Study of some physiological mechanisms mediating the cytoprotective effect of clarithromycin on induced gastric mucosal injury in rats

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ABSTRACT

Objective: *Helicobacter pylori is the main cause of gastritis, gastroduodenal ulcer and gastric cancer and should be considered as a major public health issue. According to several international guidelines first line therapy for treating Helicobacter pylori infection consists of the usage of macrolide antibiotic (clarithromycin) in combination with other anti secretory agents which has shown to be related to eradication of the microorganism. Although clarithromycin, has been used successfully with antiulcer agents to prolong duodenal ulcer remission it is not well known if it possess cytoprotective effects as well. The aim of the present study was to examine whether clarithromycin may have gastroprotective effect against 96% ethanol induced gastric lesion in rats and to elucidate the role played by opiate receptors, afferent sensory nerve fibers, α and β-adrenoceptores, endogeneous prostaglandins, sulfhydryls, fluid volume and mucous volume retained in the gastric lumen, in the mechanism of protection offered by intragastric clarithromycin against ethanol-induced mucosal injury.* ***Methods:*** *Gastric mucosal lesions were induced by 96% ethanol in rats, then the effect of intragastric clarithromycin (in a doserange: 50-400 mg/kg b.wt.) on the ethanol-induced lesion was studied. The effect of blochage of opiate receptors was studied using opiate receptor blocking agent naloxone (8 mg/kg.b.wt. intraperitoneal),denervation of the sensory afferent nerves was done by usage of capsaicin (125 mg/kg b. wt. Subcutaneous),the effect of α adrenergic receptor was done by using α1 adrenergic receptor antagonist prazosin (0.5 mg/kg b. wt. subcutaneous), while the effect of α 2 adrenergic receptor was examined by usage of α 2 adrenergic receptor antagonist yohimbine (5 mg/kg b. wt. subcutaneous), the influence of β1 adrenoceptores was tested by using β1 adrenoceptores antagonist metoprolol (2 mg/kg b. wt. intraperitoneal), while the effect of β 2 adrenoceptores was done by using of β 2 adrenoceptores bloker butoxamine (4 mg/kg b. wt. intraperitoneal), the effect of endogenous prostaglandins was assassed by application of cycloxygenase inhibitor indomethacin (5mg/kg b. wt. subcutaneous) and sulphhydryls blocking agent is used (iodoacetamide) in a dose of (100 mg/kg. b.wt. subcutaneous). In addition, the effect of clarithromycin on the volume of gastric content was also investigated. Each study was carried out using six rats per group.* ***Results:*** *It has been found that intragastric administration of clarithromycin protected the rat gastric mucosa against 96% ethanol-induced lesion in a dose dependent manner. The inhibition of lesions was 31.86, 51.33, 79.65 and*

91.15% at doses of 50, 100, 200 and 400 mg/kg b.wt. respectively. The gastroprotective effect of clarithromycin was not significantly modified by pretreatment with either opiate receptor blocking agent; or sensory nerve fiber denervation. Subcutaneous pretreatment of rats with α1 blocker or intraperitoneal pretreatment with β1 or β2 blocker did not significantly modify the gastroprotective effect of clarithromycin, however, clarithromycin protection was significantly diminished, although not completely abolished by subcutaneous α 2 blocking agent. Clarithromycin protection was not significantly modified by pretreatment with either subcutaneous cycloxygense inhibitor, or sulfhydrls blocker. In addition there was a dose dependent increase in fluid volume for clarithromycin and in the mucous volume at 100, 200 and 400 mg/kg b. wt. of clarithromycin at 30 min. α 2 blocking agent significantly reduced both basal and clarithomycin-stimulated gastric mucous secretion. ***Conclusion:*** *It could be concluded that the mechanism mediating the intragastric clarithromycin protective effect against 96% ethanol induced mucosal lesion is independent of opiate receptors, capsaicin-sensitive afferent sensory nerve fibers, α1, - β1-,β2-adrenoceptors, endogenous prostaglandins, and sulfhydryl compounds of the gastric mucosa. However, the increase in luminal gastric mucous and fluid volume may contribute to the protective effect of intragastric clarithromycin against 96% ethanol-induced gastric lesion, α2-adrenoceptors possibly are involved in such protection by a mucous dependent mechanism.*

INTRODUCTION

The remarkable resistance of the mucosa of the upper gastrointestinal tract to concentrated gastric acid remains one of the biggest unsolved mysteries of upper gastrointestinal physiology[(1)]. It is assumed that an overproduction of gastric acid is the most important factor in the development of peptic ulcer, however it has also been demonstrated that gastric defense mechanisms which prevent mucosal injury are enhanced by same factors that increase acid secretion[(2).]

Helicobacter pylori is the main cause of gastritis ,gastroduodenal ulcer and gastric cancer and should be considered as a major public health issue .According to several international guidelines first line therapy for treating Helicbopacter pylori infection consists of the usage of the macrolide antibiotic clarithromycin in combination with antisecretory agents usually proton pump inhibitor or H_2 receptor antagonist. Clarithromycin decreases the relapse of duodenal ulcer, and eradicates Helicobacter pylori from the gastric mucosa[(3,4)]. However, it is not well known if clarithromycin does have any cytoprotective effect against necrotizing agents on gastric mucosa[(3,4)].

A systemic evaluation of the various gastric protective mechanisms indicates that, a number of mechanisms have been postulated to play a role in defending the gastric mucosa against injury by noxious agents. These include: stimulation of opiate receptors,[(5)] activation of capsaicin-sensitive afferent sensory nerve fibers,[(6)] enhancement of endogenous prostaglandins[(7)] &

sulfhydryl agents,[7,8] increase in mucosal blood flow,[9] stimulation of mucous synthesis [10] and increase in gastric fluid volume and hence dilution of the injurious agent.[11]
Moreover, the histochemical studies have demonstrated adrenergic innervation of the gastric mucosa in rat and guinea pigs.[12] Stimulation of α- or β-adrenoceptores has been demonstrated to play a role in defending the gastric mucosa against injury by noxious agents and stress.[13,14] The present study was undertaken to examine whether clarithromycin may have any gastroprotective effect against 96% ethanol induced gastric mucosal lesions in rats, and to test the hypothesis that one or more of the following may mediate the gastric protection induced by intragastric administration of clarithromycin:

1- Activation of opiate receptors.
2- Stimulation of capsaicin-sensitive afferent sensory fibers.
3- Activation of α & β-adrenoceptores.
4- Synthesis of endogenous prostaglandin.
5- Synthesis of endogenous sulfhydryls.
6- Increase in mucous and fluid volume retained in the gastric lumen at the time when ethanol is administrated.

