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Vitamin E ameliorates oxidative stress in the stomach of l-arginine induced acute pancreatitis rat model

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Abstract

Gut dysfunction is a major outcome during acute pancreatitis (AP). Some studies have reported a positive association between acute pancreatitis and peptic ulcer. The major cause of peptic ulcer is due to ischaemia reperfusion leading to oxidative stress. This study assessed the oxidative status of the stomach during AP and the role of vitamin (Vit.) E administration. Twenty male Wistar rats were divided randomly into 4 groups; Control, Vit. E (500 mg/kg) only, AP and Vit. E + AP. Acute pancreatitis was induced by administering four doses of l-arginine (100 mg/100 g) intraperitoneally at 1-hour interval. The rats were sacrificed 72 hours later and stomach obtained for mucin content and biochemical assays (MDA, MPO, NO, CAT, GST, and GSH). Mucin content was depleted following AP induction while, vit. E significantly increase mucin content. AP resulted in significant reduction of antioxidant enzymes (GSH and GST) and increase in oxidative stress markers (MDA, MPO and NO). Vitamin E reverse these changes. In conclusion, the oxidative status of the stomach was greatly altered following AP induction with an increase in oxidative stress markers and decrease in antioxidants. Vitamin E was able to protect the stomach from the damaging effect of acute pancreatitis.

Keywords: L-arginine; Acute pancreatitis; Oxidative stress; Stomach; Mucin

1. Introduction

Acute pancreatitis (AP) is a major disease of the pancreas, which occurs because of pancreatic inflammation thereby leading to the destruction and loss of exocrine (acinar) and ductal cells majorly as well as the endocrine (islet cells) of the pancreas (Pham & Forsmark, 2018).

The disease condition presents itself with variable degree of severity and outcome which makes it life threatening. Some of the outcome associated with AP is onset of epigastric pain radiating to the back, nausea and vomiting and very infrequently but highly dangerous is gastrointestinal bleeding (Banks et al., 2013; Shah et al., 2018).

Very commonly associated with acute pancreatitis is multiple organ failure, which occurs due to systemic inflammation resulting from the surge of oxidative stress, pancreatic necrosis and local hemorrhage accompanying acute pancreatitis (Liu et al., 2023).

About 20 to 40% of individuals have been reported to die from multiple-organ failure and systemic inflammatory response syndrome (Boxhoorn et al., 2020) during acute Pancreatitis. Gut dysfunction has been reported severally as a very common occurrence in AP, even more common than respiratory dysfunction (Uhl et al., 1996). Some of the gut dysfunction associated with AP includes gut dysmotility (delayed gastric emptying and increase transit time),

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disruption of mucosal epithelial integrity, decreased mucosa immunity, dysbiosis, gastrointestinal fistula, paralytic ileus, perforation and increased intestinal permeability (Fishman et al., 2014; Omayone, 2020; Wang et al., 2003).

It was recently reported by (Omayone, 2020) that there was delayed gastric emptying in the stomach of rats in l-arginine induced acute pancreatitis, which was ameliorated by diet containing seeds or leaves of *Moringa oleifera*. It is well known that the gut plays a significant role in the progression of acute pancreatitis thereby leading to high morbidity and mortality. Thus protecting the intestinal architecture and maintaining normal function of the gut is very relevant to decreasing the systemic effect of acute pancreatitis (Capurso et al., 2012). Increase in reactive oxygen species (ROS) leading to oxidative stress has been reported to be a major disruptor to intestinal architecture especially in ischemia (Suzuki et al., 2012). This calls for better treatment options that can manage both acute pancreatitis and protect the gut epithelium.

Vitamin E, which is a well-known vitamin for its antioxidant and anti-inflammatory properties (Rychter et al., 2022). It has been reported as an essential vitamin for the development and proper functioning of the gut as well as healing of gastric mucosa injury (Kamisah et al., 2014). In this study, it was shown that Vitamin E prevents oxidative stress and preserve the mucus content in the stomach, which have been compromised due to acute pancreatitis.

