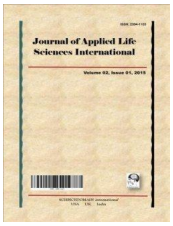

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Anti-ulcerogenic, Antioxidant and Mucogenic Effects of L-cysteine in Gastric Tissue of Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the effect of L-cysteine pretreatment on indomethacin induced ulceration in male wistar rats.
Study Design: Experimental animal study.
Place and Duration of Study: Department of Physiology (inflammation and Gastrointestinal secretion unit), College of Medicine, University of Ibadan, Nigeria, between January and July 2017.
Methodology: 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 and MDA), Nitric oxide (NO), mean ulcer score and gastric blood flow (GBF), 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 as follows: ulcer control, L-cysteine (100 mg/kg, 300 mg/kg and 500 mg/kg), cimetidine (50 mg/kg). Results were analysed using ANOVA and reported as Mean ± SEM. Values were considered significant at $P \leq 0.05$.
Results: The results of this study showed that L-cysteine (100 mg, 300 mg, and 500 mg respectively) pretreatment significantly reduced mean ulcer score (9.5±1.9; 7.5±1.5; 4.5±0.9) and MDA level (7.2±0.23; 7.49±0.3; 6.54±0.55), and increased SOD activity (10.69±0.1; 10.12±0.29;

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14.76±0.07) when compared with the mean ulcer score, MDA and SOD in the ulcer control group (39.5±7.9; 10.62±1.11; 5.02±0.74). Also, NO level (10.8±0.44; 10.37±0.18; 8.41±0.06), gastric mucus secretion (0.92±0.008; 0.94±0.001; 0.99±0.001) and GBF (2.08±0.02; 2.11±0.06; 2.11±0.01) were significantly ($p<0.05$) higher in the L-cysteine pre-treated animals when compared with NO, mucus secretion and GBF in the ulcer control (7.86±0.09; 0.82±0.01).

Conclusion: This study shows that L-cysteine pre-treatment has anti-ulcer potential which might be mediated through increased antioxidant enzymes, increased mucus secretion and enhancing gastric blood flow. This will be of immense advantage in the treatment of peptic ulcer.

Keywords: L-cysteine; cimetidine; antioxidants; mucus secretion; anti-ulcer.

1. INTRODUCTION

Peptic ulcer, a common gastrointestinal disorder, is a multifactorial and complex disease that involves imbalance between gastric offensive factors (e.g. lipid peroxidation) and defensive mucosal factors e.g. antioxidant enzymes [1,2]. However, diverse factors such as non-steroidal anti-inflammatory drugs (NSAIDs), stressful lifestyle, alcohol consumption, *Helicobacter pylori* (H. pylori) infection, smoking, and family history can contribute to its pathogenesis [3,4]. The NSAIDs such as indomethacin are commonly prescribed drugs for the treatment of pain and inflammation in rheumatic disorders and osteoarthritis [5], but are associated with peptic ulcer as their major complications. The mechanisms underlying the pathogenesis of NSAIDs-induced ulcers are complex and multifactorial. It involves both prostaglandin-dependent (through Cyclooxygenase inhibition) and prostaglandin-independent mechanisms. The independent mechanisms include inflammatory, immunogenic, genetic, and stress response pathways [6].

In an attempt to protect the gastric mucosa from ulceration, enhance ulcer healing and prevent ulcer recurrence, pharmacological control of gastric acid secretion has long represented a desirable goal. Thus, there is an increasing need to develop more potent therapeutic agents for the treatment of peptic ulcer and several experimental studies have shown the effectiveness of some nutrients and food supplements in the management of peptic ulcer.

