attention of researchers to safer, alternate therapies. This study aims to assess the role of a high-protein diet in mitigating acute pancreatitis caused by L-arginine. Two groups of twenty male rats were randomly assigned; groups fed with normal diet (NP), and groups fed with high-protein diet (HPD). Acute pancreatitis was induced with L-arginine monohydrochloride at dose of 250mg/Kg. It was administered 3 times at interval of one hour. After induction, the groups were further grouped into subgroups upon observations on day 3 and day 7. Lipid peroxidation (MDA level), total protein and antioxidants parameters such as hydrogen peroxide, nitric oxide. sulfurhydric acid concentration, total antioxidant capacity (TAC), CAT, GPx and NO were evaluated using spectrophotometry. Every data set was shown as mean ± SEM and as an ANOVA with a post-hoc analysis at $\alpha = 0.05$. Findings revealed that the high protein diet administered significantly increased the protein level, sulfurhydryl concentration, TAC, CAT, GPx and NO compared to the acute pancreatitis model on day 3, and had no significant effect in most parameters on day 7. Lipid peroxidation substantially decreased in the treatment groups when compared to pancreatitis model. High protein diet improves the amelioration of acute pancreatitis by enhancing the antioxidant enzymes, nitric oxide and inhibiting the process of lipid peroxidation.

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1. Introduction

An inflammatory condition of the pancreas known as acute pancreatitis (AP) is caused by the pancreatic auto-digestion process, in which the pancreatic enzymes damage the pancreatic tissue and cause the gland, as well as remote systems and organs, to fail [1]. Acinar cell death and local and systemic inflammation are the hallmarks of acute pancreatitis, and they are caused by well-known factors like alcohol, endoscopic retrograde cholangiopancreatography (ERCP), pancreatic ductal obstruction secondary to gallstones (the most common cause), and various drugs. These factors also trigger pathological cellular pathways and organelle dysfunction [2–4] . Management of acute pancreatitis has been a major challenge, as of right now, there are no proven therapeutics that directly

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treat or prevent acute pancreatitis. Most medications employed are purposely targeted at decreasing the pain sensation or inhibiting the activities of pancreatic enzymes.

Fluid recovery or intravenous hydration also referred to as nourishment support, has been a major therapy to enhance the flow of the pancreatic and intestinal microcirculation following detection of acute pancreatitis. It is said to prevent bacterial translocation and Secondary pancreatic infection caused by ischemia [5, 6].

In the clinical setting, acute pancreatitis was also treated with enzyme therapy, which attempts to normalize pancreatic output in order to reduce discomfort. Therefore, morbidity and long-term consequences continue to be significantly elevated, amounting to around ten percent [7,8]. As a matter of fact, following clinical therapy, approximately eight percent of individuals with acute pancreatitis go on to develop chronic pancreatitis, and Eighteen percent of patients have a recurrence [7–10].

In acute pancreatic , several factors has been implicated in pathophysiology, expression of pro-inflammatory and inflammatory factors pathological calcium overload, early trypsinogen activation, pancreatic microcirculation abnormalities, NF-kB activation, leucocyte cell infiltration, and defective autophagy.

Research has demonstrated how oxidative stress plays a factor in the inflammatory responses that are acute, especially in cases of acute pancreatitis resulting from pancreatic damage [11, 12]. The imbalance between free radicals and antioxidants, known as oxidative stress, may be a harmful catalyst for the onset due to severe pancreatitis.

However, ROS can also trigger leukocyte relocation as well as the discharge of many chemokines and inflammatory cytokines. Conversely, Que *et al*. [13] found that oxidative species like H_2O_2 and alpha-beta-unsaturated aldehydes possessed a direct role in the inflammation as second messengers. Utilizing L-arginine is one of the modern method used in inducing acute pancreas. The most widely used amino acid-induced AP model in rats and mice at the moment is L-arginine-induced AP (ARG-AP).

Since 1984, when L-arginine is administered intraperitoneally (i.p.) at a dosage of 5 g/kg caused prolonged pancreatic adipose tissue necrosis and PAC rats, without affecting the islets or other organs, Many studies have been conducted on the impact of L-arginine on the pancreas [14].

Taking a proper or balanced Diet has been one of the ways patients tend to improve the disease condition. Diet is actually pattern of food intake that obeys certain demands that are relevant to health and weight. A nutritious diet is one that delivers sufficient macronutrients in the proper proportions to maintain energetic and physiological demands without overeating, as well as adequate water and micronutrients to meet the body's needs [15].

High protein diets, characterized by elevated protein intake at the expense of carbohydrates and fats, have garnered considerable attention due to their potential impact on health and physiology [16]. These diets have been extensively studied for their role in weight management, muscle growth, and metabolic regulation. A diet classified as high protein contains at least 20% of its calories from protein. The majority of high-protein diets severely limit carbohydrate consumption and are heavy in saturated fat [17].

In light of the complex relationship between dietary protein and health, this study examines the possible benefits of a high-protein diet in treating male Wistar rats' acute pancreatitis caused by L-arginine.

2. Resources and procedure

2.1. Ethical approval

The research was approved by the Federal University of Technology Akure Ethical Committee.According to the guidelines provided in the National Academy of Science's Guide for the Care and Use of Laboratory Animals [18], the study was completed. In conventional laboratory settings, animals were raised in homes with a 12-hour light/dark cycle and provided with unlimited access to food and water.