2. Material and methods

Twenty (20) male Wistar rats weighing 180 to 200 g were used for this study. They were housed in the animal house of the Department of Physiology, Federal University of Technology Akure. The animals were acclimatized for one week and allowed continuous access to food and water. They were then grouped into 4 groups;

- **Control:** They received olive oil for 3 days and intraperitoneal administration of normal saline.
- **Vitamin E (Vit. E) Only:** They received vitamin E (500 mg/kg) dissolved in olive oil for three days and intraperitoneal administration of normal saline.
- **Acute pancreatitis (AP):** They received olive oil for 3 days and thereafter, intraperitoneal administration of l-arginine for induction of acute pancreatitis.
- **Vitamin E (Vit. E) + AP:** They received vitamin E (500 mg/kg) dissolved in olive oil for three days and thereafter, intraperitoneal administration of l-arginine for induction of acute pancreatitis.

2.1. Acute Pancreatitis induction

L-arginine monohydrochloride was purchased from Loba Chemie India. Acute pancreatitis was induced by modification of the method used by (Omayone et al., 2021). Four doses of 100 mg/100 g l-arginine monohydrochloride were administered intraperitoneally at 1 hour interval to induce acute pancreatitis. The animals were sacrificed 72 hours later and the stomachs obtained for biochemical assays.

2.2. Biochemical assays

The stomach tissues collected were rinsed in ice-cold phosphate buffer saline and thereafter homogenized in sodium phosphate buffer pH 7.0. The homogenates were centrifuge and the supernatant collected for the following assays;

Total protein determined Biuret reagent as described by (Gornal et al., 1949), Malondialdehyde (MDA) was determine by the method describe by (Varshney & Kale, 1990). Nitric oxide was determine by determining nitrite concentration using Griess reagent as described by (Ignarro et al., 1987),. Glutathione reductase (GSH) level was determined by the method of (Beutler et al., 1963). Catalase was determined by (Sinha, 1972). Glutathione-S-Transferase (GST) activity was determine according to the method of (Habig et al., 1974).

2.3. Statistical Analysis

Statistical analysis was carried out using Graph Pad prism 9.3. The results were analyzed using one-way ANOVA and Independent T-test to compare the differences among variables and thereafter, expressed as Mean±SEM. P-value was set at 0.05 to determine the level of significance.

3. Results

3.1. Vitamin E effect on Mucin content and antioxidant status in the stomach following l-arginine induce acute pancreatitis

There was no significant difference in mucin content (Figure 1A) of the stomach between AP group and Control. However, both Vitamin E groups (Vit. E only and Vit. E +AP) had significantly higher mucin content compared to AP group but not to control. Catalase activity (figure 1B) was significantly increased in Vit. E only group compared to other groups. There was no significant difference among other groups. Glutathione reductase (GSH) and glutathione-S-Transferase (GST) were significantly decreased in AP group compared to control and Vit. E only. Vit. E+ AP significantly reverse this effect as shown in figure 1C&D respectively.