Thus, the aim of the present study was focused on the underlying mechanisms related to the possible cytoprotective effect of intragastric clarithromycin against the damage induced by 96% ethanol in rats other than its antibacterial action.

METHODS

Male albino rats weighing 180-220 gm, aging six months were utilized for this study. The animals were kept in cages with wide meshed galvanized wire bottoms to decrease coprophagy as much as possible. The rats were fasted for 24 h before the experiments but water was allowed adlibitum. Each study was carried out using six to eight rats per group.

Ethanol-induced gastric mucosal lesions[15,16]

Gastric lesions were induced by the oral administration of 96% ethanol (by a gavage needle, 1 ml/rat) one hour later the animals were sacrificed. The stomachs were removed, opened along the greater curvature, stretched out and fixed into cardboard with insect pins. The luminal debris was washed off with saline. The mucosal injury was scored on the basis of lesion diameter according to Abou Zeit Har *et al*.[17]

In this injury study, only gross lesions were assessed, because it has been demonstrated that there is a significant linear correlation between the extent of gross and histologic deep necrotic lesions induced by ethanol in the gastric mucosa.[18]

Study I: Effect of intragastric clarithromycin (at different doses) on gastric mucosal injury induced by 96% ethanol:

Six groups of rats were used (6 rats each): Group 1 (control): received intragastric vehicle (distilled water, 10 ml/kg b. wt.). Groups 2, 3, 4, 5: calrithromycin was given intragastricaly at 400, 200, 100, and 50 mg/kg b. wt.[15] Group 6:

calrithromycin was given subcutaneously at 400 mg/kg b. wt.[15] Calrithromycin was given half an hour before 96% ethanol (10ml/kg b. wt. administration in all groups).

One hour after ethanol administration, the rats were sacrificed and examined as mentioned before for evaluation of lesions.

Study II: Effect of blockade of opiate receptors on clarithromycin protective effect (Naloxone study):

A specific antagonist of μ, k and δ opiate receptors naloxone hydrochloride was dissolved in deionized water.[19] Rats were divided into four groups: Group 1 and 2 corresponded to strict controls and they received vehicle (deionized water2 ml/kg b. wt) intraperitoneally. Group 3 and 4 received opiate receptor antagonist 8 mg/kg b. wt. intraperitoneally[20]. After half an hour, group 1 and 3 were treated with vehicle (intragastric deionized water) and group 2 and 4 were treated with clarithromycin (groups 2 and 4 were subdivided into two subgroups (n=6 each) to allow for intragastric use of clarithromycin at 100 and 400 mg/kg b. wt.) after another one hour, 96% ethanol was administered intragastrically to all the rats. One hour after ethanol treatment, rats were sacrificed and the gastric mucosal lesions were examined as described in study. I.

Study III: Effect of denervation of capsaicin afferent sensory nerve fibers on clarithromycin protective effect (capsaicin study):

Capsaicin was dissolved in the vehicle which consisted of 10% ethanol and 80%saline[20]. All rats received a total dose of 125 mg/kg b. wt. of capsaicin subcutaneously over two days[20]. After confirming the functional denervation of the capsaicin-sensitive afferent sensory fibers (by the eye test)[21]. The injury study was carried out (after 10 days) in these sensory denervated and control rats using the same design as in the pervious study (naloxon study).

Study IV: Effect of blockade of α adrenoceptors on clarithromycin protective effect:

This study was performed to test the effect of blockade of α_1 and α_2 adrenoceptors by prazosin or yohimbine, respectively, on the intragastric clarithromycin protection against 96% ethanol-induced gastric mucosal injury. The doses of α adrenoceptors blocking agents which were chosen in this study had been shown previously to block α_1 and α_2 adrenoceptors[22,23]. Rats were divided into six groups. Groups 1 and 2 were controls, and they received control pretreatment (deionized water 5 ml/kg b. wt. subcutaneously).

Groups 3 and 4 were pretreated with α_1 blocking agent (0.5 mg/kg b. wt., 5 ml/kg b. wt. subcutaneously)[22]. Groups 5 and 6 pretreated with α_2 bloking agent (5 mg/kg b. wt., 5 ml/kg b. wt. subcutaneously)[23].

After 30 min., rats in groups 1, 3, 5 were treated with vehicle (deionized water 10 ml/kg b. wt. intragastric) and rats in groups 2, 4, 6 were treated with clarithromycin intragastrically (groups 2, 4 and 6 were subdivided into subgroups to allow for the use of clarithromycin at doses of 100 and 400 mg/kg b. wt, n=6 for each dose). After another hour, 96% ethanol (10 ml/kg b. wt.) was administered

intragastrically to all rats. One hour later, the rats were sacrificed and examined as mentioned before for evaluation of lesions.

Study V: effect of blockade of β adrenoceptors on clarithromycin protective effect:

A selective β_1-adrenoceptor antagonist[24]. Metoprolol tartrate, or a selective β_2 adrenoceptor antagonist[25] butoxamine hydrochloride, was dissolved in deionized water at a concentration of 1 or 2 mg/ml respectively. Rats were divided into six groups: group 1 and 2 received control treatment (deionized water 2 ml/kg intraperitoneally). Groups 3 and 4 received pretreatment with β_1-adrenoceptor antagonist (2 mg/kg b. wt., 2 ml/kg b. wt. intraperiloneally).[24] Groups 5 and 6 were pretreated with β_2 adrenoceptor antagonist (4 mg/kg b. wt., 2 ml/kg b. wt. intraperitoneally).[26]

Subsequent procedures were similar to those in the previous study, after pretreatment with the β adrenoceptor antagonists.

