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Anti-ulcer Mechanisms of L-lysine in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author CJO provided updated literature on the study and executed most parts of the bench work. Author FSO developed the design of the study and prepared the manuscript and author AD did the data analysis and proof-reading of the draft manuscript. All authors read and approved the final manuscript.

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ABSTRACT

L-lysine is an essential amino acid found in most protein food sources, in particular high-protein foods such as eggs, meat, soybean, milk and fish. This amino acid has been reported to have an indirect antioxidant property. Antioxidants are known to have gastroprotective property. This study examined the effect of L-lysine pre-treatment on indomethacin induced ulceration in male Wistar rats.

Fifty male Wistar rats were used for this study and were randomly divided into two study groups of twenty five (25) animals each. The first sub-group was used for the anti-ulcer studies; antioxidant enzymes (SOD, CAT, and MDA), Nitric oxide, parietal cell count, and the mean ulcer score, while the second sub-group was used for the gastric mucus secretion study. Each sub-group was divided into five groups with five animals per group namely: Control, Omeprazole (20 mg/kg), L-lysine (100 mg/kg, 200 mg/kg and 400 mg/kg).

The results showed that l-lysine pre-treatment significantly increased SOD activity and reduced MDA levels but with no significant change in catalase activity. NO levels in the treated groups were significantly higher than in the control. Gastric mucus secretion was significantly increased and the

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parietal cell count significantly reduced in L-lysine pre-treated animals.
The findings from this study reveal that l-lysine supplement has some anti-ulcer properties which might be mediated through increased antioxidant enzymes, increase gastric mucus secretion and inhibition of parietal cell synthesis. This will be beneficial in the treatment of peptic ulcer.

Keywords: Antioxidants; nitric oxide; omeprazole; L-lysine; anti-ulcers.

1. INTRODUCTION

Peptic ulcer is a common disorder of the gastrointestinal system, which causes much discomfort to patients, disrupting their daily routines and causes mental agony [1]. The aetiology of peptic ulcer disease includes a complex imbalance between gastric offensive factors like gastric acid secretion, Non-steroidal anti-inflammatory drugs (NSAIDs), lipid peroxidation, as well as smoking [2] and defensive mucosal factors like prostaglandins (PG's), gastric mucus secretion, bicarbonate secretion, and antioxidant enzymes in combating free radicals [3,4]. In an attempt to protect the gastric mucosa from gastric acid, enhance ulcer healing and prevent ulcer recurrence, pharmacological control of gastric acid secretion has long represented a desirable goal [5]. Various pharmaceuticals, such as histamine H_2 receptor (H_2R) antagonists and H^+/K^+-ATPase (acid pump) inhibitors have been developed and utilized for the treatment of acid-related peptic diseases [6]. Furthermore, there is also an increasing need to develop more potent therapeutic agents for the treatment of peptic ulcer.

L-lysine is an essential amino acid, i.e. it is not synthesized in the body hence it must be ingested from our diets to meet up the body's requirement [7]. Good sources of lysine are high-protein foods such as eggs, meat, soybean, milk, poultry, cheese and certain fish. L-lysine administration in rats has been reported to cause an increase in antioxidant enzyme activities thereby protecting the cells against the destructive effects of reactive oxygen species [8]. Many experimental studies have shown the efficacy of some nutrients and food supplements in the treatment of peptic ulcer. A recent study reported that L-lysine has gastro protective effect [9]. This study therefore aimed at evaluating the effect of L-lysine pre-treatment on indomethacin induced ulceration in male wistar rats and to further investigate the mechanism of action of this amino acid in the prevention of peptic ulcer.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals Used

L-lysine, Solgar, Inc. 500 Willow Tree Road, Leonia, NJ 07605 U.S.A, Indomethacin (Sigma), Omeprazole, Ulcertret-20 (Swiss Pharma Pvt. Ltd. 3709, GIDC, Phase IV, Vatva, Ahmedabad-382 445, Gujarat, India, Sodium thiopental (Abbot Laboratories), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Ellman reagent (5', 5' dithio-bis-2-nitrobenzoic acid), Sodium azide, 1-2, 4-dinitrobenzene.

2.2 Experimental Design

Fifty adult male Wistar rats weighing 180-200 g were used for the study. The animals were obtained from Central Animal house, College of Medicine, University of Ibadan. The experimental animals were acclimatized for two weeks and were fed on rats' pellets and water given ad libitum.

