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Full Length Research Paper

Gastroprotective Mechanisms of Imipramine on Indomethacin-Induced Gastric Ulcer in Male Wistar Rats

***Oluwole, F.S., Ayoade, J.T. and Busari, A.M**

Department of Physiology, College of Medicine, University of Ibadan. Ibadan, Nigeria.

ABSTRACT

Tricyclic antidepressant drugs were used in the past for the treatment and management of endogenous depression and mood disorders. They have also been used in the treatment of gastric ulcer as far back as five decades ago. However, there is scanty information on the effect of imipramine on gastric acid secretion, gastric mucus secretion and antioxidative activity as a basis for its anti-ulcer effect. In this study, the antisecretory and gastroprotective effects of imipramine pretreatment in male Wistar rats were investigated. One hundred rats (180-250g) were used in this study. The animals were divided into four study groups. Each group was further divided into five subgroups of five rats each. Groups 1 (Control), Groups II, III, and IV had 10, 25,40mg/kg Imipramine respectively, while group V served as positive control; 20mg/kg Omeprazole, a proton pump inhibitor. All drugs were administered orally for 14 days. Gastric antisecretory, mucogenic and antioxidative effects of imipramine were determined using standard methods in the text. Level of statistical significance were determined using the one-way analysis of variance (ANOVA), followed by students't-test. $P< 0.05$ was considered significant. Imipramine significantly decreased basal acid secretion when compared with the control. There was significant increase in gastric acid secretion in all the pretreated groups after intravenous administration of carbachol. Gastric mucus secretion and gastric mucus cell count increased significantly in all imipramine pretreated groups when compared with the control. It also significantly decreased parietal cell count per field and MDA activity when compared with the control. The SOD and CAT activity was significantly increased at all doses when compared with the control. Imipramine significantly reduced indomethacin induced ulcers when compared with the control. This study suggests that imipramine possesses gastroprotective activity via multiple mechanisms, among which are antisecretory, mucogenic and antioxidant activities.

Keywords: *Imipramine, Gastric acid, Indomethacin, Gastric Mucus*

*Author for correspondence: *E-mail:* franwole@yahoo.com; *Tel:* +234-8057074993

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INTRODUCTION

Peptic ulcer is a disorder of the gastrointestinal tract presenting features of mucosal damage perhaps as a result of exposure of the gastric mucosa to harsh substances such as pepsin and gastric acid. The luminal surface of the stomach is covered by a visco elastic mucus gel layer that acts as a protective barrier against the harsh luminal environment. The structural characteristics of this barrier are primary indicators of its physiological function, and the changes of its composition have been identified in gastrointestinal pathologies. An imbalance between these harsh factors and mucosal defensive factors leads to gastric damages such as gastritis and peptic ulcer disease (Mota *et al*., 2009).Other causes of gastric ulcer include alcoholic beverages, stress, nutritional deficiencies, smoking and *Helicobacter pylori* (O'Malley, 2003).

Stress can be beneficial to human because it helps to keep us alert, motivated and primed to respond to danger. However, too much of it may lead to major depression in susceptible people. Stress has the consequential effect of increased generation of reactive oxygen species (ROS), while reactive oxygen species have contributed enormously to the etiology and pathophysiology of different kinds of diseases and of particular interest is gastrointestinal inflammation and gastric ulcer (Repetto and Llesuy, 2002). Over the years

various drugs; H2 receptor blocker, hydrogen ion channel inhibitors and antacids have been used for the treatment of peptic ulcers.

The family of tricyclic antidepressant (TCA) have been found to exhibit antiulcer activity (Sen *et al*., 2002). Desipramine, an active metabolite of imipramine and trimipramine had been shown to be gastroprotective and inhibit gastric acid secretion and in addition reduce ulcer index in several ulcer models (Hano *et al*., 1983; Nobrega and Weiner, 1983; Aguwa and Ramhanujam, 1984). Also, imipramine, mainly used in the treatment of depression (Vukkum *et al*., 2014), has been reported to have antiulcer effects (Hamid and Taghi, 1997). Many scientific reports have equally highlighted these positive effects of TCAs in healing ulcer, but the mechanism still remains unclear (Dursun *et al*, 2009). The aim of this study therefore, is to examine the anti-ulcerogenic effect of imipramine and its possible mechanisms of action.