2.2. Experimental protocols

Twenty (20) male Wistar strain with a mass of 180 to 280g were employed in the research. They were provided with unfettered access to food and water at the Department of Physiology's animal home, FUTA. Animals have been separated into several categories.The animals were fed with high protein diet for 10 weeks, after feeding the animal for 10 weeks, they were made to fast for 24hrs before induction of acute pancreatitis with L-arginine, it was administered at dosage of 250mg/100g rat thrice at an hour interval between each dose. Animals were sacrificed at 72hrs and 7 days later to check disease severity.

2.3. Induction of acute pancreatitis

After dissolving L-arginine monohydrochloride powder (LOBA chemie PVT Ltd, India) in regular saline, the pH was brought to 7.0. A modification of the approach described by Omayone *et al*.[19] was used to induce acute pancreatitis. At 1-hour intervals, four doses of L-arginine 200mg/100g were delivered intraperitoneally. No mortality was recorded in this study. On days three and seven following the injection of L-arginine, the animals were sacrificed. Blood samples were collected using heart puncture and transferred to a plain bottle. After centrifuging the blood samples for ten minutes at 4000 rpm, the serum was obtained. It was then quickly chilled till the levels of serum amylase and oxidative stressors were ascertained.

Table 1: Component of normal rat pellet and composition of high protein diet.

S/N	Feed composition	Control feed (kg)	High protein diet (kg)
1	Maize	7.00	3.25
2	Soya	3.75	3.75
3	Bone meal	1.00	1.00
4	PKC	1.60	1.60
5	Wheat offal	5.25	4.00
6	Rice bran	1.25	1.25
7	GNC	2.85	2.85
8	Grower premix	0.10	0.10
9	Methionine	0.10	0.10
10	Lysine	0.03	0.03
11	ToxiMos	0.03	0.03
12	Salt	0.08	0.08
13	Fish meal	2.00	7.00
	TOTAL	25.03	25.03

2.4. Biochemical parameters

The Biuret procedure, first developed by Gornall *et al*.[20] in 1949, was used to assess the protein contents of the various samples. However, there was a little adjustment made to the reagent: potassium iodide was added to stop Cu2+ ions from precipitating as cuprous oxide.

Using the approach of Varshney and Kale [21], the thiobartutic reactive substances (TBARS) generated during lipid peroxidation were measured in order to identify malondialdehyde (MDA) as a marker of lipid peroxidation. MDA produces a pink solution, the absorbance of which may be measured at 532 nm.

Using K2Cr2O7/acetic acid reagent, the Sinha technique was followed to assess the catalase activity (CAT) [22].

The method Ignarro *et al*. [23] developed was used to determine nitrite. The test is based on a diazotization reaction that Griess first reported [24]. The process is based on a chemical reaction that takes place in an acidic environment using sulfanilamide and naphthyethylenediamine dichlorate (NED). In the Griess process, nitrite is competed for by sulfanilamide and NED. The GPx activity was calculated using the Rotruck *et al*. [25] technique. The method outlined by Ellman [26] was used to determine the tissue sulfhydryl level. After mixing everything together, it was left to sit at room temperature for fifteen minutes [27].

2.5. Statistical analysis

Graphpad Prism 9 was used to create the statistics, and the findings were presented as Mean+SEM. Analysis of variance (ANOVA) was used to evaluate the differences between each group, and Turkey's multiple comparisons test. The level of significance was determined by setting the P-value at 0.05.

3. Result

3.1. High protein diets enhances protein concentration following L-arginine induced acute pancreatitis

The impact of a high-protein meal on pancreatic protein concentration after L-arginine-induced pancreatitis is depicted in Figures 1(a) and 1(b). The Protein concentration was noticably increased compared to the groups fed with the normal diets(ND+AP DAY 3 group),P<0.01. However, on day 7, there was no significant difference in the two groups.

3.2. Following acute pancreatitis caused by L-arginine, a high-protein meal raises the catalase level

Figures 2(a) and 2(b) illustrate how a high-protein diet affects the pancreas' catalase level after L-arginine-induced pancreatitis. In comparison to the ND+AP DAY 3 group, the HPD+AP on DAY 3 shows a substantial increase in catalase activities, while on day 7, a considerable increase in ND+AP DAY 7 groups shown a considerable rise, P<0.05.

3.3. High protein diet upregulate Nitric oxide expression after post L-arginine acute pancreas

Figures 3(a) and 3(b) illustrate how a high-protein diet affects the pancreas' production of nitric oxide after L-arginine-induced pancreatitis. Nitric oxide activity was significantly higher in the HPD +AP Day 3 compared to ND+ AP,P<0.05,while on day 7, the nitric oxide increased significantly in both groups, hence no significant difference in the groups .