After the period of acclimatization, the experimental animals were pre-treated with l-lysine for twenty eight days. They were divided randomly into two groups each containing twenty-five animals and each group were subdivided into five groups containing five animals each and treated as follows; Group 1 - (control); normal rats that had access to clean water and rat pellets; Group 2- animals pre-treated with 20 mg/kg body weight omeprazole; Group 3- animals pre-treated with 100 mg/kg body weight of l-lysine; Group 4- animals pre-treated with 200 mg/kg body weight of l-lysine; Group 5- animals pre-treated with 400 mg/kg body weight of l-lysine. The first sub-group was used for the anti-ulcer studies; antioxidant enzymes (SOD, CAT, and MDA), Nitric oxide, parietal cell count, and the mean ulcer score, while the second sub-group was used for the gastric mucus secretion study. All procedures used in this study conformed to the guidelines on the care and use of animals in research and teaching [10].

2.3 Indomethacin Gastric Ulcer Induction

Experimental gastric ulceration was induced in the animals using indomethacin at a dosage of 40 mg/kg body weight in accordance with previously described method by [11]. Indomethacin was used to induce this at the dosage of 40 mg/kg body weight. The animals were sacrifice by cervical dislocation 4 hours after ulcer induction.

2.4 Assessment of Ulcer Spots

Macroscopic examination of the stomach was carried out and scored using the method described by [12] modified by [13]. Ulcer index was calculated using the formula.

Ulcer index = Mean Ulcer Score x Number of animals in a group/100

2.5 Determination of lipid peroxidation

MDA assessment was done according to the method of [14]. MDA which is the unit for lipid peroxidation is calculated in units/mg protein, using the formula:

MDA (units/mg proteins) = (Absorbance x Volume of mixture)/ (E532nm x Volume of sample x mg protein)

2.6 Assay of Superoxide Dismutase (SOD)

SOD activity was measured by assessing the inhibition of autoxidation of adrenaline at 30℃ with the pH raised from 7.8-10.2 using the method described by [15].

2.7 Assay of Catalase

Catalase activity was determined according to the method of [16]. This was determined by measuring the rate of H_2O_2 Absorbance at 480 nm within 30-60 seconds against distilled water. Homogenized sample of stomach tissue (0.5 ml) was mixed with equal volume of 30M of Hydrogen peroxide, 1ml of 6M H_2SO_4 and 7 ml of 0.01 M of Potassium permanganate. Absorbance was then read. The result was expressed in µmol/mg protein.

2.8 Determination on Nitric Oxide Levels

Nitrite was determined as an oxidation product and indicator of NO synthesis as described by [17]. The method is based on the addition of Griess reagent to the sample which converts nitrite into deep purple azo chromophore. The colour intensity was measured using a UV-visible spectrophotometer. Nitrite level was expressed as mol/g tissue.

2.9 Gastric Mucus Secretion Study

This study was carried out using the spectrophotometry method described by [18].

The weight of dye was expressed over the weight of the stomach, to give the weight of mucus secreted.

Thus,

Gastric mucus secretion (mg/g tissue) =

$$\frac{\text{Weight of dye (mg)}}{\text{Weight of stomach (g)}}$$

2.10 Parietal Cell Count Study

Stomach sections were prepared from strips removed from the fundic area of the stomach and stained using Hematoxylin and Eosin. This method was earlier described by [19], and modified by [20].

2.11 Statistical Analysis

Data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was carried out with Graph Pad Prism 5.0 while means were compared using one way analysis of variance (ANOVA). Differences in means were considered statistically significant at P=0.05.

3. RESULTS

3.1 Effect of L-lysine Pre-treatment on Gastric Mucus Secretion

Animals pre-treated with various doses of L-lysine showed significant increase in gastric mucus secretion when compared to the control. The positive control; 20 mg/kg Omeprazole and 100 mg/kg l-lysine had the highest secretion of gastric mucus as shown in Fig. 1.

3.2 Effect of L-lysine Pretreatment on Mean Ulcer Score

The results presented in Fig. 2 shows that pre-treatment of animals with L-lysine significantly

reduced the mean ulcer score in the groups when compared to the control (except for the 200 mg/kg L-lysine group).The percentage inhibition obtained from this study are comparable to 20 mg/kg Omeprazole, the standard drug at doses of 100 mg/kg L-lysine and 400 mg/kg L-lysine. However, the lowest dose (100 mg/kg) L-lysine appears more effective.