Study VI: Effect of a cyclooxygenase inhibitor (indomethacin) and sulfhydryl blocker (iodoacetamide) on clarithromycin protection:

Gastric endogenous prostaglandins (PGs), and sulfhydryl compounds were postulated to be involved in the mechanism of mild irritant or other agents.[8,27] Therefore, the participation of PGs and sulfhydryls in the mechanism of the protective effect of clarithromycin was examined. Three groups of rats were used: group 1: the vehicle was given to the control rats subcutaneously (10 ml/kg b. wt.). Groups 2 and 3: either cycloxygenase inhibitor or sulfhydryl bloker dissolved in distal water) was given subcutaneously at 5 or 100 mg/kg b. wt. respectively in a volume of 10 ml/kg b. wt. One hour later, 96% ethanol was given intragastrically and the animals were sacrificed 60 min. later. Clarithromycin (100, 400 mg/kg b. wt.) was given intragastrically half an hour before ethanol.

Groups 2 and 3 were subdivided into two subgroups (n=6 each) to allow for the administration of the two doses of clarithromycin (100 and 400 mg/kg b. wt.).

Study VII: Effect of clarithromycin on the volume of gastric content:

This study examined the effect of intragastric clarithromycin on gastric fluid volume and gastric mucous volume retained in the gastric lumen.[20] In this study, rats were divided into five groups treated with vehicle or clarithromycin at different doses as in study I. Thirty min. later, rats were sacrificed (ethanol was not administered). After laparotomy, the pylorus and the esophagogastric junction were ligated and the stomach was removed. Gastric content was gently expressed from the stomach via incision made in the fore stomach by pressing the stomach between cotton tip applicator and the wall of a plastic funnel and letting the gastric juice flow into the graduated test tube. The volume of the gastric juice to the nearest 0.01 ml was measured. The gastric mucous volume to the nearest 0.01 ml was also assessed by placing the mucous in a 1 ml graduated syringe[28]. All volume measurements were confirmed by an unbiased observer who was unaware of the treatment.

Study VIII: Effect of varying gastric fluid volume on 96% ethanol-induced lesions:

It was found that 30 min. after administration of vehicle or clarithromycin 100 or 400 mg/kg b. wt., the vehicle group had about 51 µl gastric fluid, the clarithromycin (100 mg/kg b. wt.) group had 396.5 µl of gastric fluid and the clarithromycin (400mg/kg b. wt.) group had 840 µl of gastric fluid retained in the stomach. Accordingly the animals were divided into three groups. Group 1 received 60 ul, group 2 received 400 ul, group 3 received 900 ul of vehicle (distilled water) intragastrically immediately before treatment with 96% ethanol (gavage needle, 1 ml/rat) one hour later, the animals were sacrificed and the gastric lesions were evaluated as indicated in study I.

Study IX: The effect of clarithromycin on gastric mucous volume, gastric juice volume and titratable acid in gastric juice after subcutaneous α_2-blockade.

The animals were divided into four groups. Groups 1 and 2 received distilled water, subcutaneoulsy (10 ml/kg b. wt.). Groups 3 and 4 received α_2-blocker (5 mg/kg b. wt. (10 ml/kg b. wt.) subcutaneously after 30 min, animals in groups 1 and 3 were treated with vehicle (distilled water 10 ml/kg b. wt. intragastric) and animals in groups 2 and 4 were treated with clarithromycin (400 mg/kg b. wt., 10 ml/kg b. wt intragastric). After another 30 min, the animals were killed. The volume of gastric mucous and the volume of gastric fluid were measured separately as indicated in study VII. Acid content in the gastric juice was determined by titration of aliquots of the gastric juice with 0.1 N NaOH to pH 7.0. Total acid output was then calculated in units of micro equivalents.[28]

Statistical Analysis

Student's t test or Anova test (F-test) and LSD test were used for the evaluation of statistical significance. Differences were considered significant at $p<0.05$ level. Values were expressed as mean ± S.D.[29]

RESULTS

Effect of intragastric clarithromycin at different doses on gastric mucosal lesion induced by 96% ethanol

- Intragastric 96% ethanol produced 100% induction of ulcer in the used rats with a mean ulcer score of 18.83±1.16 (Fig.1)
- Intragastric clarithromycin produced a significant protection against ethanol-induced ulcer in a dose dependent manner with a protective index of 31.86, 51.33, 79.65,and 91.15% at doses of 50, 100, 200, 400 mg/kg b.wt. respectively.
- On the other hand, the lesions score was not significantly changed as compared with that of the control when clarithromycin at (400mg/kg b. wt.) was given subcutaneously (18.500±1.048 vs 18.66±1.211 $p> 0.05$) (Fig. 1).

Effect of blockade of opiate receptors on clarithromycin protection (Naloxone study):

As presented in Fig. 2, despite the blockade of the opiate receptors by intragastric clarithromycin at doses of 100, and 400 mg/kg b.wt. produced a significant reduction in the lesion

score (9.166±0.752 and 1.500±0.547 respectively vs 19.000, $p< 0.05$). These data indicated that pretreatment with opiate blocker did not abolish the protective effect of the intragastric clarithromycin against ethanol-induced gastric mucosal lesion (Fig. 2).