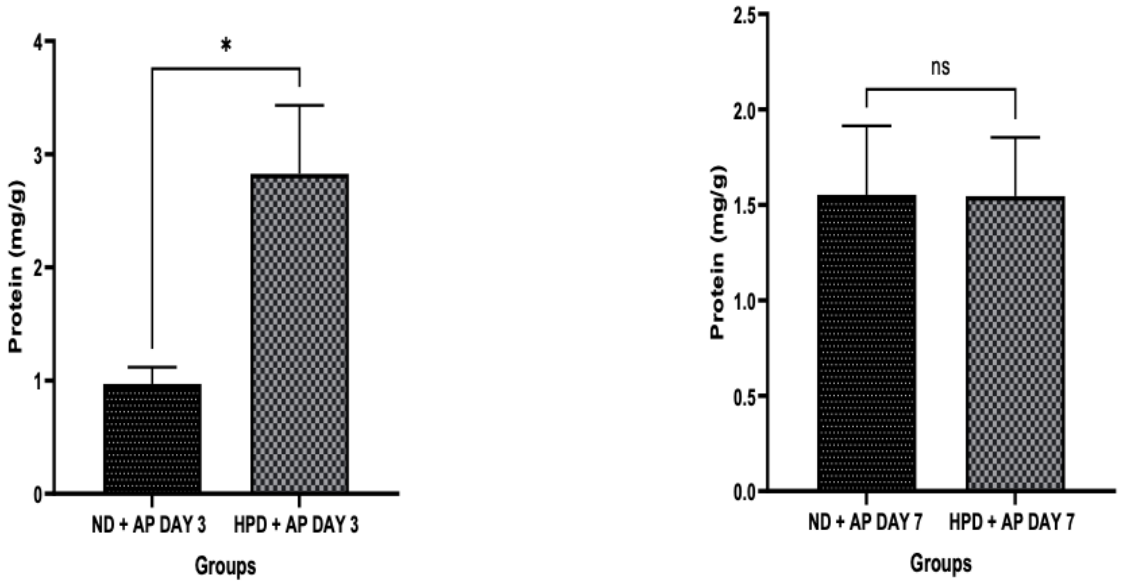

Figure 1: (a) After acute pancreatitis caused by L-arginine, the impact of a high-protein diet on pancreatic protein concentration on days 3. (b) After acute pancreatitis caused by L-arginine, the impact of a high-protein diet on pancreatic protein concentration on day 7. The values are given as mean±SEM for the number of six. Acute pancreatitis is represented by AP and normal diet by ND. * Significant in relation to ND + AP DAY 3, # Significant in relation to HPD + AP DAY 7.

3.4. Impact of a high-protein diet on pancreatic MDA activity after acute pancreatitis caused by L-arginine

After L-arginine-induced pancreatitis, the impact of a high-protein meal on MDA on the pancreas is displayed. There was a substantial reduction in MDA concentration in groups fed with normal diets(. ND+AP Day 3 and NP + AP Day 7) compared to high protein diets fed groups(HPD + AP day 3 and HPD +AP day 7) on day 3 and day 7 as shown in Figures 4(a) and 4(b).

3.5. High-protein diet's effect on the pancreas's hydrogen peroxide activity after acute pancreatitis triggered by L-arginine

The impact of a high-protein meal on the pancreatic hydrogen peroxide concentration after L-arginine-induced pancreatitis is depicted in Figures 5(a) and 5(b). A significant decrease was seen in HPD +AP, Day 3 compared to ND+ AP , Day 3, $P<0.05$, although on day 7, no significant change in the two groups.

3.6. Impact of a high-protein diet on pancreatic GPx function after acute pancreatitis caused by L-arginine

Glutathione peroxidase (GPx) activity in the pancreas after L-arginine-induced pancreatitis is affected by a high-protein diet in Figures 6(a) and 6(b). Comparing the HPD+AP Day 3 group to the ND+AP Day 7 and HPD+AP Day 7 groups revealed a substantial increase, $p<0.05$.

3.7. High protein diet increases TAC activity on pancreas following L-arginine induced acute pancreatitis

The effect of high protein diet on total antioxidant capacity on pancreas following L-arginine induced pancreatitis as seen in Figures 7(a) and 7(b) indicate HPD+AP Day 3, ND+AP Day 7 and HPD+AP Day 7 groups showed a significant increase compared to ND+AP group on day Day 3,$P<0.05$

3.8. High protein diet increases sulphur hydryl activity on pancreas following L-arginine induced acute pancreatitis

The impact of a high-protein meal on the pancreatic sucrose content after L-arginine-induced pancreatitis is depicted in Figures 8(a) and 8(b). Compared to the ND+AP DAY 3 group, there was a notable rise in SH activity in the HPD+AP DAY 3 and ND+AP DAY 7 groups. When comparing HPD+AP Day 3 to ND+AP Day 7, there was a substantial increase ($p<0.05$).

3.9. Histological studies

From histological studies, on day 3 and 7 post AP induction. ND+AP showed severe vacuolation, edema and inflammation. HPD+AP showed a fairly intact acinar cells with mild blood vessel constriction on day 3 and 7 as shown in Figure 9.