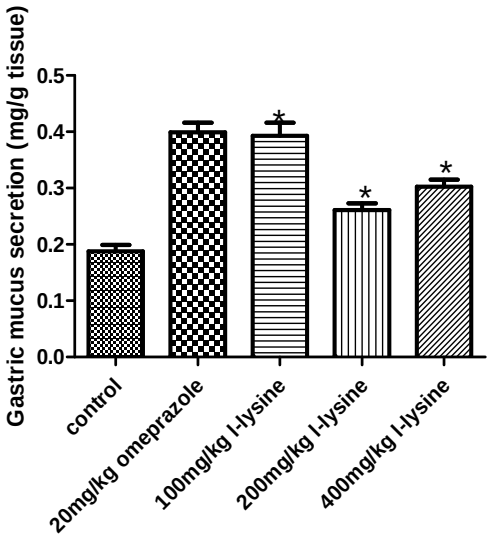

Fig. 1. Effect of l-lysine pre-treatment on gastric mucus secretion

**P = 0.05 when compared with control group*

3.3 Effect of L-lysine Pre-treatment on Antioxidant Enzymes

3.3.1 Lipid peroxidation

Fig. 3 shows gastric mucosal malondialdehyde (MDA) levels recorded in L-lysine pre-treated animals. All the pre-treated animals showed significant decrease in the lipid peroxidation when compared to the animals in the control group. (P=0.05).

3.4 Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) levels obtained from this study are presented in Fig. 4. The control had a value of 0.67 ± 0.44 (units/mg protein). There was a general increase in the SOD levels of all the pre-treated rats, however, only 400 mg/kg L-lysine pre-treated groups that was significant when compared to the control.

3.5 Effect of L-lysine on Catalase Activity

Catalase activity in the various pre-treated groups is presented in Fig. 5. The control group had a value of 1893±541.1. There was no significant difference in the values of the pre-treated animal groups when compared to the control.

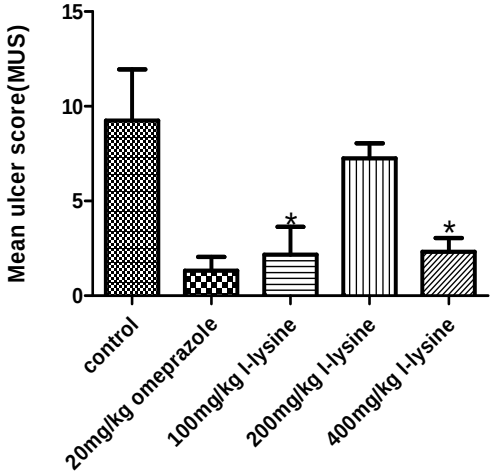

Fig. 2. Effect of l-lysine pre-treatment on mean ulcer score

**P = 0.05 when compared with control group*

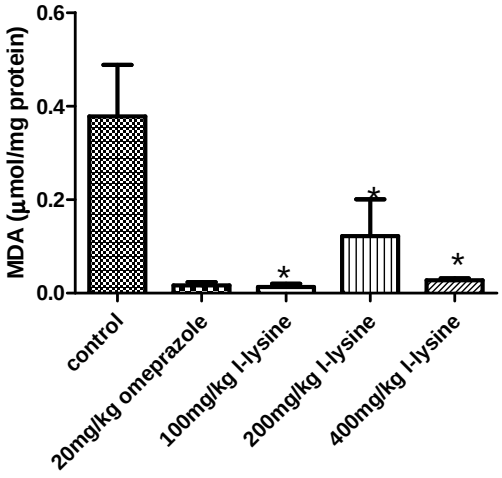

Fig. 3. Effect of l-lysine pre-treatment on malondialdehyde

**P = 0.05 when compared with control group*

3.6 Effect of L-lysine on Gastric Nitric Oxide Level

The values obtained from the nitric oxide level are presented in Fig. 6. In this study, the lowest dose of L-lysine (100 mg/kg) pre-treated rats recorded significantly higher value of gastric NO when compared to the control group.