MATERIALS AND METHODS

Animals: One hundred male Wistar rats weighing between 180 and 250g obtained from the Central Animal House, Department of Physiology, College of Medicine, University of Ibadan, Nigeria were used for the study. The animals were housed under standard laboratory conditions and were fed with standard rat pellet (Ladokun Feeds, Nigeria) and water *ad libitum*. Five different studies were carried out namely; measurement of gastric acid secretion, estimation of gastric mucus secretion, Parietal and mucus cell counts, indomethacin-induced gastric ulceration and antioxidant enzymes assay. Each study has five subgroups with five (5) animals treated as follows: Distilled water (Control), Imipramine 10 mg/kg; Imipramine 25 mg/kg; Imipramine 40 mg/kg and Omeprazole 20 mg/kg

Animals were handled in accordance with the guidelines of the National Institute of Health (NIH) for laboratory animal care and use. Proposal of the research work had earlier been presented and approval received from the department of physiology committee.

Test and Standard Drugs: Imipramine hydrochloride (Dalkeith Laboratories Limited, Woburn, MK179PG). Omeprazole (TEVA UK Ltd, Eastbourne, BN229AG). Carbachol (Sigma Chemical Co. Ltd, UK). All drugs were administered for a period of 14 days. All drugs were administered orally. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and National Institute of Health (Suleyman *et al*.,2001),

Gastric acid secretory study: The animals were starved for 24 hours prior to the investigation to allow for clear stomach without debris. The rats were anaesthetized with urethane given intraperitoneally at 0.6ml/100g body weight. The stomach was prepared for perfusion using the continuous perfusion technique described by Ghosh and Schild (1958) as modified by Adeniyi and Oluwole (1990).

Basal secretion: The stomach was continuously perfused at1.0 ± 0.1 ml/min with 0.9% normal saline warmed to rat body temperature. Then 10ml effluent was collected for 1 hour and titrated against 0.0025N NaOH to the end point of phenolphthalein. Gastric acid secretion was stimulated with intraperitoneal injection of 50 µg/kg b.wt carbachol which was given via a cannulated femoral vein.

Gastric mucus secretory study: Following fourteen days of imipramine and omeprazole pretreatment, the rats were fasted overnight and sacrificed. Midline laparatomy was performed on each rat to enable identification and removal of the stomach. The stomachs were removed and the glandular portion excised. The stomach tissues were opened along the lesser curvature to expose the luminal portions. The everted stomachs were soaked in 0.1% Alcian blue dissolved in 0.16 M sucrose buffered with 0.05M sodium acetate for two hours. The solution was adjusted to pH 5.8 with HCl. Uncomplexed dye was removed by two successive washes at 15 and 45 mins in 0.25 M sucrose. Dye complexed with mucus was diluted by immersion in 10 ml aliquots of 0.5M $MgCl_2$ for two hours. The resulting blue solutions were shaken briefly with equal volume of diethyl ether and absorbance of aqueous phase was measured at 605nm with Spectrophotometer (Corney, 1974). The absorbance of each solution was used to calculate the various concentrations of dye and the weight of dye (expressed in mg) deduced, using a standard curve. Gastric mucus secretion in mg/kg was expressed as the weight of the dye against the weight of the stomach as earlier reported by Oluwole *et al* (2007).

Gastric mucus cell count study: The gastric mucus cells were counted using an improvised calibrated microscope. This was an improvement over the foremost blind manner approach for counting (Li *et al*, 2002). Twenty-five squares, each measuring 2mm by 2mm, were drawn faintly on a transparent nylon. The nylon was then affixed onto the eyepiece of the microscope. The gastric mucus cells were counted using Periodic Acid Schiff stain. The mucus cells were counted in five squares during each view. The number of gastric mucus

cells in each microscopic view was recorded and the mean number of gastric mucus cells in each square millimeter of gastric tissue was calculated.

