

Actions of L-citrulline on gastric mucus secretion and gastric mucous cell counts in albino rats

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Abstract: Oral L-citrulline is efficiently converted to L-arginine, the precursor for endothelial nitric oxide (NO) synthesis. Nitric oxide NO enhances gastric mucus production. This study investigates the role of nitric oxide as up regulator of mucus secretion and mucous cell count in the stomach. Sixty Adult male albino rats, weighing between 180-210 g were used for the experiment. They were divided into two study groups, (gastric mucus secretion study group, gastric mucus cell count study group), of thirty rats per group and each group was further subdivided into five groups with six (n=6) rats in each group. Group (I) served as control. Group (II) was treated with L-Citrulline (600mg/kg) for five days. Group (III) received L-Citrulline (900mg/kg) for five days. Group (IV) received L-Arginine (200mg/kg) and group (V) was treated with misoprostol (100µg/kg). Gastric mucus wall content was determined by Alcian blue method and the rate of secretion was determined by the quantity of Alcian blue extract per gram wet stomach (mg/g). The tissues were fixed in 10% formalin and embedded in Paraffin wax, then sectioned with microtome at 4microns. The mucus cell count was counted using calibrated microscope in five randomly selected areas of the gastric mucosal tissue on each slide. The results show that in the first study group, L-citrulline increases the rate of gastric mucus secretion from 7.4 ± 1.58 mg/g tissue in the control to 12.7 ± 5.05 mg/g tissue in the 600mg/kg treated in group 2, while group 4 treated with L-arginine the secretion is about 11.30 ± 2.64 mg/g per tissue while in the second study group, the mucus cell count in the control is 14.50 ± 2.56 cells/mm² and it decreases to 10.75 ± 1.80 and 14.20 ± 3.80 cells/mm² in 600mg/kg and 900mg/kg l-citrulline treated group while it increases to 15.60 ± 2.27 cells/mm² and 17.96 ± 2.73 cells/mm² in the L-arginine and misoprotol treated group. It is therefore concluded that L- citrulline increases gastric mucus secretion which could be as a result of up-regulation of endothelial Nitric oxide via the citrulline-NO cycle.

Key words: L-citrulline, gastric mucus, gastric mucous cells, L-arginine, nitric oxide

I. Introduction

L-arginine is the substrate for endothelial nitric oxide (NO) production, which is protective factor that maintains the gastric mucosa integrity by increasing the mucosal barrier, mucus secretion, blood flow, cell regeneration. (Singha *et al.*, 2008, Taddei *et al.*, 2003). From previous studies, L-arginine can be synthesized from L-citrulline via the citrulline-NO cycle resulting in increased endothelial NO production (Flam *et al.*, 2007). Oral L-citrulline (6 g/day) provides greater circulating L-arginine levels than a similar dose of L-arginine due to an efficient conversion of L-citrulline to L-arginine (Schwedhelm *et al.*, 2008, Waugh *et al.*, 2001). Similarly, L-citrulline from watermelon is efficiently converted to L-arginine (Collins *et al.*, 2007). Peptic ulcers are open sores in mucous lining of the stomach and duodenum. The annual mortality rate due to peptic ulcer is not low and deaths are largely due its complications such as hemorrhage and perforation (Isemberg and Soll, 1996). The risk of developing an ulcer depends upon an imbalance between aggressive factors such as acid and pepsin, and defensive mechanisms like gastric mucous, high mucosal blood flow and high mucosal. The pathogenesis of peptic ulcer is far from clear and so also the mechanism of anti-ulcer drugs (Akimoto *et al.*, 1998). There are many factors that is involved in gastric and duodenal ulcer pathology such as stress, smoking, nutritional deficiencies, alcohol ingestion and non-steroidal anti-inflammatory drugs (Basil and Howard, 1995). For instance, the use of NSAID stimulates HCl secretion and cause weakness of mucous gel layer which act as barrier by decreasing mucin production and increasing the secretion of bicarbonate from gastric and duodenal mucosa (Huang *et al.*, 2002). These studies suggest that gastric mucus content, increase in gastric mucous cells count could contribute significantly to maintaining normal gastric integrity. In this study, actions of L-citrulline on gastric mucous secretion and mucus cell count is evaluated.