L-cysteine is an essential amino acid that is ingested from diet to meet up the body's requirement and is majorly found in most dairy foods (e.g. milk, egg, meat and spices). L-cysteine contains sulfhydryl group and serves as a precursor of hydrogen sulphide [7]. Hydrogen sulphide is a potent mediator of vascular smooth muscle relaxation, exhibit anti-inflammatory activities and contribute to gastric mucosal defence [8,9]. It has also been shown to reduce the severity of NSAIDs and also protective in a number of models of acute gastric injury, but the mechanism underlying this action is unclear [10]. Therefore, the present study aimed at evaluating the mechanisms of action of this amino acid in the prevention of peptic ulcer.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals Used

Cimetidine, Ulcertret-20 (Swiss pharma pvt.Ltd. 3709, GIDC, Phase IV, Vatva, Ahmedabad- 382 445, Gujarat, India. Indomethacin**,** Omecet (Medibios Laboratories PVT Limited. J-76, M.I.D.C, Tarapur, Taluka-Palghar Dist, Thane-401 506, India), L-cysteine**, (**Solgar, Inc. 600 Willow Tree Road, Leonia, NJ 07605 U.S.A. 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 100-130 g were used for this study. The animals were obtained from Central Animal house, College of Medicine, University of Ibadan. The experimental animals were acclimatised for two weeks and were fed on rats' pellets and water given *ad libitum*. After the period of acclimatisation, the experimental animals were divided 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 100 mg/kg body weight of L-cysteine; Group 3- animals pre-treated with 300 mg/kg body weight of L-cysteine; Group 4- animals- pre-treated with 500 mg/kg body weight

of L-cysteine; group 5- animals pre-treated with 50 mg/kg body weight of cimetidine.

The first sub-group was used for the anti-ulcer studies; antioxidant enzymes (SOD and MDA), Nitric oxide, Gastric blood flow and the mean ulcer score, while gastric mucus secretion study was performed with the second sub-group. All procedures used in this study conformed to the guidelines on the care and use of animals in research and teaching [11].

2.3 Indomethacin Gastric Ulcer Induction

Gastric ulcer was induced in the experimental animals using indomethacin at a dosage of 40 mg/kg body weight in accordance with the previously described method by Oluwole and Bolarinwa [12]. Afterwards, the animals were sacrificed 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 Alphin and Ward [13] modified by Elegbe and Bambgose [14]. Ulcer index was calculated using the formula.

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

2.5 Assay of Superoxide Dismutase (SOD)

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

2.6 Determination of Lipid Peroxidation

MDA (marker for oxidative stress) assessment was done according to the method of Varshney and Kale [16]. 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).

2.7 Gastric Mucus Secretion Study

This study was carried out using the spectrophotometry method described by Corney et al. [17]. 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) =

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

2.8 Determination on Nitric Oxide Levels

Nitrite was determined as an oxidation product and indicator of NO synthesis as described by Moshage et al. [18]. 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 Determination of Gastric Blood Flow

Gastric blood flow was measured as a component of abdominal aortic blood flow. Abdominal aortic blood flow was measured by placing an ultrasonic Doppler flow probe (Transonic# 11RB) around the abdominal aorta between the diaphragms and celiac artery. Flow rates were obtained with the Transonic T206 Blood Flow Meter (Transonic Instrument, Ithaca, NY).

Animals were fasted, but not deprived of water for 24 hours before the onset of the experiment. 1 hour before ulcer induction, the test substance were administered to their respective group after which indomethacin was given to induced ulcer in all groups except group 1 (control group). Animals were anaesthetised with ketamine (1ml/kg) intraperitoneal. A midline laparotomy was performed to expose the abdominal aorta for the placement of probe. The intestine of the rats was deflected to the right to expose the abdominal aorta. Adjacent fats were removed for proper acoustical coupling. The recorded blood flow was expressed in ml/min.

2.10 Statistical Analysis

Data were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed with Graph Pad Prism 5.0. Comparison between mean were done using one way analysis of variance (ANOVA) and differences between means were considered statistically significant at $P=0.05$.

3. RESULTS

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

Animals pre-treated with various doses of L-cysteine showed a significant increase in gastric mucus secretion when compared with the ulcer control group. The groups treated with the standard drug; cimetidine and 500 mg/kg L-cysteine showed the highest secretion of gastric mucus. There was also a dose-dependent increase in gastric mucus secretion in the L-cysteine treated group as shown in Fig. 1.