Parietal cell count study: The animals were sacrificed and the stomach removed as quickly as possible into normal saline. The stomach was opened along the greater curvature, washed and transferred into a beaker containing 10% formalin. Sections were prepared from strips removed from the fundic area of the stomach and stained using the method of Adeniyi(1991) as modified by Oluwole *et al*, (2007), using the Hematoxylin and Eosin stain. The various gastric mucosal secretory cells were clearly differentiated, taking up different colours. The nuclei of the parietal cells were stained deep blue while the mucous cells were clearly vacuolated. Five counts from randomly selected fields were made on each section and the average count per unit area was calculated for each stomach by dividing the number of cells seen by the number of counts made.

Indomethacin-induced gastric ulceration study: The rats were deprived of feed twenty-four hours prior to the commencement of ulcer induction though there was free access to water. Indomethacin was then administered (40 mg/kg orally). After four hours of administration, the animals were sacrificed and the stomach removed surgically and opened along the greater curvature to determine ulcer score. Gastric ulcer score was measured using the stipulated criteria while percentage ulcer inhibition was calculated with the formula below. Assessment of gastric mucosal lesion was expressed in terms of the ulcer index according to the method of Alphin and Ward (1967) as modified by Elegbe and Bamgbose (1976).

$$\text{Ulcer index (U.I)} = \frac{\text{Mean degree of ulceration} \times \% \text{ of group of ulcerated}}{100}$$

$$\% \text{ Inhibition} = \frac{\text{Ulcer index in control} - \text{Ulcer index in test}}{\text{Ulcer index in control}} \times 100$$

Antioxidant enzymes' assay

Malondialdehyde (MDA) assay-Lipid peroxidation
MDA was determined by spectrophotometry of the pink coloured product of thiobarbituric acid (TBA) reactive substances complex. Briefly, 0.1ml of the test sample was mixed with 0.5ml of 10% TCA, and 0.5ml of 75% TBA was then added to it. The mixture was then placed in a water bath at 80^{o}C for 45minutes.The absorbance of the resulting pink solution was measured against a reference blank of distilled water at 532nm.The test sample was calibrated using the MDA as a standard and the results was expressed as the amount of free MDA produced. The MDA level was calculated according to the method of Adam-Vizi and Seregi (1982). The Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x $10^{5}M^{-1}Cm^{-1}$.

Superoxide dismutase (SOD) activity: The levels of SOD activity was determined by the method of Misra and Fridovich (1972). This involves inhibition of epinephrine autoxidation, in an alkaline medium at 480nm in a UV vial spectrophotometer. For the determination of specific activity of SOD in homogenate sample of stomach tissue, the rate of autoxidation of epinephrine was noted at 30 seconds intervals in all groups. The enzyme activity was expressed in arbitrary units considering inhibition of autoxidation, as 1 unit of SOD specific activity.

Catalase activity: This was determined by measuring the rate of H_2O_2 absorbance at 480nm within 30 to 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 7ml of 0.01M of potassium permanganate. Absorbance was then read. The result was expressed in μmol/mg protein.

Statistical analysis: Statistical analysis was done with Graph Pad Prism 5.0 and Microsoft Excel using One-way Analysis of Variance (ANOVA) and student's t-test. Data were expressed as Mean ± SEM with $P<0.05$ considered statistically significant.

RESULTS

Effect of Imipramine on gastric acid secretion
The mean basal gastric acid secretion in the control animals was 0.68 ± 0.04 mEq/L/10min. There was significant reduction in basal gastric acid secretion in 10 mg/kg Imipramine treated animals (0.39 ± 0.03 mEq/L/10min) when compared with the control ($p<0.05$). Imipramine pre-treated groups showed normal secretory response to carbachol stimulation of gastric acid secretion (Fig 1).

Effect of Imipramine on gastric mucus secretion
The mean gastric mucus secretion in the control animals was 0.19 ± 0.03 mg/g tissue as against 0.38 ± 0.03, 0.39 ± 0.01, 0.30 ± 0.01 mg/g tissue in animals treated with 10, 25 and 40 mg/kg Imipramine respectively, showing a significant increase in gastric mucus secretion ($p<0.05$).