II. Method

Drug Preparation

L-citrulline (L-Cit) was dissolved in distilled water. It was prepared freshly each time and given at different doses (600, and 900 mg/kg) by gavage. L-arginine was also dissolved in distilled water and given at 200mg/kg intraperitoneally. Misoprostol was also dissolved in distilled water and given at 100µg/kg by

intra-gastric gavage. Drugs were administered 2 hours before the animals were sacrificed and their stomachs harvested.

Animals

The study protocol was approved by the Institutional Animal Ethical Committee, University of Ibadan. Male Wistar rats weighing 180-210 g were procured from the institutional animal house and were housed in groups of 5 in each study group. The animals were acclimatized for a duration of 7 days at 25 ± 1 °C and 12:12 h light-dark cycle with free access to food and water. The animals were deprived of food 24 h before the study and transferred to metabolic cages so as to avoid coprophagy.

Group I: serves as control and were given 1ml of normal saline.

Group II: were treated with L-Citrulline (600mg/kg) for five days

Group III: were treated with L-Citrulline (900mg/kg) for five days

Group IV: were treated with L-Arginine (200mg/kg)

Group V: were treated with misoprostol (100µg/kg)

Measurement of mucus production

Gastric mucus production was measured in the rats that were treated with different drugs. The gastric mucosa of each rat was gently scraped using a glass slide. The gastric barrier mucous was estimated by the method of Corne *et al* (1974). Briefly, the excised stomachs from the rats were soaked for 2 hours in 0.1% Alcian blue dissolved in buffer solution containing 0.1M sucrose and 0.05M Sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25M sucrose (15 and 45 min), the dye complexed with mucous was eluted by immersion in 10ml aliquots of 0.5M MgCl₂ for 2 hours. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase measured at 580nm using a spectrophotometer. The quantity of Alcian blue extract per gram wet stomach (mg/g) was then calculated from a standard curve.

$$\text{Gastric Mucus Secretion (mg/g)} = \frac{\text{Weight Of stomach}}{\text{Weight of Wet Stomach (g)}}$$

Histological studies

The tissues were fixed in 10% formalin. They were then processed in Automatic tissue processor. The tissues were then embedded in Paraffin wax. They were thereafter sectioned with microtome at 4 microns. The sections were then floated on frosted end slide. The slides were fixed on hotplate for about thirty minutes. The sections were then stained by Periodic Acid Schiff.

Mucus Cell count.

The mucus cell count was counted using calibrated microscope in five randomly selected areas of the gastric mucosal tissue on each slide. Each selected area assessed five cubic boxes (Li *et al*, 2002). The microscope was calibrated by inserting into its eyepiece a transparent nylon with drawn squares (2mm by 2mm each) on its surface. The squares were faintly drawn such that the gastric mucus cell could be seen properly. The nylon was cut out in such a way that it directly fixes into the round hole of the microscope eyepiece.

Statistical analysis

All the values were tabulated and presented in the tables and were expressed as mean \pm standard error mean (SEM) of six animals. Significant difference among the means were calculated at the level of $*P < 0.05$ when compared with controls. The statistical significance was calculated using students 't' test.

III. Result

L-citrulline shows a dose dependent up regulation of mucous secretion and mucus cells. Maximum production of mucous was seen in the 600mg/kg L-citrulline treated group. Even though L-citrulline produced a significant production of mucous secretion in the higher dose treated groups (600 and 900 mg/kg body weight), all other test groups also produce significant increase in gastric mucous secretion as compared to the control. L-citrulline in a dose of 600 mg/kg body weight was more efficacious than the dose of 900mg/kg body weight, L-arginine (200mg/kg body weight) and misoprostol (100 µg/kg), $p < 0.05$. L-Citrulline also showed a significant increase in mucus cell count in the stomach tissue. The mucus cell count was significantly high in misoprostol treated (standard group) as compared with the control, L-citrulline, L-arginine treated group, $p < 0.05$ as shown table 1 and 2.