Effect of denervation of capsaicin afferent sensory nerve fibers on clarithromycin protection (Capsaicin study)

- All the capsaicin treated rats failed to show the wiping response, indicating that the pretreatment was effective in functionally denervating the afferent sensory fibers.
- Despite denervation of capsaicin afferent sensory nerve, intragastric clarithromycin at doses of 100 and 400 mg/kg b.wt. produced a significant reduction in lesion score as compared to the vehicle value (9.00±0.752 and 1.667±0.816 respectively vs 19.000, $p< 0.05$). Thus, denervation of capsaicin afferent sensory nerve did not abolish the protective effect of intragastric clarithromycin at low and high doses.(Fig. 3)

Effect of blockade of α-adrenoceptors on clarithromycin protection:

- In the control rats the lesion scores in the clarithromycin groups were (at 100 and 400mg/kg b. wt.) significantly lower than that in the vehicle group.(Fig. 4)
- In rats pretreated with α_1-adrenoceptor blocker, the lesion score in clarithromycin groups was significantly lower than that in the vehicle group and both of them showed insignificant change compared to the respective control.(Fig. 4)
- In the α_2-blocker pretreated rats although the lesion score in the clarithromycin groups (100-400 mg/kg b. wt.) was significantly lower than that in the vehicle group, they were significantly higher than those of the clarithromycin groups (16.166±0.752 and 14.000±0.894 vs 9.166±0.752 and 1.66±0.816 respectively, $p< 0.05$) in controls not given α_2-blocker pretreatment.(Fig. 4)
- There was insignificant difference in the lesion scores (19.000±0.894 vs 18.833±1.1690, $p> 0.05$) between the vehicle-treated rats with α_2-blocker pretreatment and the vehicle-treated rats in the control group, indicating that α_2-blocker alone did not aggravate the lesions.(Fig. 4)

Effect of blockade of β-adrenoceptors on clarithromycin protection:

- The lesion score in the clarithromycin groups (not given any β-adrenoceptors antagonist) were significantly lower than that in the vehicle group.(Fig. 5)
- In rats pretreated with either β_1 or β_2 adrenoceptor blocking agents, the lesion scores in the clarithromycin groups were significantly lower than those in the respective vehicle groups. This indicated that pretreatment with β_1 or β_2 antagonist did not abolish the protective effect of intragastric clarithromycin against 96% ethanol-induced gastric mucosal injury. (Fig. 5)

Effect of cycloxygenase inhibitor and sulfhydryle blocking agent on clarithromycin protection:

Subcutaneous administration of cycloxygenase inhibitor (5mg/kg b. wt.) or sulfhydryle blocking agent (100mg/kg b. wt.) alone did not significantly modify the gastric lesions score induced by 96% ethanol. The protective effect of clarithromycin intragastrically administered at 100 or 400 mg/kg b. wt. was not affected by pretreatment with cycloxygenase inhibitor or sulfhydryle blocking agent (Fig. 6). These data indicated that these agents did not suppress the protective effect of intragastric clarithromycin at low or higher doses against ethanol-induced lesions. (Fig. 6)

Effect of intragastric clarithromycin on volume of gastric content:

- Clarithromycin in a dose dependent manner increased the fluid volume retained in the gastric lumen. Rats treated with clarithromycin at doses of 50, 100, 200, 400g/kg b. wt. had a significantly higher fluid volume as compared to the vehicle control groups (Table I). Rats treated with clarithromycin at 400 mg/kg b. wt. had a higher fluid volume than rats treated with clarithromycin at doses of 50, 100 and 200 mg/kg b. wt. (Table I).
- The gastric mucous volume in the rats treated with clarithromycin at doses of 100, 200 and 400 mg/kg b. wt. were significantly higher than that in the rats treated with vehicle (Table I).
- These results indicated that intragastric clarithromycin dose-dependently increased gastric fluid volumes and that there was a significant increase in gastric mucous volume for clarythromycin 100, 200 and 400 mg/kg b. wt.

Effect of varying gastric fluid volume on 96% ethanol induced lesion:

It was found that half an hour after the administration of vehicle or clarithromycin at doses of 100 or 400mg/kg b. wt., the gastric fluid retained in the stomach were 51.98 ± 21.02, 369.50 ± 3.21 and 840.50 ± 4.32 µl respectively. Accordingly, 60, 400, 900 µl of vehicle (distilled water) was instilled into the rat stomach immediately before ethanol. It was found that the lesion score in the rats treated with 60 µl of vehicle was significantly higher than that in the rats treated with 400 µl of vehicle and in rats treated with 900 µl of vehicle. This indicated that the higher gastric fluid volume retained in the gastric lumen of rats treated with different doses of clarithromycin accounted for the reduction in lesion.(Fig. 7)

The effect of clarithromycin on gastric mucous volume, gastric juice volume and titratable acid in gastric juice after subcutaneous α_2-blocker:

- It was found that both in the control or α_2 blocker-pretreated rats, the gastric mucous volumes in the clarithromycin group were significantly higher than those in the respective vehicle groups (control groups 222.00 ± 6.0992 µl vs 80.667 ± 5.921µl ,α_2 blocker pretreated groups 110.666µl ± 3.2660µl vs 57.666 ± 4.501µl respectively, $p<0.05$). However, gastric mucous volumes in the, α_2 blocker - pretreated animals were significantly lower than those of

the respective controls (vehicle: 57.666 ± 4.50 µl vs 80.667 ± 5.9 µl, clarithromycin (400mg/kg b.wt.): (110.667 ± 3.266 µl vs 222.00 ± 6.099 µl, $p< 0.05$). These results indicated that ,α_2 - blocking agent significantly reduced basal and clarithromycin stimulated gastric mucous secretion.(Fig. 8)

- The gastric juice volumes both in the control or ,α_2 blocker - pretreated rats in the clarithromycin groups were significantly higher than those in the respective vehicle groups (control: 0.6950±8.044 vs 0.1683±1.472 ml, ,α_2 blocker pretreated group: 0.706±1.966 vs 0.2150 ml, $p<0.05$). These results indicates that ,α_2 blocker pretreatment did not abolish the increase in gastric juice volume induced by intragastric clarithromycin and that the reduction in the protective effect of intragastric clarithromycin by, α_2 blocker was not related to an effect on gastric juice volume.(Fig. 9)
- There was no significant difference in titratable total acid in the gastric juice in the control rats (vehicle treated vs clarithromycin treated, 3.918 ± 0.4504 vs 4.9500 ± 0.3728 µeq. respectively, $P>0.05$) or the, α_2 blocker pretreated rats (vehicle vs clarithromycin treated, 14.303 ± 0.4683 vs 15.4000 ± 0.7616 µeq. respectively, $p>0.05$). Gastric acid output values were significantly higher in the, α_2 blocker pretreated rats than those in the respective controls.(Fig. 10).