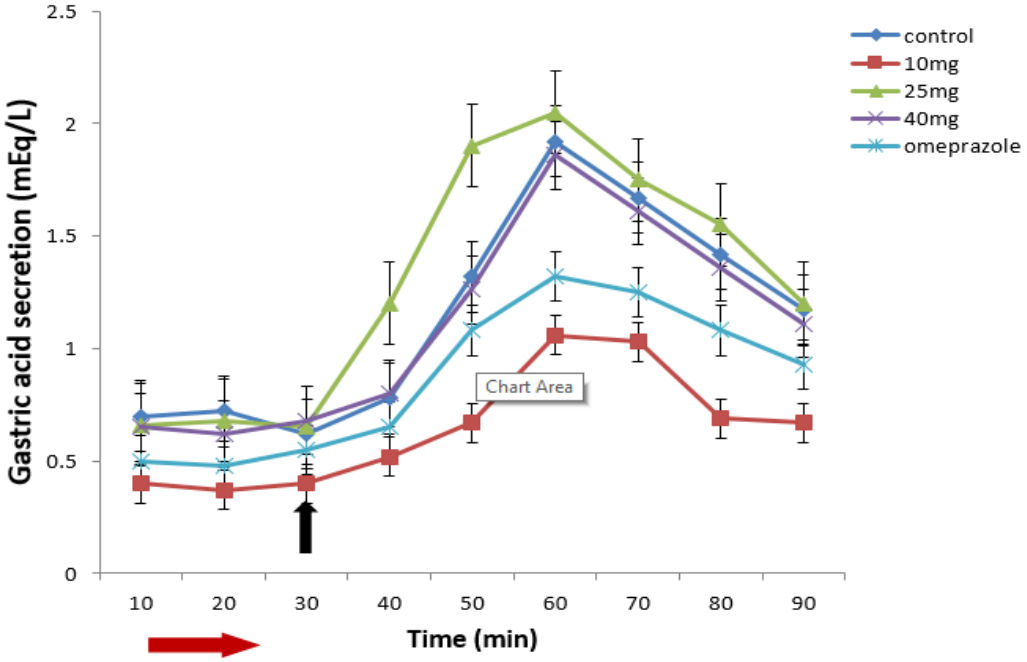

Figure 1: Gastric acid secretion in pretreated rats in response to carbachol. Each point represents Mean±SEM of the gastric acid secretion in five animals per group. Peak acid output (PAO) was recorded at post 30 minutes of stimulation. Horizontal (red) arrow= basal secretion; Vertical arrow represents point of intravenous administration of carbachol

The gastric mucus secretion in omeprazole treated animals showed significant decrease when compared to imipramine treated groups (0.26 ± 0.02 mg/g tissue), but significantly increased when compared with the control ($p<0.05$) (Table 1).

Table 1:
Effect of imipramine on gastric mucus secretion.

Grouping	Gastric mucus secretion (mg/g)	Mean gastric mucus cell count/field
Control	0.19 ± 0.03	436.3±26.52
10mg/kg Imipramine	0.38 ± 0.03*	675.5±50.97*
25mg/kg Imipramine	0.39 ± 0.01*	1029±27.65*
40mg/kg Imipramine	0.30 ± 0.01*	590.3±38.18*
Omeprazole	0.26 ± 0.02*	548.8±23.14*

Values are in mean ± S.E.M. n = 5. * Significantly different from control at P<0.05.

Effect of Imipramine on parietal cell count

The mean parietal cell count in the control animals was 256.5 ± 4.05 as against 133.8 ± 3.33, 179 ± 9.04 in animals treated with 10 and 40 mg/kg Imipramine respectively, showing a significant decrease in parietal cell count ($p<0.05$). The mean parietal cell count in omeprazole treated animals also showed significant decrease (229.5 ± 6.90) when compared with the control ($p<0.05$). 25 mg/kg imipramine showed no significant difference in the mean parietal cell count per field (247.0 ± 4.42) when compared with the control ($p<0.05$) (Table 2).