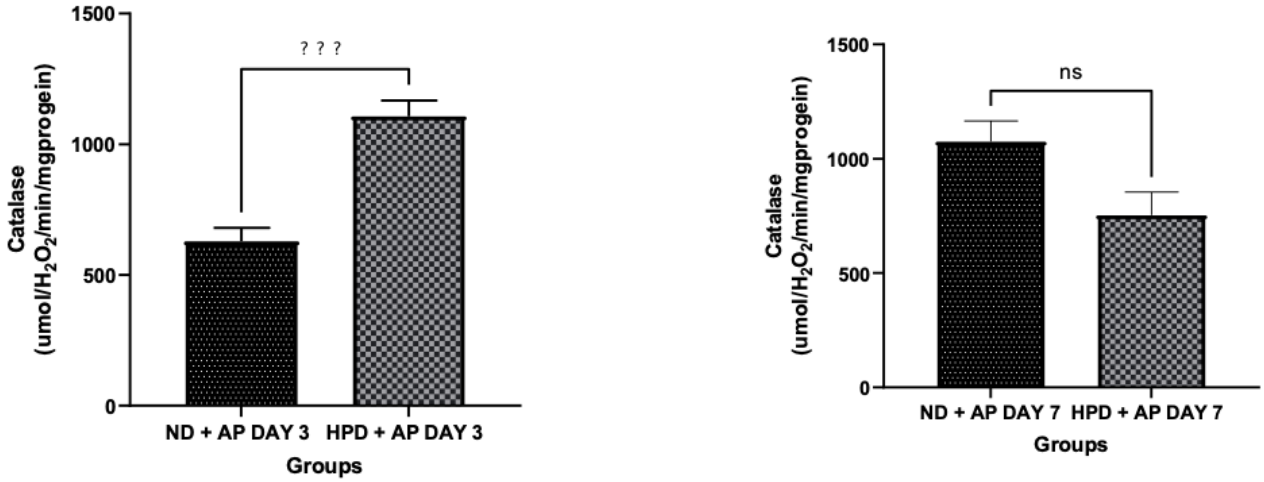

Figure 2: (a) Impact of high-protein diet on Catalase levels in the pancreas after acute pancreatitis produced by L-arginine on day 3. * Notable in relation to ND + AP DAY 3. (b) Impact of high-protein diet on Catalase levels in the pancreas after acute pancreatitis produced by L-arginine on day 7. The values are given as mean±SEM (n = 6). ND = Normal diet, AP = Acute pancreatitis #Notable in relation to HPD + AP DAY 7.

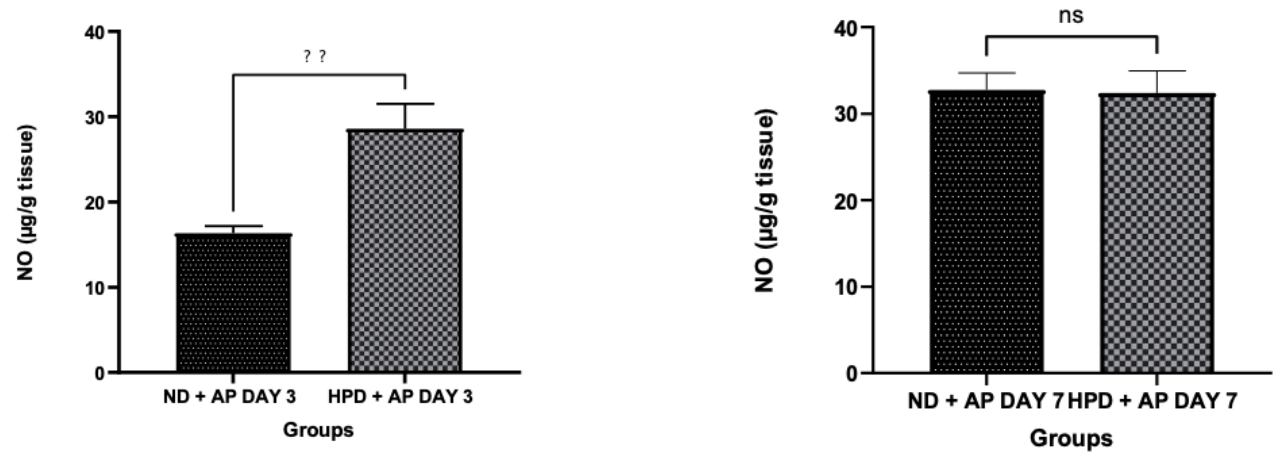

Figure 3: (a) he impact of a high-protein meal on pancreatic nitric oxide activityafter L-arginine-induced acute pancreatitis. The values (n=5) are reported as mean±SEM. Acute pancreatitis (AP) and normal diet *Significant in comparison to HPD + AP DAY 3. (b) The impact of a high-protein meal on pancreatic nitric oxide activityafter L-arginine-induced acute pancreatitis. The values (n=5) are reported as mean±SEM. Acute pancreatitis (AP) and normal diet (ND)* Significant in comparison to ND + AP DAY 7 *Significant in comparison to HPD + AP DAY 7.