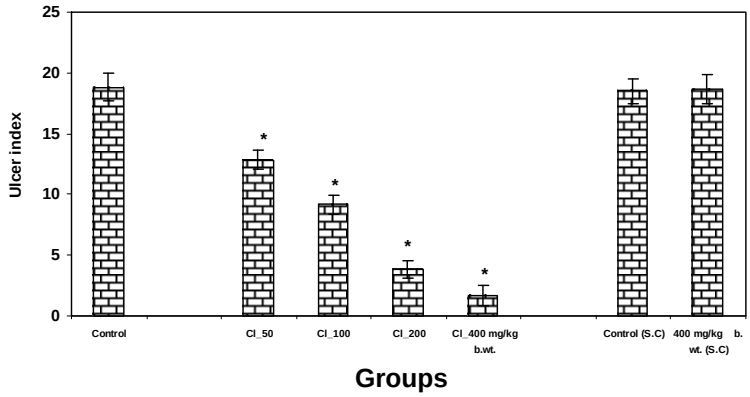

Fig. (1): Effect of clarithromycin (Cl) given either intragastrically or subcutaneously on 96% ethanol induced gastric lesion in rats. Cl was given 30 min. before ethanol administration and the rats were sacricified 1hr after ethanol administration. Data represent the mean ± SD (n = 6 rats/group).
* Statistically significant as compared to the control at $p < 0.05$.

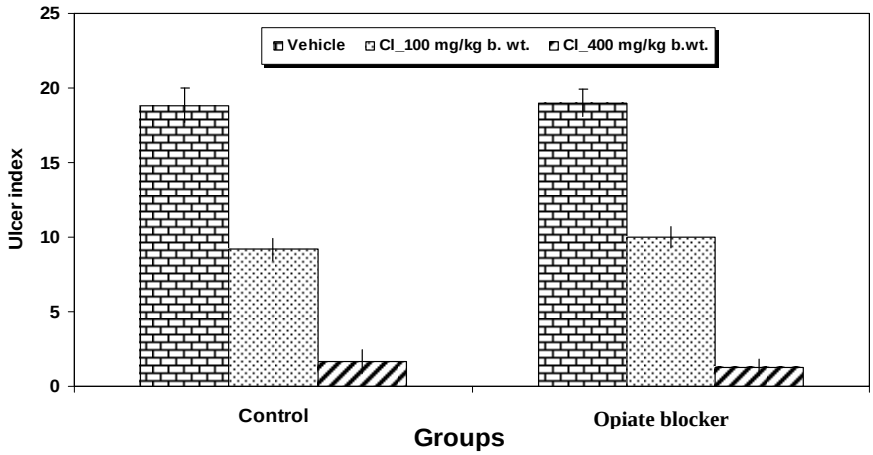

Fig. (2): Effect of blockade of opiate receptors by naloxone on the protective effect of intragastric clarithromycin against 96%ethanol induced gastric mucosal lesion. Thirty min prior to the injury study rats were pretreated with intraperitoneal deionized water (control) or opiate blocker (8mg/Kg b. wt.). The rats in each group were then given either intragastric vehicle or clarithromycin at 100 and 400 mg/Kg followed by 96% ethanol. Data represent the mean ± SD (n = 6 rats/group).

***** Statistically significant from the vehicle at $p < 0.05$.

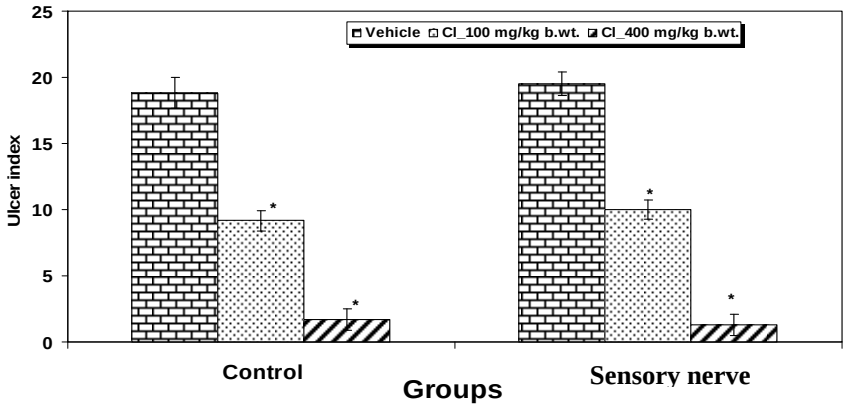

Fig. (3): Effect of sensory denervation by capsaicin on the protective effect of intragastric clarithromycin against 96% ethanol induced gastric lesion in rats. Capsaicin was given in a dose of 125 mg/Kg S.C 10 days prior to the injury study. Data represent the mean ± SD (n = 6 rats/group).

* Statistically significant from the vehicle at $p < 0.05$.

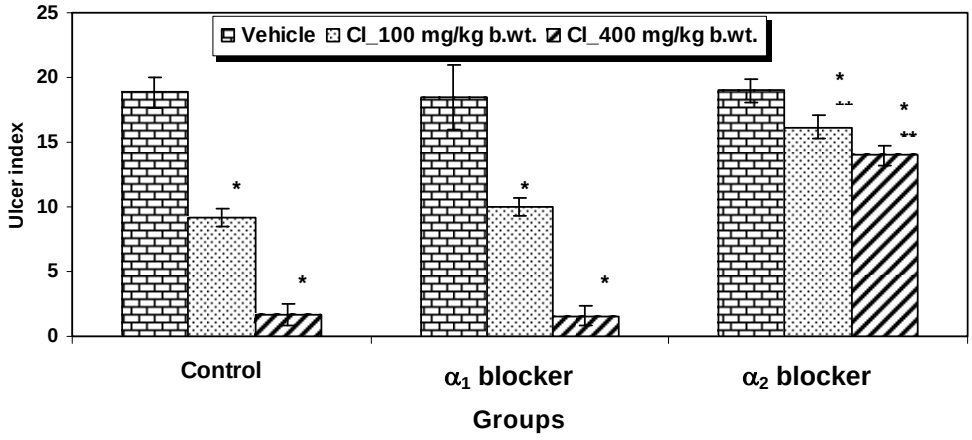

Fig. (4): Effect of blockade of α adrenoceptors by α_1 or α_2 blocker on the protective effect of intragastric clarithromycin against gastric mucosal lesions induced by 96% ethanol. Thirty min prior to the injury study rats were retreated with α_1 blocker (0.5 mg/Kg S.C) or α_2 blocker (5 mg/Kg S.C) or vehicle (5 ml/Kg). The rats in each group were then given either intragastric vehicle (10 ml/Kg) or clarithromycin (100 and 400 mg/Kg, 10 ml/kg) followed by 96% ethanol 10 ml/Kg intragastric). Results are expressed as mean ± SD (n = 6 rats/group).