3.2 Effect of L-cysteine Pre-treatment on Antioxidant Enzymes

3.2.1 Superoxide dismutase

The levels of superoxide dismutase obtained from this study are presented in Fig. 2. There was a significant increase in super oxide dismutase levels in all the L-cysteine and cimetidine pre-treated animals when compared with the control.

3.2.2 Lipid peroxidation

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

3.3 Effect of L-cysteine on Gastric Nitric Oxide Level

The results obtained from the nitric oxide study are presented in Fig. 4. In this study, pre-treatment with L-cysteine caused a significant increase in nitric oxide concentration similar with the standard drug cimetidine.

3.4 Effect of L-cysteine Pre-treatment on Gastric Blood Flow in Indomethacin induced Ulceration in Rats

The gastric blood flow was significantly increased in all the treated groups compare to the ulcer control group as shown in Fig. 5.

3.5 Effect of L-cysteine Pre-treatment on Mean Ulcer Score

The mean ulcer score recorded in this study is presented in Table 1. The ulcer control group had a mean ulcer score of 39.5±7.90, which was significantly reduced in all the L-cysteine treated groups in a dose-dependent manner.

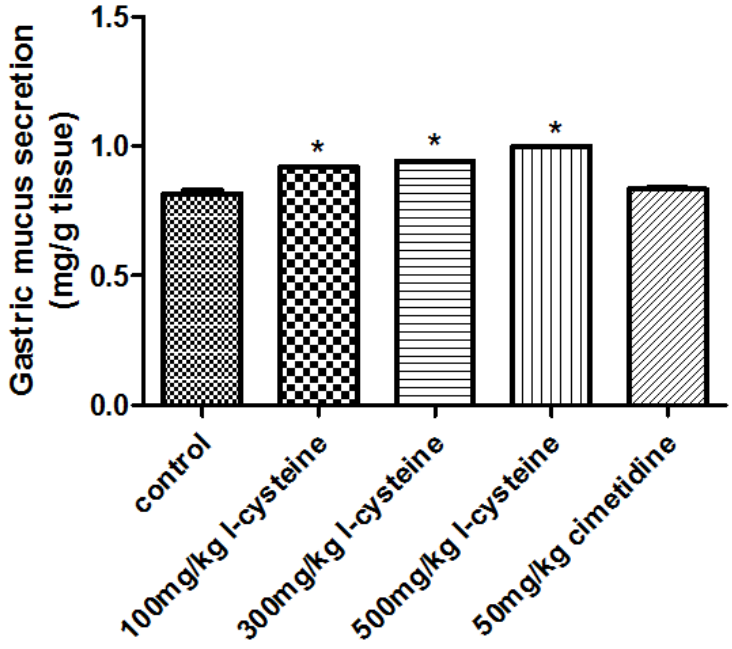

Fig. 1. Effect of Cysteine on mucus secretion in Indomethacin induced ulceration in rats
**p<0.05, when compared with ulcer control*

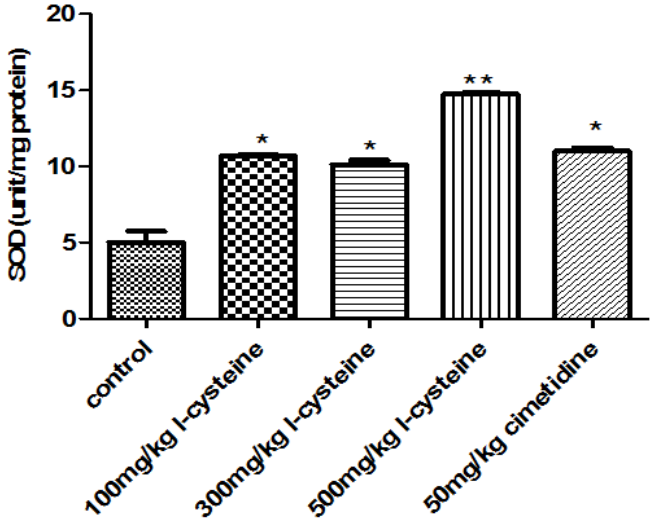

Fig. 2. Effect of L-cysteine on Superoxide Dismutase (SOD) in Indomethacin-induced ulceration in rats