4. Discussion

Acute pancreatitis (AP) caused by L-arginine is one of the most popular animal models for studying the biochemical and histological alterations related to the disease. L-arginine produces free radicals that cause AP by rupturing the zymogen granules' cell membranes, allowing digestive enzymes and cellular proteins to leak into the interstitial space [28]. An increase in the presence of free radicals promotes the activation of pro-inflammatory mediators, which leads to the development of acute inflammation [29]. The role of nutrition especially in the prevention and management of disease is very crucial and has been implicated by some research that

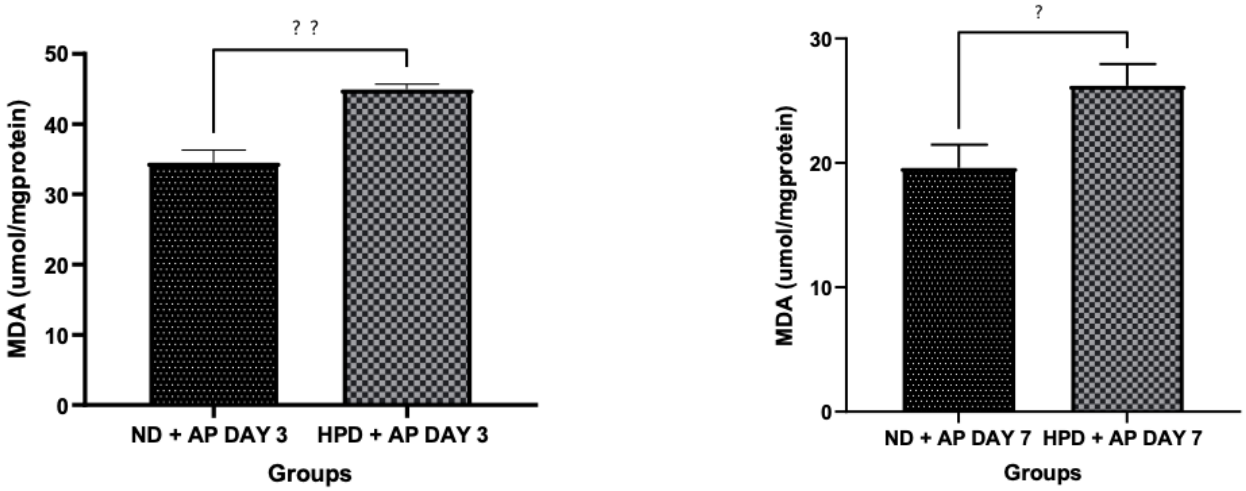

Figure 4: (a) Effect of a high-protein diet on pancreatic MDA activity after acute pancreatitis produced by l-arginine on day 3. The values (n=5) are reported as mean±SEM. Acute pancreatitis (AP) and normal diet (ND)* Significant to ND + AP Day 3; # significant compared to HPD + AP Day 7. (b) Impact of a high-protein diet on pancreatic MDA activity after acute pancreatitis produced by l-arginine on day 3. The values (n=5) are reported as mean±SEM. Acute pancreatitis (AP) and normal diet (ND)* Significant compared to HPD + AP Day 7.

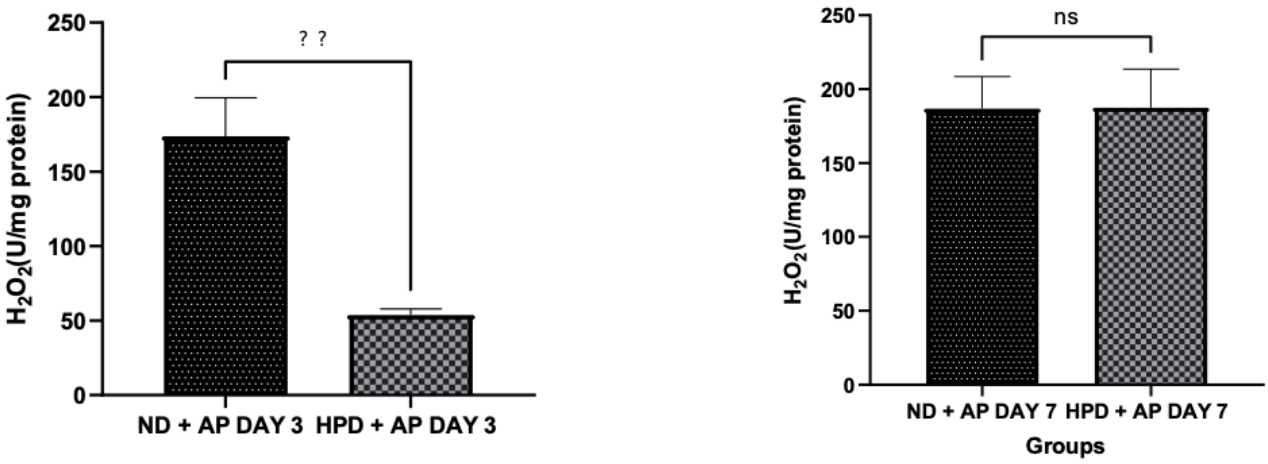

Figure 5: (a) The impact of a high-protein diet on hydroxyperoxide activity in pancreas following L-arginine-induced acute pancreatitis on day 3. The values (n=5) are reported as mean±SEM. * Significant in comparison to ND + AP DAY 3. (b) The impact of a high-protein diet on hydroxyperoxide activity in pancreas following L-arginine-induced acute pancreatitis on day 7. The values (n=5) are reported as mean±SEM. **Significant in comparison to HPD + AP DAY 7; AP = Acute pancreatitis; ND = Normal diet. NS-not significant.

diet plays a significant role in the development of acute pancreatitis [30]. The initial traditional way of managing acute pancreatitis is fasting which is necessary to obtain "pancreatic rest." This arises due to the complains of pain from patients presenting with AP after the consumption of large meal or after a period of starvation. This pattern is fast changing as patient who suffer from acute pancreatitis are already malnourished. Thus, fasting will further worsen the situation [30].