* Statistically significant from the vehicle at $p < 0.05$

** Statistically significant from respective control at $p < 0.05$

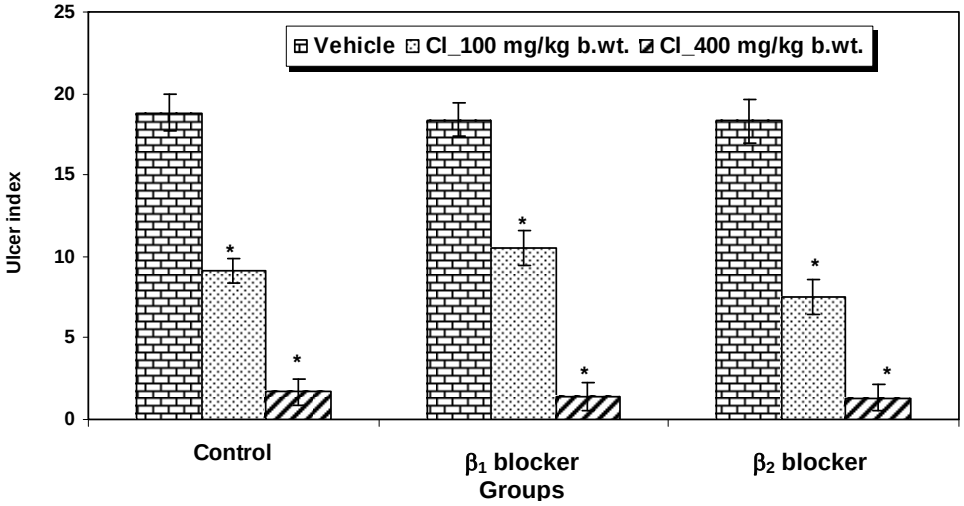

Fig. (5): Effect of blockade of β--adrenoceptors by β_1 or β_2 on the protective effect of intragastric clarithromycin against mucosal lesions induced by 96% ethanol. Thirty min. prior to the injury study rats were pretreated with intraperitoneal vehicle (2ml/Kg) or β_1 blocker (2mg/Kg) or β_2 blocker(4mg/Kg). The rats in each group were then given either intragastric vehicle or clarithromycin (400 and 100 mg/Kg, 10 ml/Kg) followed by 96% ethanol. Results are expressed as mean ± SD (n = 6 rats/group).

* Statistically significant as compared to vehicle.

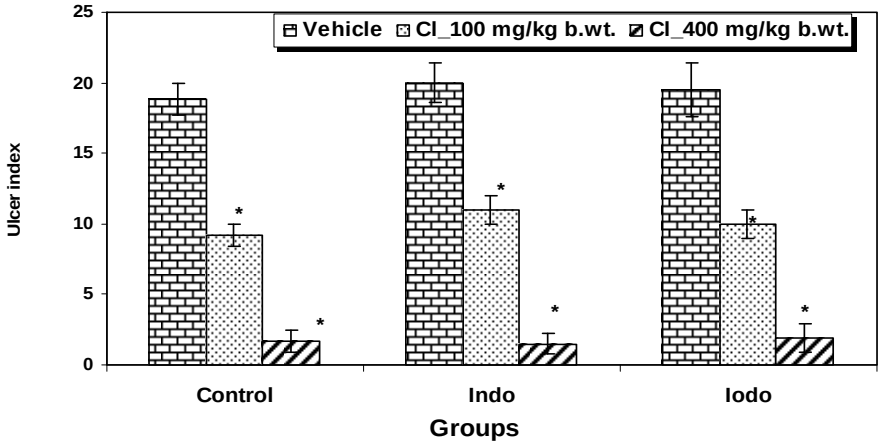

Fig. (6): Effects of pretreatment with cycloxygenase inhibitor or sulfhydryle blocking agent on clarithromycin (Cl) protection against mucosal injury induced by 96% ethanol. Subcutaneous cycloxygenase inhibitor (5 mg/Kg) or sulfhydryle blocking agent (100 mg/Kg) was given 1 hour before ethanol administration. Cl was given intragastrically 30 min before ethanol administration. Data represent the mean ± SD (n = 6 rat/group).

* Statistically significant from vehicle at $p < 0.05$

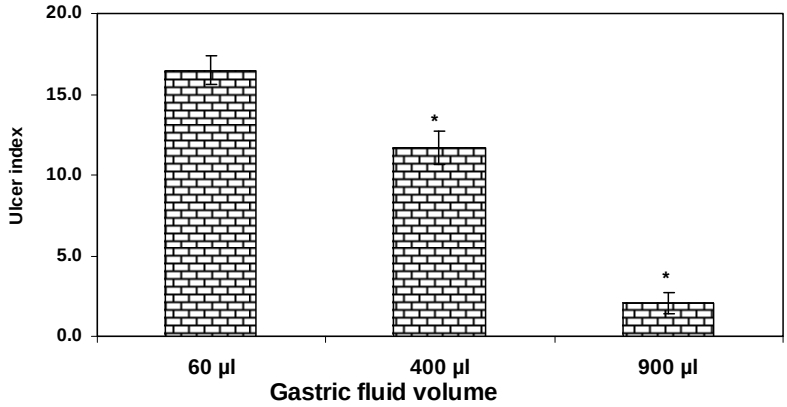

Fig. (7): Effect of varying gastric fluid volume on gastric mucosal lesion induced by 96% ethanol.
* Significantly different as compared to the first column, $p < 0.001$.