** p<0.05, **p<0.005, when compared with ulcer control*

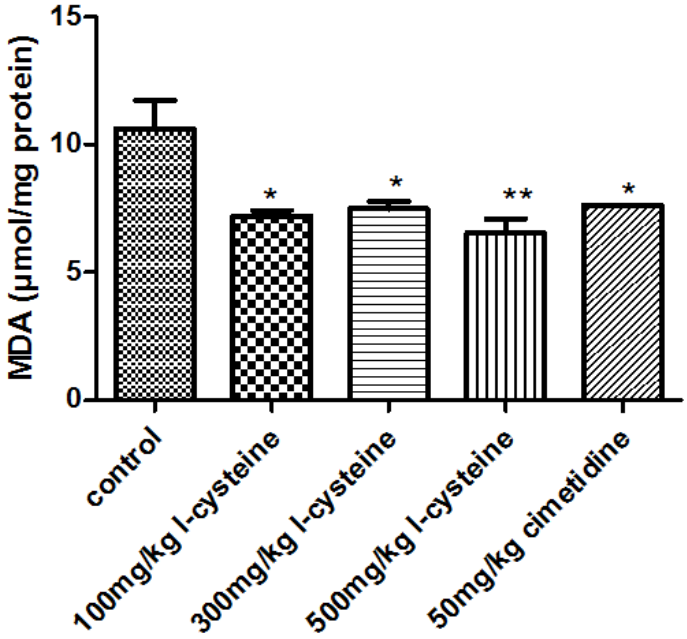

Fig. 3. Effect of Cysteine on Malondialdehyde (MDA) in Indomethacin induced ulceration

**p<0.05, **p<0.005 when compared with ulcer control*

Table 1. Effect of L-cysteine pretreatment on mean ulcer score

Groups	Mean ulcer score
Control	39.5±7.9
L-cysteine (100 mg/kg)	9.5±1.9*
L-cysteine (300 mg/kg)	7.5±1.5*
L-cysteine (500 mg/kg)	4.5±0.9**
Cimetidine	7.0±1.4*

**p<0.05, **p<0.005, when compared with ulcer control*

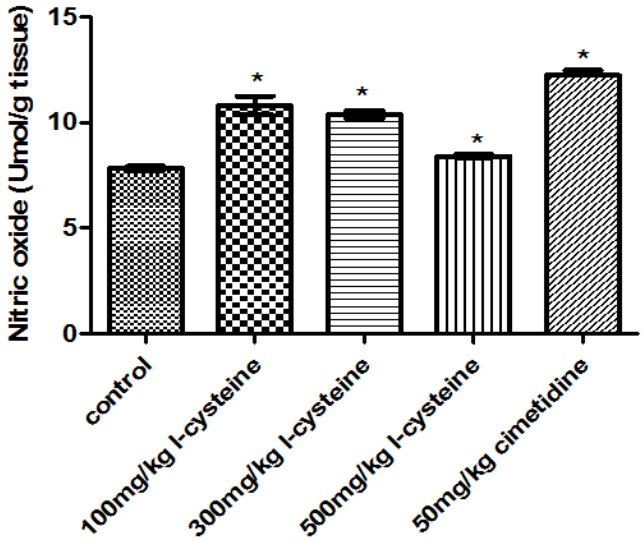

Fig. 4. Effect of L-cysteine on Nitric Oxide activity in Indomethacin induced ulceration
**p<0.05 when compared with ulcer control*