Table 1: Analysis of L-Citrulline activity on mucous secretion in the stomach of albino rats
Study model 1

Grouping	Treatments	No of animals	gastric mucus secretion mg/g tissue
I	Control	5	7.4±1.58*
II	l-citrulline 600mg/kg	5	12.7±5.05**
III	l-citrulline 900mg/kg	5	10.11±1.83**
IV	l-arginine 200mg/kg	5	11.30±2.64**
V	Misoprostol 100ug/kg	5	8.71±2.0*

All values are expressed as mean± SEM, *P<0.05,
 **=highly significant compared with the control,
 *= level of significance is not very high compared with control

Table 2: Analysis L-Citrulline activity on mucous secretion in the stomach of albino rats
Study model 2

Grouping	Treatments	No of animals	Gastric counts(cells/mm2 mucus
I	Control	5	14.50±2.56***
II	l-Citrulline 600mg/kg	5	10.75±1.80*
III	l-citrulline 900mg/kg	5	14.20±3.80**
IV	l-arginine 200mg/kg	5	15.60±2.27***
V	Misoprostol 100ug/kg	5	17.96±2.73***

All values are expressed as mean± SEM, ***P<0.05,*p>0.05
 ***=highly significant compared with the control,
 **= have the same level of significance as the control group,
 *= level of significance is not very high compared with control

IV. Discussion

This present experiment studied the effect of L- citrulline on the gastric mucous content rats and the mucous cell count in albino rats and observed that L-citrulline increases the rate of mucus secretion at dose of 600mg/kg-900mg/kg of L-citrulline when administered orally. The microscopic observation also shows that L-citrulline is also effective in up regulation of mucus cell count and not directly related to rate of mucus secretion. At the dose of 600mg/kg of L-citrulline, there is higher secretion of mucous, compared with the dose of 900mg/kg, ***p<0.05 and the mucus cell count was not significantly high compared with the 900mg/kg treated, control and the other tests group. This suggests **at a high dose** of L-citrulline, its effective in boosting secretion will be affected by increase in the number of mucus cell counts. From previous report, the gastric wall mucus content has been found to play important role in the protection of gastric barriers plays a critical role in the protection of gastric barriers (Gou *et al.*,2011,Brown *et al.* 1992). Any decrease in gastric mucus renders the mucosa susceptible to injuries induced by aggressive factors such as acid and pepsin (Nair *et al.*,2010). Also, studies have shown a protective effect of gastric mucus against oxygen radicals (Cross *et al.*, 1984). Recently, reports indicated that impaired gastric mucus synthesis and secretion occur through a decrease in gastric mucosal cNOS activity in rats subjected to Ishemic Reperfusion injury (Kim and Hwan ,2001; Ohta and Nishida, 2001). L-citrulline, is a non-essential amino acid in mammals, is closely related to biosynthesis of L-arginine. It can be readily converted to L-arginine, in the kidney, vascular endothelium and other tissues, thus raising its plasma and tissue levels.(Solomonson *et al.*,2003).From this study, it was found that L-arginine