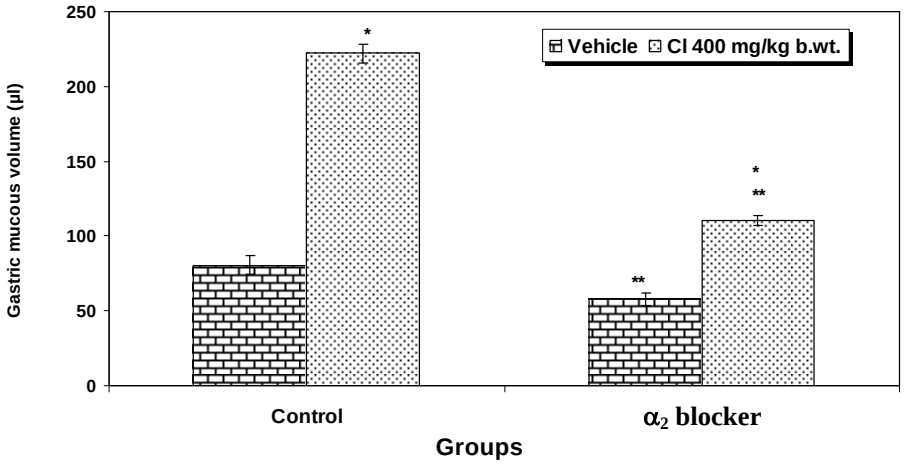

Fig. (8): Effect of vehicle or clarithromycin (Cl) on gastric mucous volume in control and α_2 blocker pretreated rats. Thiry min after subcutaneous distilled water (control 10 ml/Kg) or α_2 blocker (5mg/Kg) rats were treated with intragastric vehicle (10 ml/Kg) or Cl (400 mg/Kg, 10 ml/Kg). Thirty minutes later, gastric mucous was collected and its volume was measured. Values are expressed as mean ± SD (n = 6 rats/group).
* Significantly different from the vehicle at $p < 0.05$.
** Significantly different from the control at $p < 0.05$.

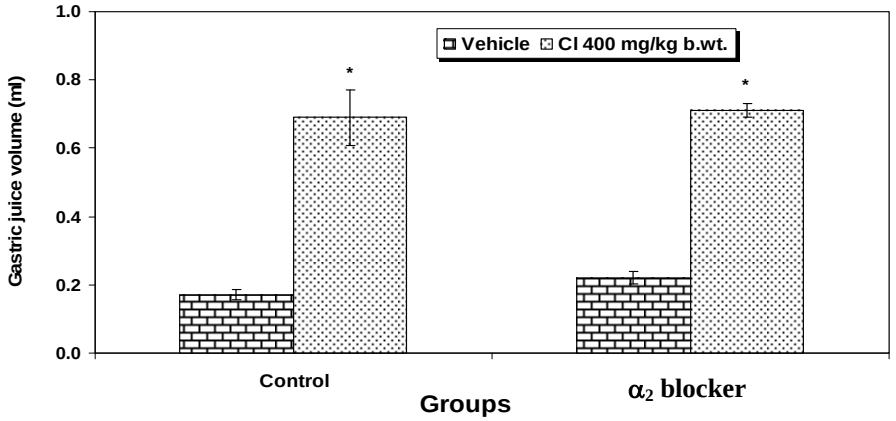

Fig. (9): Effect of vehicle or clarithromycin (Cl) on gastric juice volume in control and α_2 blocker pretreated rats. Thirty minutes after subcutaneous distilled water (control, 10 ml/Kg) or α_2 blocker (5 mg/Kg, 10 ml/kg), rats were treated with intragastric vehicle (10ml/Kg) or Cl (400 mg/Kg, 10 ml/Kg). Thirty min. later, gastric juice was collected and its volume was measured. Values are expressed as mean ± SD (n= 6 rats/group).
* Significantly different as compared to vehicle.

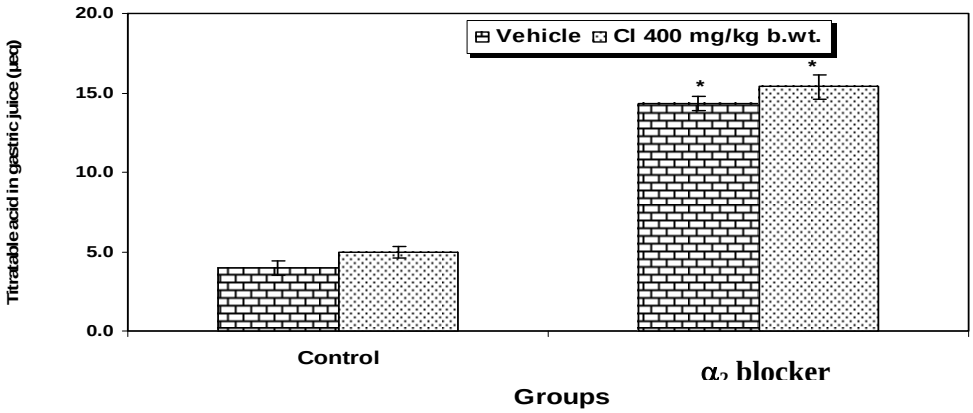

Fig. (10): Effect of intragastric vehicle or clarithromycin (Cl) on the titrable acid in gastric juice in control and α_2 blocker pretreated rats. Rats were pretreated with subcutaneous distilled water (control, 10ml/Kg or α_2 blocker 5 mg/Kg, 10 ml/Kg). Thirty min. later the rat were treated with intragastric vehicle (10 ml/Kg) or Cl (400 mg/Kg, 10 ml/Kg). Thirty min. later, gastric juice was collected and its volume was measured using graduated test tube. Acid content in the gastric juice was determined by titration of aliquots of the gastric juice (0.03-0.1ml depending on total acid output) with 0.1N NaOH to pH 7.0. Values represented as mean ± SD (n= 6 rats/group)
* Significantly differnt as compared to the respective control.