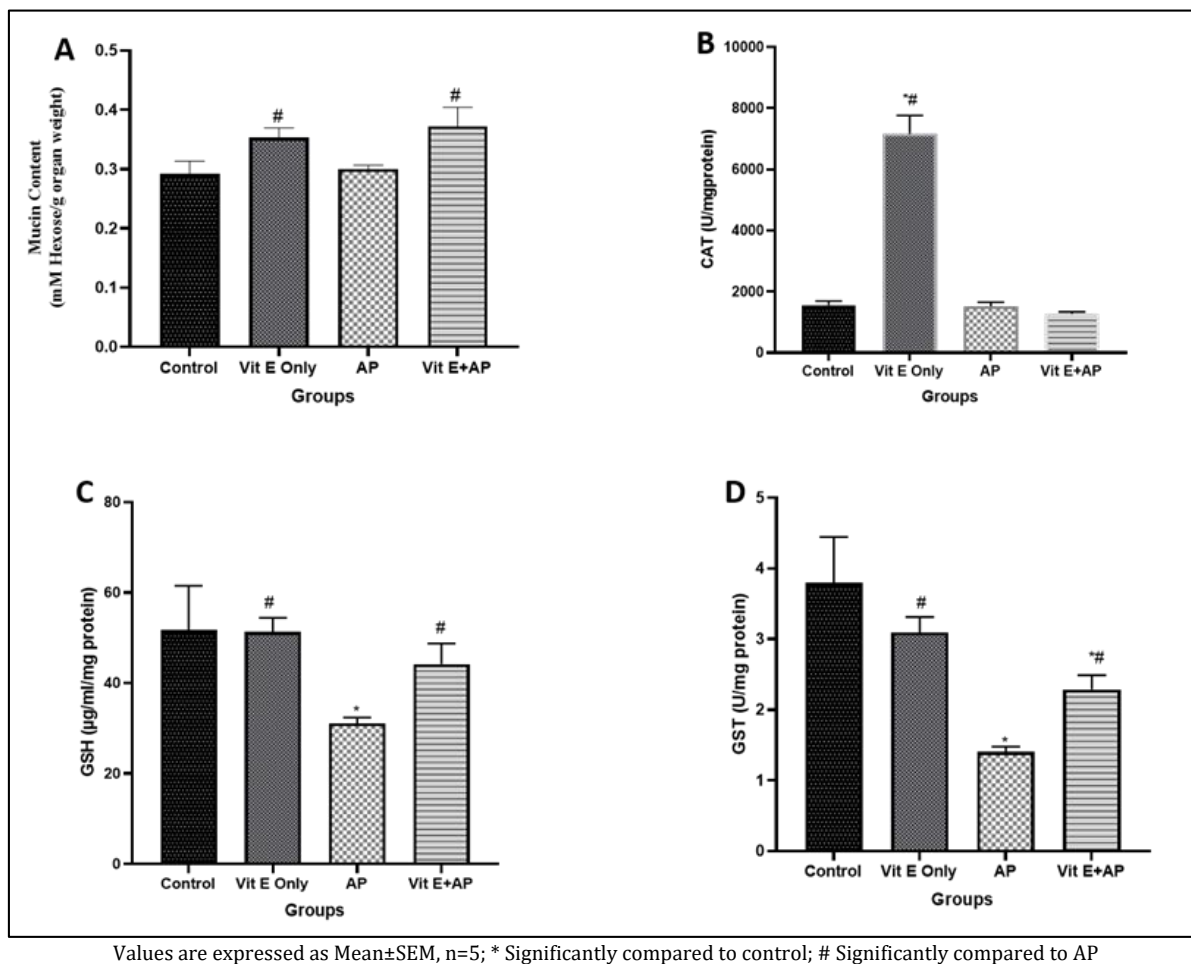


Figure 1 Effect of Vitamin E on (A) Mucin content (B) Catalase (C) Glutathione reductase (D) Glutathione -S-Transferase in the stomach in l-arginine acute pancreatitis

3.2. Effect of Vitamin E on protein concentration and oxidative stress markers in the stomach following l-arginine induced acute pancreatitis.

Protein concentration was significantly increased following induction of acute pancreatitis in the AP and Vit. E + AP groups compared to control and Vit. E only as show in figure 2A. Figures 2B, C & D represent malondialdehyde levels (MDA), myeloperoxidase (MPO) activity and nitric oxide (NO) level respectively. There was a significant rise in MDA, MPO and NO in AP group following acute pancreatitis induction compared to control and Vit. E only. This was reverse in Vit. E + AP group compared to AP and control group.