increases the mucus secretion to $11.30 \pm 2.64^{**}$ mg/tissue from $7.4 \pm 1.58^{*}$ in the control as shown in Table 1, which supports the report by Liu *et al.*, 2008 and Nishida *et al.*, 1997). As an indirect precursor of L-arginine, L-citrulline has a cardioprotective effect, Previous studies have observed reduction in brachial BP after oral L-arginine supplementation in adults with high BP and concurrent chronic diseases (Pallosi *et al.*, 2004). Oral L-citrulline and watermelon supplementation has been found to reduce peripheral BP and improve endothelium-dependent vasodilation in hypertensive and diabetic rats (Wu *et al.*, 2007, Koeners *et al.*, 2007). Watermelon supplementation, rich in natural L-citrulline reduced BP and improve arterial function in adults with high BP, in middle-aged individuals with prehypertension (Figueroa *et al.*, 2010). In the misoprostol (100 μ /kg), the mucus wall content was $8.71 \pm 2.0^{*}$ which is not as high as in the group treated with 600mg/kg and 900mg/kg of L-citrulline which indicates that is not as effective as the L-citrulline. Misoprostol increase the no of cells per mm³ compared with the other groups. This indicates that misoprostol raised the rates of gastric epithelial cell proliferation and this resultant increase in surface mucous cell population could increase the stomach's ability to withstand damage. Therefore, it shows that misoprostol cause hyperplastic response in the gastric mucosa which results in a significantly increased gland cell population, with increase in the mucous and bicarbonate secreting surface. This can also be seen in other groups treated with 900mg/kg L-citrulline, L-arginine and the control group and not very significant in 600 mg/kg L-citrulline treated as shown in the table 2. From the previous studies by Goodlad *et al.*, 1989, that misoprostol effectively elicits a hyperplastic response in the gastric mucosa resulting in a significantly increased gland cell population, with most of the increase occurring in the mucous and bicarbonate secreting mucous cell surface. The hyperplasia was the result of increased number of mitotic and DNA synthesizing cells in each gastric gland, which resulted in a significant increase in the gland cell production rate, gland per day. In conclusion, L-citrulline is very effective in increasing the rate of mucous secretion and mucous cell which could be as a result of up-regulation of endothelial Nitric oxide via the citrulline-NO cycle.

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Actions of L-citrulline on gastric mucus secretion and gastric mucous cell counts in albino rats

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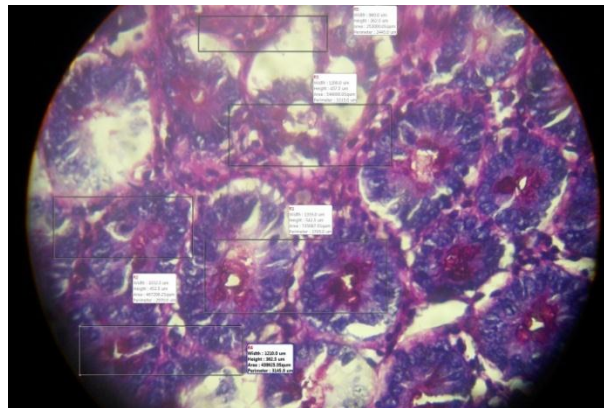


Plate 1: *Histological examination of citrulline(600mg/kg) on gastric tissue. The mucus cell count was not significant as compared with the control, $p>0.05$.the mucus cell count is $10.75\pm 1.80^*$*

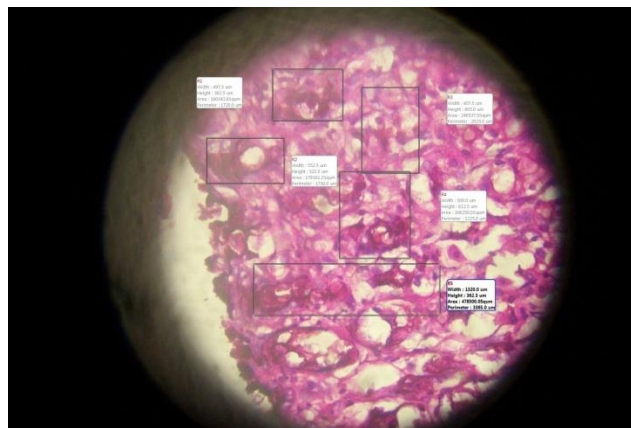


Plate 2: *Histological examination of L-arginine (200mg/kg) on gastric tissue. The mucus cell count was highly significant as compared with the control, $p<0.05$.the mucus cell count is $15.60\pm 2.27^{**}$*