This study observed the changes in oxidants and antioxidants levels during acute pancreatitis induced by administration of high

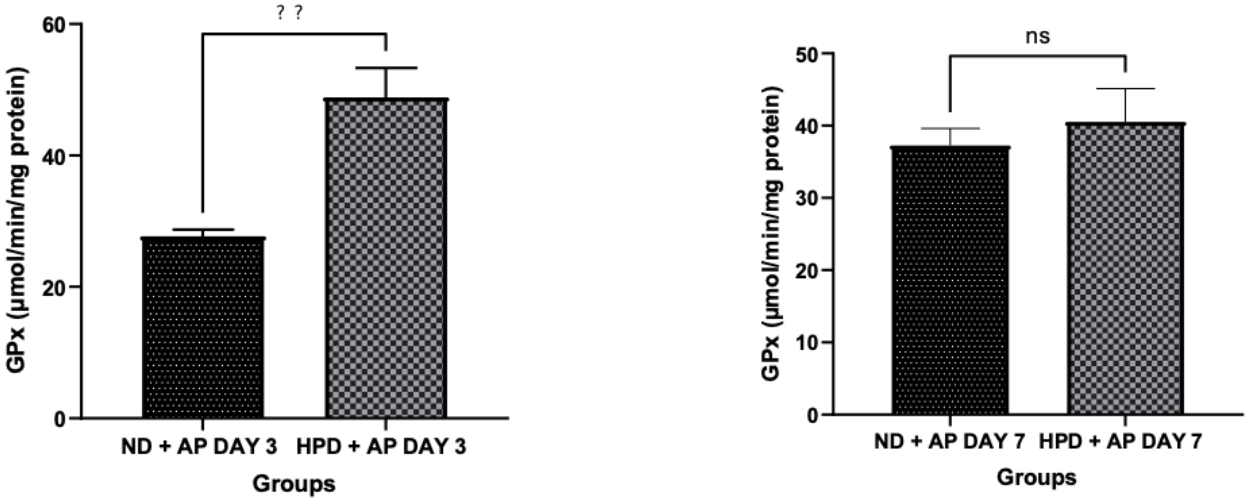

Figure 6: (a) Effect of High Protein Diet On GPX activity On Pancreas Following L-Arginine Induced Acute Pancreatitis on day 3. Values are expressed as mean±SEM (n=5). AP= Acute pancreatitis, ND= Normal diet* Significant compared to ND + AP DAY 3. (b) Effect of High Protein Diet on GPX activity On Pancreas Following L-Arginine Induced Acute Pancreatitis on day 7. Values are expressed as mean±SEM (n=5). AP= Acute pancreatitis, ND= Normal diet* Significant compared to ND + AP DAY 7,*** Significant compared to HPD + AP DAY 7.

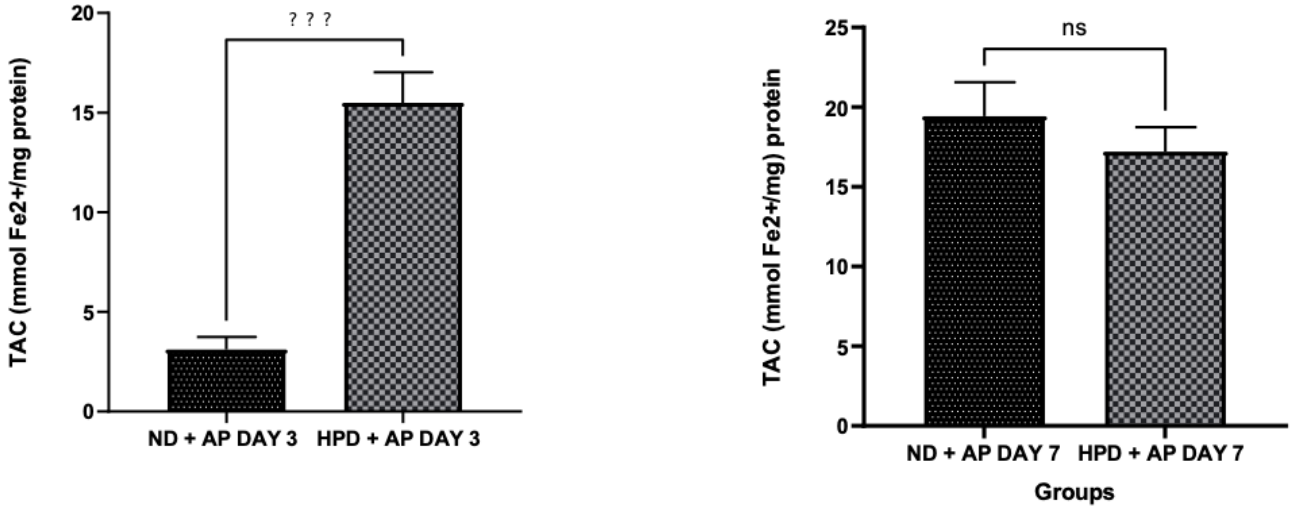

Figure 7: (a) Effect of high protein diet on total antioxidant capacity activity on pancreas following l-arginine induced acute pancreatitis on day 3. Values are expressed as mean±SEM (n=5). AP= Acute pancreatitis, ND= Normal diet* Significant compared to ND + AP DAY 3 # Significant compared to HPD + AP DAY 7. (b) Effect of high protein diet on total antioxidant capacity activity on pancreas following l-arginine induced acute pancreatitis on day 7. Values are expressed as mean±SEM (n=5). AP= Acute pancreatitis, ND= Normal diet* Significant compared to ND + AP DAY 7# Significant compared to HPD + AP DAY 7.