Table 2:
Effect of imipramine on parietal cell count

Grouping	Mean parietal cell count / field
Control	256.5 ± 4.05
10mg/kg Imipramine	133.8 ± 3.33*
25mg/kg Imipramine	247.0 ± 4.42
40mg/kg Imipramine	179.0 ± 9.04*
Omeprazole	229.5 ± 6.90*

Values are in mean ± S.E.M. n = 5. * Significantly different from control at P<0.05

Effect of Imipramine on gastric mucus cell count

The mean gastric mucus cell count in the control animals was 436.3 ± 26.52 as against 675.5 ± 50.97, 1029 ± 27.65, 590.3 ± 38.18 in animals treated with 10, 25 and 40 mg/kg Imipramine respectively, showing a significant increase in gastric mucus cell count ($p<0.05$). The values of gastric mucus cell counts in 10 and 25 mg/kg imipramine treated groups were significantly higher than omeprazole treated animals (548.8 ± 23.14) (Table 1).

Effect of Imipramine on Indomethacin-induced gastric ulceration: Imipramine significantly reduced ($p< 0.05$) ulcer scores when compared with the control .Generally there was significant reduction with all the doses of imipramine (10, 25,40mg/kg) when compared with the control. This is evident in the percentage inhibition exhibited with the three doses respectively

Effect of Imipramine on SOD and CAT

As shown in Fig. 3 and 4 there was a significant increase in superoxide dismutase and catalase activities respectively in Imipramine and omeprazole treated rats when compared with control.

Table 3:

Shows the mean ulcer score and percentage inhibition in animals pretreated with different doses of Imipramine

Groups	Mean Ulcer Score	Ulcer Index	Percentage Inhibition
Control	10.50 ±1.99	0.53	-
10 mg/kg Imipramine	5.21 ± 1.71*	0.26	50.94%
25 mg/kg Imipramine	2.0 ±1.05*	0.10	81.13%*
40 mg/kg Imipramine	4.17 ±0.64*	0.21	60.38%*
20 mg/kg Omeprazole	2.54 ±1.18*	0.13	75.47%*

***Significant when compared with the control ($p< 0.05$) n=5*

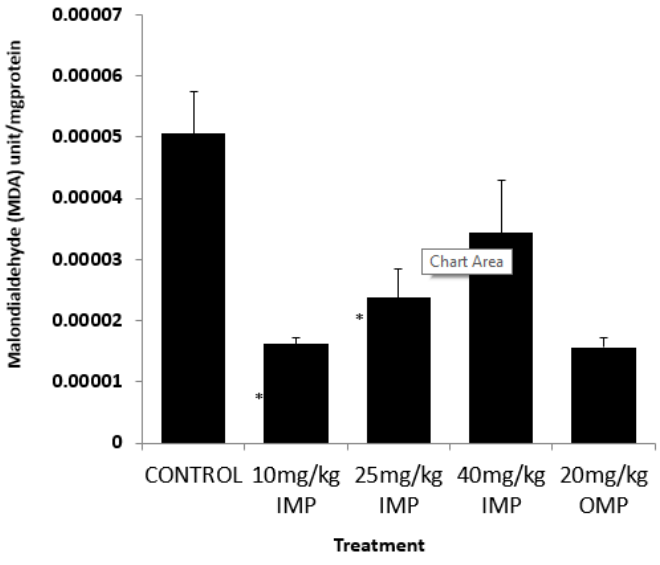

Fig. 2.

Effect of imipramine on Malondialdehyde activity.Values are expressed as mean ± S.E.M.(n=5).

*Significantly different when compared with the control at (P<0.05)