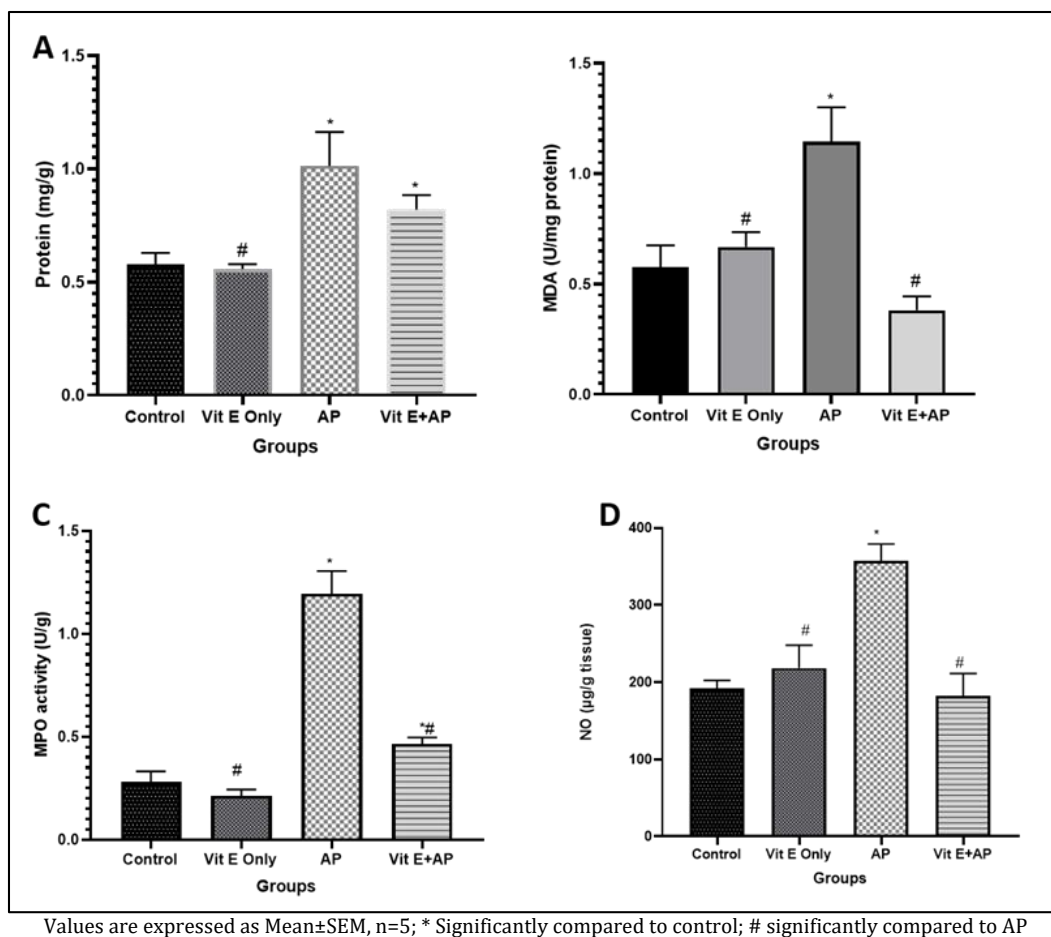


Figure 2 Effect of Vitamin E on (A) protein concentration (B) Malondialdehyde-MDA (C) Myeloperoxidase-MPO (D) Nitric Oxide-NO in the stomach in l-arginine acute pancreatitis

4. Discussions

Multiple organ failure is a major hallmark of acute pancreatitis of which the gut is greatly affected (Liu et al., 2023). Studies have reported the association between acute pancreatitis and acute gastrointestinal mucosa lesion as well as peptic ulcer disease ((Chen et al., 2007; Lee et al., 2011). The stomach was reported to have the highest prevalence (67%) combining stomach lesions alone and in association with other regions of the gastrointestinal tract (esophagus, duodenum) (Chen et al., 2007). The current study shows that acute pancreatitis increases oxidative stress in the stomach and decrease mucous content which were ameliorated upon vitamin E administration.