dose of L-arginine in rats fed with either normal diet or high protein diet for a period of 10 weeks. The disease severity was assessed at days 3 and 7 post induction of acute pancreatitis. The results of this work revealed a significant increase in protein concentration, MDA leves and NO concentration in high protein diet group especially on day 3, while only MDA was significantly increase in HPD group compared to normal diet (ND) on day 7. The increase in protein concentration and MDA are both indicative of increase in lipid peroxidation of the pancreatic organ. It has been reported that excess consumption of protein leads to increase in amino acid

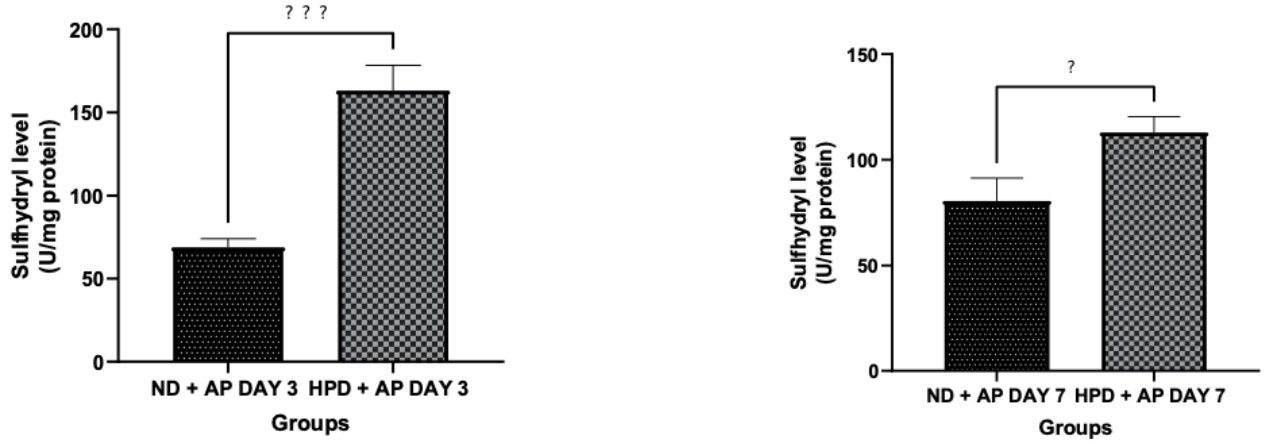

Figure 8: (a) Effect of high protein diet on SH activity on pancreas following l-arginine induced acute pancreatitis on day 3 and day 7. Values are expressed as mean±SEM (n=5). AP=Acutepancreatitis, ND= Normal diet, *Significant compared to ND + AP DAY 3, # Significant compared to HPD + AP DAY 7. (b) Effect of high protein diet on total antioxidant capacity activity on pancreas following l-arginine induced acute pancreatitis on day 7. Values are expressed as mean±SEM (n=5). AP=Acutepancreatitis, ND= Normal diet *Significant compared to ND + AP DAY 7 *** Significant compared to HPD + AP DAY 7.

oxidation and urea synthesis as well as decrease in nutritional efficiency of energy utilization [31, 32]. Also, high protein diet can distort the balance between oxidation and antioxidants in the digestive system leading to increase in reactive oxygen species in the pancreas [33].

However, it was noted in this study contrary to many studies that high protein diet resulted in increase in pancreatic antixidant enzymes (Catalase and glutathione peroxidase), total antioxidan capacity (TAC) and sulfhydryl concentration at day 3 following AP induction compared to normal diet. Nevertheless, only sulfhydryl group was significantly increase at day 7 following AP induction. This could indicate that protein diet is very essential for the production of depleted antioxidant enzymes antioxidant enzymes which during acute pancreatitis.

Several experimental investigations have documented a reduction in the body's antioxidant enzymes during acute pancreatitis. This is attributed to a rise in superoxide anion, sometimes referred to as "primary" ROS, which can subsequently interact with other molecules to produce "secondary" ROS, causing more severe damage [34, 35]. The increased antioxidant enzymes in this study can be related to the decrease observed in hydrogen peroxide which is an oxidant. Catalase help to mop up the production of hydrogen peroxide following the action of superoxide dismutase which mop up O2- free radicals to produce hydrogen peroxide [36, 37].

The endothelial nitric oxide synthase enzyme produces nitric oxide (NO), a calming substance generated from the endothelium that is a potent inhibitor of platelet activity. Numerous physiological processes are also impacted by it, including vasodilation, enhanced oxygen (O_2) transport, inhibition of pro-oxidative reactants, and leukocyte transendothelial migration [38, 39]. Nitric oxide was markedly decreased in ND group at day 3 and thereafter increase at day 7 as healing progressed. While HPD group had sustained NO level both at days 3 and 7.