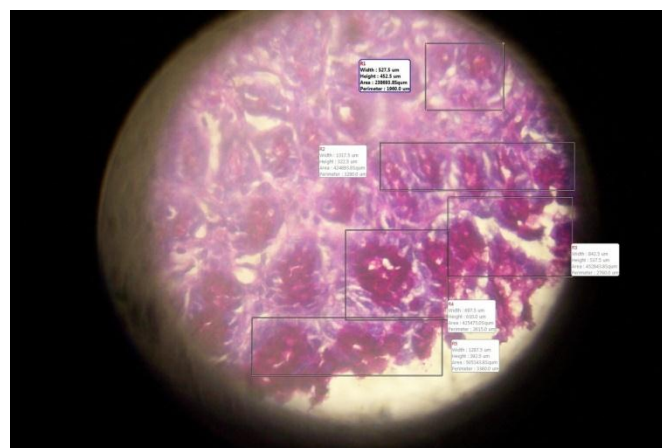


plate 3: *Histological examination of gastric tissue of the control group. The mucus cell count is not as significant as in the standard group.*

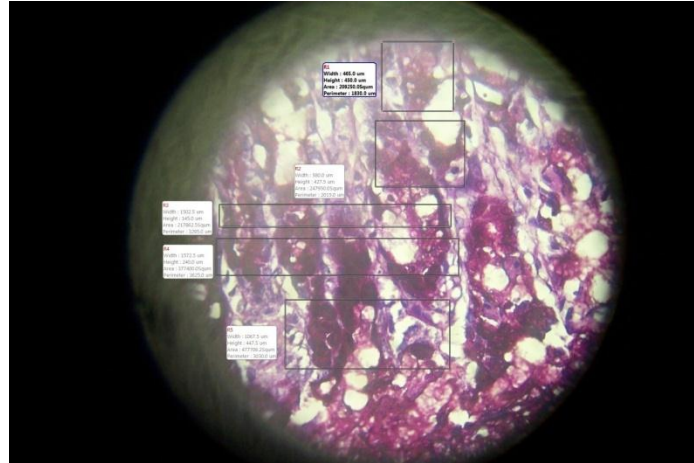


plate 4: Histological examination of Misoprotol (100µg/kg) on gastric tissue. The mucus cell count was highly significant as compared with the control, $p < 0.05$.

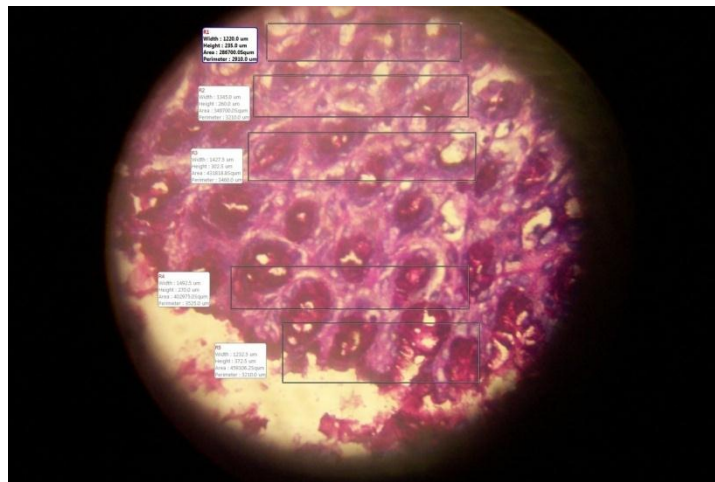


plate 5: Histological examination of L-Citrulline (900mg/kg) on gastric tissue. The mucus cell count was not as significant as compared with the control, $p < 0.05$