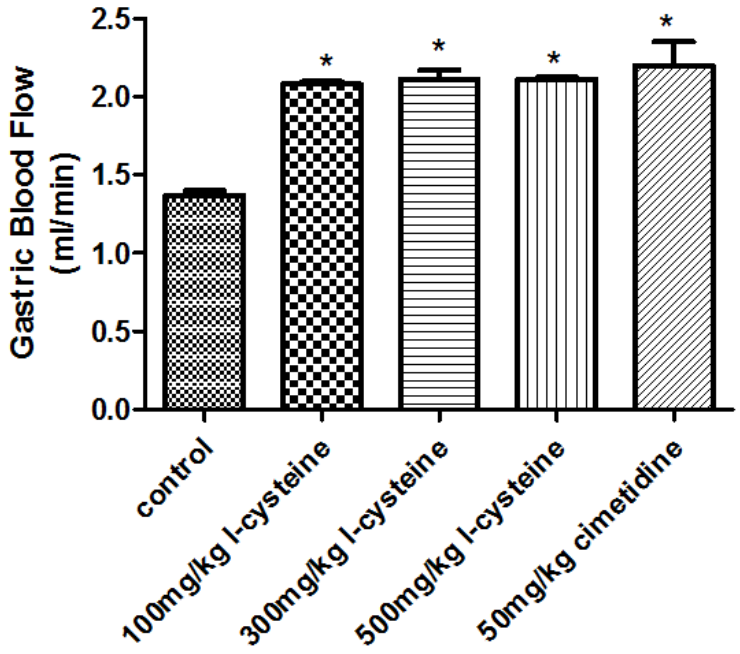

Fig. 5. Effect of Cysteine on Gastric blood flow in indomethacin-induced ulceration
**p<0.05 when compared with ulcer control*

4. DISCUSSION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat musculoskeletal disorders, and are used almost routinely long term by patients with rheumatoid arthritis but has been associated with the development of gastric ulcers [19]. It has been demonstrated that NSAIDs (e.g. indomethacin) cause peptic ulceration by a combination of direct effects in the mucous barrier and by local and systemic inhibition of the prostaglandin system. The inhibition of endogenous prostaglandins (PGs) and related compounds, decreases gastric mucosal blood flow, and carbonate synthesis as well as increased susceptibility to mucosal injury

and gastric ulceration [20,21]. In addition, sequel to the acidic nature of indomethacin [22], it enhances lipid peroxidation and generation of free radicals in the gastric mucosa [23] thereby leading to oxidative damage [24]. Thus, NSAIDs given orally or systemically will cause damage to gastric protective mechanisms, allowing gastric acid to penetrate to submucous structures and thus cause ulceration. Therefore, strategies to protect the gastric mucosa from this offensive agent has been of immense interest to various scientists.

The importance of increased mucus strength and quantity in protecting the regenerating gastric epithelium has been established [25,26]. Gastric mucus is the first protective barrier in the gastric epithelium that prevents the actions of free radical on the stomach mucosal [27] which could lead to the formation of ulcers [28]. Hydrophobicity play a significant role in protecting the gastric membrane against noxious agents in the lumen [29] as 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 [30]. In this present study, there was an increase in the gastric mucus secretion in the group of animal pre-treated with L-cysteine, which implies that L-cysteine has a promising potential in ameliorating indomethacin- induce peptic ulcer. This report is in agreement with the earlier work carried out by Amang et al. [31], where it was reported that gastric mucus secretion increased in the gastric mucosa of animals treated with aqueous extract of *Eremomastax speciose* against indomethacin-induced ulceration. Allen and Flemstrom reported that an increase in the gastric mucus secretion in stomach confers double protection on the gastric mucosal as it prevents physical damage by acting as a lubricant and chemical damage by sequestering bicarbonate and forming a pH gradient against the proteolytic and acid nature of gastric juice [32].