It is still unclear the cause of peptic ulcer in acute pancreatitis, however, gastric mucosal ischemia as well as acidity has been suggested (Fishman et al., 2014; Muddana et al., 2009). Gastric mucosal ischemia is known to result in decrease gastric mucous (Magierowska et al., 2019; Omayone, 2020). This increases intestinal permeability allowing the content of the gastrointestinal tract as well as bacteria and bacterial products to leak out of the gut, which further aggravate pancreatitis and affect other organs. It was seen in this study that stomach mucin content was significantly decreased in AP group compared control, which was ameliorated by vitamin E administration. Studies have found out that loss of mucous layer is a major contributory factor to increase in gut permeability and eventually intestinal injury in various disease conditions (Omayone, 2020; Qin et al., 2011). The mucous act as the first line of defense against external forces that can affect the gut and it consist of two layers; the inner adherent layer and the outer loose layer that is continuously shed into the gut lumen. A major constituent of the mucus is the mucin, which prevents bacteria and other substances from coming in direct contact with the epithelium. The mucus in combination with bicarbonate helps to provide protection against the corrosive action of acid produced in the stomach thereby creating a neutral pH on the mucosal surface. The mucous layer also has several other defensive proteins; lysozyme, lactoferrin, immunoglobulin (IgA), protease inhibitors, certain growth factors providing antibacterial and antioxidative action (SANTINI et al., 1992; Zagulski et al., 1998). Thus, a decrease in mucus content in the stomach predisposes it to injury, which was ameliorated

by vitamin E. The result is consistent with earlier report where vitamin E was stated to increase mucus cells in indomethacin induced gastric ulcer (Yousaf et al., 2014).

Oxidative stress is a major factor in AP formation and leading to multiple organ failure. Oxidative stress was further access in the stomach following acute pancreatitis. Malondialdehyde (MDA) a marker for lipid peroxidation and myeloperoxidase (MPO) a marker for inflammation were significantly elevated in the AP group. This was accompanied by a significant decrease in reduced glutathione and glutathione -S- Transferase. Extra pancreatic ischemia, which results from hypovolemia associated with AP, has been reported to be a causative factor in peptic ulcer disease linked with acute pancreatitis (Banks & Freeman, 2006; Lee et al., 2011; Muddana et al., 2009). Several studies have reported rise in oxidative stress and decreased antioxidant status during ischemia reperfusion gastric ulcer (Magierowska et al., 2019; Omayone et al., 2020; Salami et al., 2018). This was attributed to increased activation of neutrophils leading to hemorrhagic lesions in the gastric mucosal. The activation of neutrophils results also in activation of the enzyme myeloperoxidase thereby causing inflammation. The ability of Vit. E to decrease lipid peroxidation and inflammation in the stomach as observed in this study supports previous reports on its antioxidant and anti-inflammatory properties. Vitamin E has been reported to act as a scavenger to free radicals thereby sparing glutathione (Kamisah et al., 2014; Odabasoglu et al., 2008). Additionally, intestinal ischemia promotes intestinal permeability resulting in stimulation of cytokine release and increases the level of nitric oxide. Similar result was observed in this study, as there was a significant increase in nitric oxide in the AP group, which was ameliorated by vitamin E. During ischemia, there is increase production of induce nitric oxide synthase (iNOS) which results in excess production of nitric oxide (Omayone et al., 2020). The excessive production of nitric oxide makes it available to react with superoxide ion to form peroxynitrite, which is more stable than NO and caused more damage. This can in turn inflict more damage to the pancreas and cause organ failure. The result is consistent with the report of (Donia et al., 2019) who reported that vitamin E significantly decrease NO in doxorubicin-induced cardiomyopathy. This action further strengthened the protective effect of vitamin E on the gastric mucosa.

5. Conclusion

In conclusion, there is decreased mucous production, increase lipid peroxidation and decreased antioxidant defense in the stomach during acute pancreatitis. This can predispose the stomach to gastric ulcer formation. However, vitamin E served as a good protecting agent for acute pancreatitis induced gastric damage.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Animals were handled according to the Guide for the Care and Use of Laboratory Animals by the National Research Council (US), 2011. The experiments were approved by the Ethics Committee of the Federal University of Technology Akure.

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