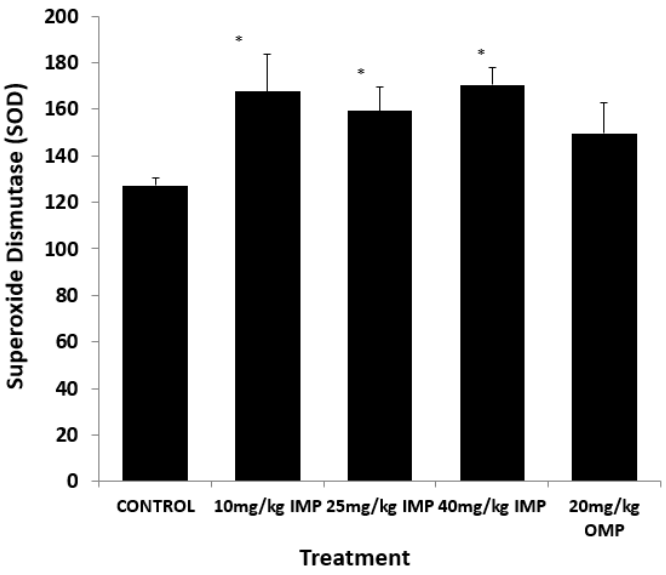

Fig. 3.

Effect of imipramine on Superoxide dismutase activity.Values are expressed as mean ± S.E.M.(n=5).

*Significantly different when compared with the control at (P<0.05)

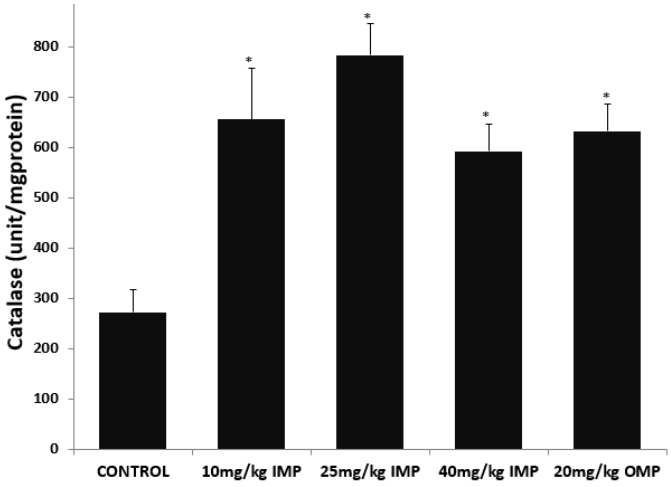

Fig. 4

Effect of imipramine on Catalase activity. Values are expressed as mean ± S.E.M.(n=5).

* Significantly different when compared with the control at (P<0.05)

DISCUSSION

This study was carried out to investigate the gastroprotective and antioxidant effects of Imipramine, a tricyclic antidepressant on indomethacin-induced gastric ulcer in male rats. Our data showed significant decrease in ulcer indices with the three doses of imipramine pretreatment. This is found to be consistent

with the report by Hamid and Taghi, (1997). The suggested mechanism had been attributed to reduction of gastric acid secretion (Roland *et al*., 1977; Bohman *et al*., 1978; Bohman *et al*., 1980; Leitold *et al*., 1984), which may be due to its anticholinergic properties that could cause an inhibition of gastric acid secretion (Snyder and Yamamura, 1977; Eberlein *et al*., 1987). These drugs have been shown to produce healing of duodenal ulcers in cases where cimetidine was not effective (Daneshmend *et al.,* 1981; Mangla *et al.,* 1982).

In the gastric acid secretory study, animals pretreated with 10 mg/kg imipramine showed significant reduction in basal acid secretion when compared to the control. This agrees with other reports where Imipramine was reported to suppress gastric acid secretion in pyloric ligated rats (Hernandez and Xue, 1989; Hernandez *et al*., 1990). Also, Sen *et al*., (2002) using amitriptyline, another member of TCAs showed significant reduction in gastric volume and total acidity in rats.

In our study with carbachol stimulation of gastric acid secretion, there was no change in the secretagogue secretory response to carbachol on gastric acid secretion with all doses administered. This suggests that imipramine did not inhibit carbachol-stimulated gastric acid secretion, but might be doing so via other receptors such as histamine (H_2-receptor) and gastrin (CCK-gastrin receptor). This conflicts with previously suggested cholinergic pathway involvement (Eberlein et al, 1987). Gastric acid secretion was observed to be higher at 25mg than at 40mg of imipramine. This may possibly be due to early and prompt sensitization of muscarinic receptors at lower dose. This would have activated maximally the peak response required during secretagogue pathway of carbachol