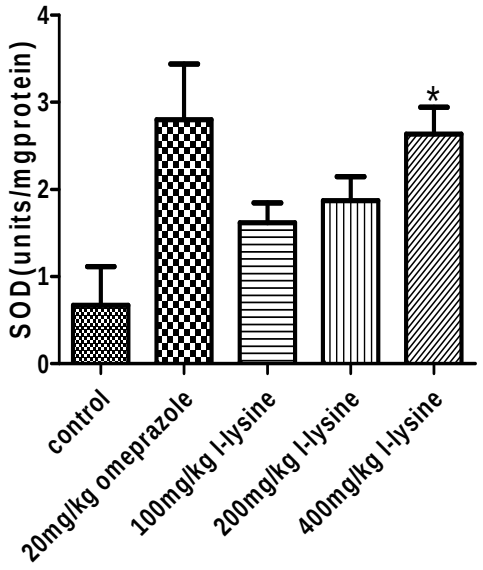

Fig. 4. Effect of l-lysine pre-treatment on superoxide dismutase

**P = 0.05 when compared with control group*

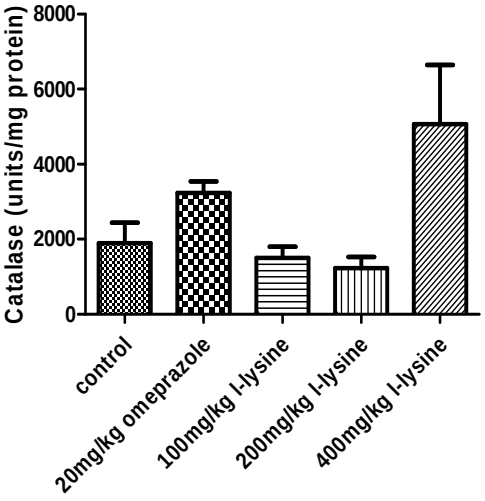

Fig. 5. Effect of l-lysine pre-treatment on catalase activity

3.7 Effect of L-lysine Pre-treatment on Parietal Cell Mass

Fig. 7 shows the results obtained when the parietal cells were counted. There was significant reduction in parietal cell count in all the pre-treated groups when compared to the control.

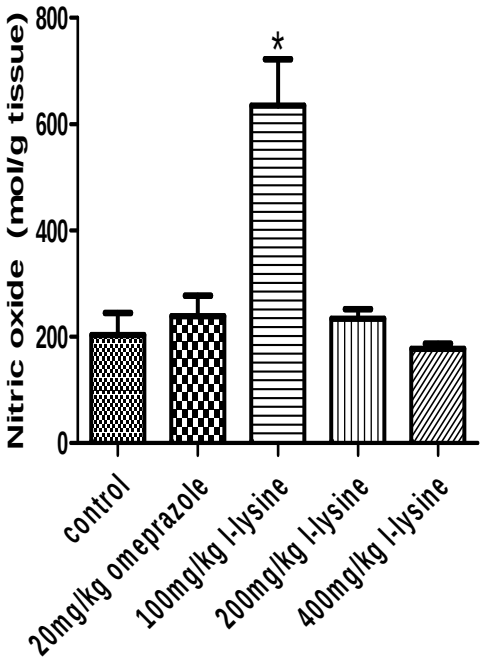

Fig. 6. Effect of l-lysine pre-treatment on nitric oxide level

**P = 0.05 when compared with control group*

4. DISCUSSION

Gastric mucus has been found to protect the stomach, and is capable of acting as an antioxidant, which can reduce the oxidative damage mediated by oxygen free radicals [21,22]. A decrease in gastric mucus renders the mucosa susceptible to injuries induced by acid, aspirin or cold restraint stress [23]. In this present study, it was observed that there was an increase in the gastric mucus secretion of the L-lysine pre-treated animals, which implies that the increase in gastric mucus secretion in these findings is a positive factor in NSAID induce peptic ulcer. The protective property of the mucus barrier depends not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface [24]. This is in agreement with the earlier work carried out by [25], who reported that gastric mucus secretion increased in the gastric mucosa of animals pre-treated with monosodium glutamate (MSG).Goel and Bhattachaya reported that an increase in the gastric mucus secretion in stomach prevents physical damage and back diffusion of hydrogen ions [26]. Similarly, the

work of Abd El-kader et al. using alpha lipoic acid (ALA) revealed that the gastric mucosa was protected from injury through increasing the mucus in the stomach wall [27].