It has been reported that biochemical with antioxidant properties exerts gastroprotective function [33]. Studies have demonstrated that these compounds can scavenge free radicals and are also involve in inhibition of lipid peroxidation, mucus production, decrease of histamine levels and inhibition of gastric acid secretion [34,35]. Studies have demonstrated that L-cysteine supplementation in rats reduces reactive oxygen species (ROS) thereby demonstrating its antioxidant property [36,37] and also contains sulfhydryl group which is a precursors of hydrogen sulfide [38] that mediates various biological functions.

In this study, the antioxidant activity of L-cysteine against indomethacin-induced ulcer in rats was observed to determine the possible mechanism of action of this amino acid. L-cysteine mediates its protective role against indomethacin-induced ulcer by reducing the level of malondialdehyde (marker for oxidative stress) and also enhancing the superoxide dismutase activity (antioxidant enzyme). Antioxidant compounds are able to protect the gastric mucosa by binding to acetylcholine muscarinic receptors inhibiting acid secretion [39] and attenuating blood flow, thereby diminishing the hemorrhagic lesions [40].

In this study, it was confirmed that the standard drug cimetidine caused a significant increase in nitric oxide. Similar result was recorded when the animals were pre-treated with L-cysteine compared with the control group. Nitric oxide is one of the most important defensive endogenous agents in the gastric mucosa [41]. It is essentially important in the regulation of gastric blood flow and also increases mucus secretion in the gastric mucosa [42]. It inhibits the activation of leukocytes within the microcirculation, and inhibits the inherent release of reactive oxygen metabolites and proteases [43]. On the other hand, suppression of NO production has been reported to delay healing process and this effect was accompanied by a decrease in the gastric blood flow, mucosal growth parameters and attenuated angiogenic response [44]. Also, data obtained from in vitro and in vivo studies suggested that nitric oxide exerts an anti-apoptotic effect on rat gastrointestinal mucosal cells [45]. In addition, L-cysteine contains sulfhydryl group (SH) and serves as a precursor of hydrogen sulphide. Hydrogen sulphide is a potent mediator of vascular smooth muscle relaxation, exhibiting anti-inflammatory activities and contributing to gastric mucosal defense [46]. The SH groups are also responsible for increasing the production of and maintaining mucus stability, through the disulfide bridges, and are involved in maintaining gastric integrity, thereby limiting the production of free radicals involved in tissue damage [47]. The relatively high concentrations of SH have been implicated as in gastroprotection [48].

In this study, pre-treatment with L-cysteine caused a significant reduction in the mean ulcer score. The percentage ulcer inhibition in animal pre-treated with L-cysteine were comparable to

the standard drug cimetidine and appears to be dose-dependent. Cimetidine is a histamine H_2 receptor antagonist which markedly inhibits gastric acid secretion [49,50]. This supports the earlier study that cimetidine significantly reduces the effect of NSAIDs-induced peptic ulcer [51]. Thus, L-cysteine could also exert its anti-ulcerogenic effect via the inhibition of H_2 receptors in the gastric epithelia cells.

Despite the potent therapeutic effect of non-steroidal anti-inflammatory drugs (NSAIDs), it has been classically established that NSAIDs, such as indomethacin, significantly reduces prostaglandin levels and blood flow to gastric mucosa and thus are considered ulcerogenic agents in long-term use [52]. Therefore, it is important to assess the gastroprotective effects of different doses of L- cysteine against indomethacin-induced gastric ulcer. The increase in gastric blood flow facilitated by L-cysteine contributes to protection by supplying the mucosa with oxygen and $HCO3^-$, and by removing H^+ and toxic agents diffusing from the lumen into the mucosa. Thus, the results showed that L-cysteine has anti-ulcer potential against different ulcerogenic agents which may be due to the high sulfhydryl content of this amino acid.

5. CONCLUSION

The result from this study shows that L-cysteine possess antiulcer activities which can be attributed to its antioxidant properties, its ability to enhance gastric mucus secretion as well as its sulfhydryl content. L-cysteine which is usually taken as a supplement might be beneficial to people with peptic ulcer disease.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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