The antihistaminic inhibition hypothesis of gastric acid secretion was likely by the report of Batzri 1984; (1985) who showed that imipramine and amitriptyline inhibited the action of histamine on gastric cells isolated from rabbits and guinea pigs. Similarly, Batzri *et al*., (1988) equally revealed that subcutaneous administration of imipramine significantly inhibited histamine-stimulated gastric acid secretion when compared with the conventional antiulcer drugs; ranitidine and omeprazole. Several studies have equally shown that tricyclic antidepressants bring about antiulcer effect by reducing histamine secretion from mast cells thereby reducing gastric acid secretion, blocking Leukotriene receptors such as LTc_4, D_4 and E_4 (Hano *et al.,* 1978; Theoharides *et al*., 1982; Sen *et al*., 2002).

The results from this study showed that imipramine, at doses of 10 and 40 mg/kg significantly decreased parietal cell count when compared with the control. This further supports the fact that decreased acid output by the parietal cells in the animals pretreated with 10 mg/kg imipramine.

The results from gastric mucus secretion study revealed that oral administration of imipramine significantly increased the gastric mucus secretion, as well as mucus cell count at all doses used when compared with the control .This findings conform with the concept that increased amount of mucus secreted by the gastric mucosal cells prevents ulcer formation by acting as an effective barrier to the back-diffusion of hydrogen ions (Goel and Bhattacharya, 1991), improving the buffering of gastric juice, and reducing the stomach wall friction during peristalsis (Venables, 1986). 10 and 25 mg/kg Imipramine significantly increased mucus secretion and number of mucus cells when compared to the omeprazole group. This supports the point that one of the possible mechanisms of gastroprotection elicited by imipramine is as a result of enhancement of gastric mucosal defense barrier via improved gastric mucus secretion and gastric mucus cell count.

The study on mean ulcer score (MUS) showed that there is a significant reduction in MUS when compared with the control. Imipramine (25 mg/kg) showed the highest percentage inhibition (81.13%) of MUS. The gastroprotective nature of imipramine is in line with the earlier work by Hernandez and Xue, (1989; 1990) who reported that intraperitoneal administration of imipramine inhibited gastric lesions induced by cold restraint stress model, pyloric ligation model in rats and by intracisternal administration of Thyrotropin–releasing hormone (TRH) (TRH-induced gastric lesions). In the present study however, Imipramine 10 mg/kg though reduced MUS but the reduction in the MUS was not significantly different from the control group.

MDA is a compound useful as a biomarker of lipid peroxidation because it can be measured in body fluids (Michel *et al.,* 2008). Since reduction in MDA is beneficial to tissue protection, lower dose of imipramine is important in cytoprotection. This study showed a dose dependent decrease in MDA levels (Figure 2).This conforms to the work of Mokoena *et al.,* (2010) who reported a reversal in lipid peroxidation in the brain of rats when imipramine was administered. A reduction in MDA level in rats pretreated with Fluvoxamine and Opipramol, tricyclic antidepressants had been reported (Dursun, 2009). Also other antidepressants like tiyaneptine and mirtazapine have been found to exert

antioxidative effect (Kolla *et al*., 2005; Bilici *et al*., 2009; Suleyman *et al*., 2009)

This study revealed significant increase in the level of catalase activity in the animals pre-treated with imipramine when compared to the control group (Figure 4). Catalase enzyme speeds up the conversion of hydrogen peroxide (H_2O_2) to water (H_2O) (Cheesman and Slater, 1993). ROS is continuously generated by cells. To avoid exposure to higher levels of ROS leading to cellular oxidative stress, physiologically generated ROS are normally detoxified by cellular antioxidants which as Superoxide dismutase, catalase, Glutathione and thioredoxin. The increase in SOD level (Figure 3) also accounts for gastroprotective effect of Imipramine. The findings of this study suggest that imipramine possesses antiulcer activity via multiple mechanisms. Its anti secretory, cytoprotective and antioxidant properties should spring up thoughts about clinical application of Imipramine especially because it has been established that most peptic ulcer patients are depressed. Further studies are progressing in our laboratory focusing on the detailed mechanism of action of Imipramine on gastric ulcer.

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