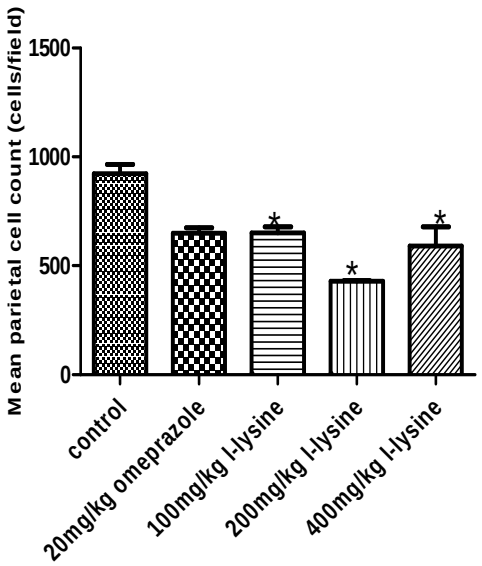

Fig. 7. Effect of l-lysine pre-treatment on parietal cell count

**P = 0.05 when compared with control group*

Naturally, the body produces free radicals as by-products (of metabolism) which can cause damage. Antioxidants act as "free radical scavengers" that prevent and repair damage done by these free radicals [21]. Antioxidants seemed to have protective role in gastric ulcers and carcinomas [28]. L-lysine in this study, showed an antioxidant capacity. MDA levels in this study were significantly reduced in all the l-lysine pre-treated animals. This indicates that, the level of lipid peroxidation was low with the administration of L-lysine in this study. The activity of SOD as an antioxidant is to provide the first defense line of action by converting superoxide radicals to form H_2O_2 and therefore preventing the damaging effect of oxygen radicals. The increase in SOD levels observed in this study is in agreement with the study [29], who reported that l-lysine attenuates the oxidative stress via increasing SOD levels and decreasing lipid peroxidation in animals with pancreatitis [8]. This study suggests that one of the actions of L-lysine is to prevent oxidative damage.

In this study, only the 100 mg/kg l-lysine pre-treated group showed a significant increase in nitric oxide when compared to the control group. Nitric oxide plays an important role in the control of gastric blood flow as well as in the maintenance of gastric mucosal integrity [30] and this pathway is a major protective system in gastric mucosa via relaxation of the arterial smooth muscles. Some studies have reported the ability of nitric oxide to protect against oxidative damage by intercepting reactive species, such as the hydroxyl radical, converting them to less damaging and more easily detoxified products [31]. In this study, the action of nitric oxide observed, also suggest that the action of l-lysine in increasing nitric oxide levels might not be dose dependent, but very efficient at very low dose.

The mean ulcer score in this study was reduced in the animals pre-treated with L-lysine. There was a comparable percentage inhibition when the low and high doses of L-lysine were compared to the standard drug Omeprazole. Omeprazole is a proton pump inhibitor, which are potent acid suppressants [32]. This supports the earlier study that omeprazole significantly decreased the effect of NSAIDS-induced gastric ulceration [33,34].

Parietal cells are the principal cells in the gastric glands which secrete gastric acid (HCl) to promote proteolytic digestion of food substances, absorption of iron, and killing pathogens. L-lysine administration in this study reduced the production of parietal cells. The action of L-lysine in reducing parietal cell can be said to be similar to that of Omeprazole in this present study.

200 mg/kg Lysine pre-treated group had the lowest parietal cell count (Fig. 7), and 100 mg/kg L-lysine appears to be the most potent in this study because the former may uniquely be acting via any of the receptors in the stomach responsible for gastric acid secretion control. For example Gatrin CCK receptors that will normally stimulate growth and secretion of HCl from the parietal cell may be inhibited causing low production of gastric acid by affecting the mitotic division of parietal cells. Also, from our study, almost the same level of response to L-lysine pre-treatment was noticed with Omeprazole in the following parameters investigated: Gastric mucus secretion, mean ulcer score and MDA concentration. These similarities in effects and activity of both drugs are pointers to suggest that they have similar protective mechanism.

5. CONCLUSION

The result from this study shows that L-lysine possess antiulcer actions which might be linked to its antioxidant properties, its ability to enhance gastric mucus secretion as well as reducing parietal cell count. L-lysine which is usually taken as a supplement might therefore be beneficial to people with peptic ulcer disease.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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