In the pancreas, nitric oxide is known to regulate normal pancreatic functions such as pancreatic exocrine and secretions as well as blood flow [35]. It has also been reported tha eNOS and nNOS which are responsible for NO production are constitutively expressed in the pancreas [35, 40]. Concerns have been raised regarding nitric oxide's impact on inflammation. According to reports, it has an anti-inflammatory protective effect [41]. Additionally, according to certain findings, it exacerbates inflammation [42, 43] while another has shown it has no significant effect [44]. However, in this study increased NO seen enhance healing of the l-arginine induced AP in all groups, hence, no significant healing effect through the NO production pathway was observed.The results of the current work also revealed that HPD group had significantly increased protein concentration compared to ND at Day 3 post induction of AP. The HPD+AP Day 7 and ND+AP Day 7 groups had no significant difference as compared to the ND+AP Day 3. From histological study, the acinar of the pancreas were not intact , there was severe vacuolation, edema and inflammation in the pancreas in L-arginine induced acute pancreas. The groups given high protein diets , the acinar cells were intact with mild blood constriction with decrease inflammation.

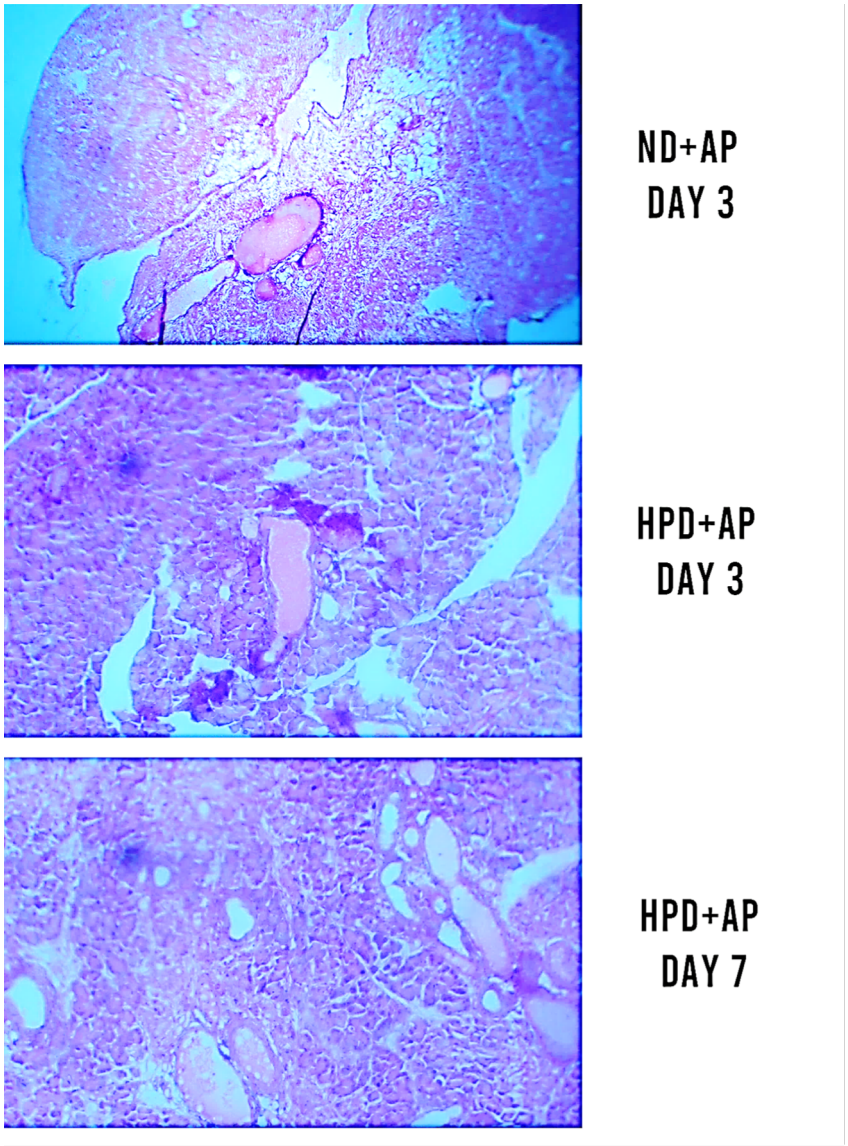

Figure 9: Photomicrograph of Pancreas, H&E (100) on day 3 and 7 post AP induction. ND+AP showed severe vacuolation, edema and inflammation. HPD+AP showed a fairly intact acinar cells with mild blood vessel constriction on day 3 and 7.

5. Conclusion

High protein diet could be said to play different roles during acute pancreatitis formation and healing as observed in this study. During formation of acute pancreatitis, high protein diet promotes the production of both oxidants and antioxidants at the same time. However, it decreases oxidative stress in general. It has no significant role during the healing phase of acute pancreatitis. The effect of high protein diet can however be enhanced by supplementing the diet with an exogenous antioxidant which will be done as a follow up to this experiment.

Data availability

The data will be made available by the corresponding author upon request.

Acknowledgement

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APPENDIX A.

Abbreviations

HPD - high protein diet
NP - Normal rat diet
MDA - Malonaldehyde
CAT - catalase
TAC - total antioxidant capacity
GPx - Glutathione peroxidase
NO - nitric oxide
SH - sulfurhydric acid
AP - acute pancreatitis