DISCUSSION

The discovery of the Helicobacter pylori has changed our understanding of the pathophysiology of peptic ulcer disease.An estimated one billion people harbour the organism worldwide but the highest prevelance is found in developing countries with up to 80% of people infected.The eradication of Helicobacter pylori is now a very important goal of treatement of gastric and duodenal ulcers.Most eradication regimens combine anti-secretory agents,usually a proton pump inhibitor or H 2 antagonist and antibiotic clarithromycin[30]. The present study demonstrated that intragastric administration of clarithromycin protected the rat gastric mucosa against ethanol-induced lesions in a dose dependent manner. These results are consistent with data of other investigators[15,31] who have found that intragastric administration of clarithromycin protected against indomethacin and ethanol induced gastric lesion. On the other hand, the present findings showed that subcutaneous administraton of clarithromycin (400 mg/kg b. wt.) did not have any significant effect on rat gastric mucosal lesion induced by ethanol, thus it seems clear that clarithromycin need to be placed in direct contact with the gastric mucosa to exert its action. These results are in accordance with the results of other studies[15,31] that provided an evidence that only the intragastric administration of clarithromycin substantially decreased the damaging effect of ethanol on gastric mucosa. It has been reported that intragastric clarithromycin may act as a mild irritant and protect the gastric mucosa against ethanol damage through adaptive protection[32]. However, this possibility seems to be unlikely since intragastric clarithromycin (400 mg/kg b. wt.) did not induce any gross mucosal damage.

The current study aimed to investigate the mechanism underlying the protective effect of clarithromycin against ethanol-induced gastric damage. In this study, the protective effect was observed when clarithromycin (50-400 mg/kg b. wt.) was given intragastrically, only once before ethanol and these doses were reported to be more than the doses of clarithromycin prescribed clinically for Helicobacter pylori eradication[1,2] .Moreover, previous studies have demonstrated that one to two weeks of clarithromycin, amoxicillin and omeprazole are effective treatment for Helicobacter pylori eradication[1,2,33]. Satoh *et al*[34] have demonstrated that antibiotic prevented indomethacin-induced gastric lesions by a protective mechanism other than its antibacterial action. Thus it could be suggested that clarithromycin prevented ethanol-induced gastric lesions by a mechanism other than its antibacterial action.

The next series of experiments were performed to investigate the mechanism by which clarithromycin protects the gastric mucosa against ethanol-induced lesions. The present study was tried to test the hypothesis that one or more of the following factors may mediate the gastric

protection induced by intragastric administration of clarithromycin:
(1) Activation of opiate receptors (2) Stimulation of capsaicin-sensitive afferent sensory nerve fibers (3) Activation of α & β-adrenoceptores (4) Synthesis of endogenous prostaglandin or endogenous sulfhydryls (5) Increase in the mucous and fluid volume retained in the gastric lumen at the time when ethanol is administrated.
Opiate receptors and endogenous opioid peptides are present in various parts of the gastrointestinal system in man and in animals[35-37]. Opiate receptors activation by morphine was reported to reduce gastric mucosal damage induced by cold restraint,[38] intragastric HCl or NaOH[5]. Naloxone, a specific opiate receptor antagonist abolishes such protection[38,39].

In the present study, naloxone was administrated in a dose of 8 mg/kg b. wt. intraperitoneal (to block the opiate receptor mechanism) and this dose was twice the dose used in the study of Glavim *et al.*[38]. The lesions were not worsened by opiate blocker treatment. The protective effect of intragastric clarithromycin was not abolished. This indicated that opiate receptor mechanism may not be involved in the protective effect of intragastric clainthromycin against ethanol-induced gastric mucosal injury. Endoh *et al.*[20] have also shown that the gastroprotective effect of intragastric nicotine against ethanol-induced gastric injury was not abolished by pretreatment with opiate blocker, indicating that opiate receptors may not be instrumental in the protective effect of a number of agents administrated intragastrically.

The afferent sensory nerve fibers mediate gastric mucosal protection.[6] Capsaicin, the major pungent ingredient of hot peppers,[40] has been used as probe to study such a protective mechanism. It was reported that after acute oral administration, low doses of capsaicin protect the rat gastric mucosal against mucosal injury induced by pylorus ligation,[41] ethanol[6] or aspirin.[42] However, systemic treatment with high doses of capsaicin functionally denervates the sensory nerve fibers and aggravates gastric mucosal injury induced by pylorus ligation,[41] acid distension, indomethacin,[43] ethanol or cysteamine.[43] In the present study, rats pretreated with capsaicin 125mg/kg subcutaneously, a dose known to produce functional denervation of the afferent sensory fibers[21] (this was confirmed by the absence of the wiping reflex when capsaicin in a low doses was introduced into the eyes of these rats).

In the current study, the protective effect of intragastric clarithromycin was not abolished by capsaicin pretreatment. This indicates that the protective effect of intragastric clarithromycin against ethanol-induced mucosal injury may not be mediated by afferent sensory nerve fibers.

The findings of the present study have demonstrated that pretreatment with (selective α_1- adrenoceptor antagonist) in a dose provided adequate α_1- blockade did not produce a significant reduction in the lesion score both in vehicle and clarithromycin treated rats (at

different doses) meaning that clarithromycin treatment still produced a significant reduction in lesion score. This observation suggested that α_1- adrenoceptors seem to have no role in the pathogenesis of ethanol-induced gastric mucosal. This finding agrees in part with the finding of previous study[44] that suggested that α_1- adrenoceptors may play no or partial role in the pathogenesis of ethanol-induced gastric mucosal injury.

On the other hand, the present findings have shown that α2-adrenoceptor antagonist) significantly reduced but did not completely block the protective effect of intragastric clarithomycin, suggesting that stimulation of α_2- adrenoceptor by intragastric claithromycin may be involved partially in its protective effect against ethanol-induced gastric mucosal injury. The mechanism may be a mucous dependent since intragastric clarithromycin (in the present study) was found to increase both gastric mucous volume and gastric juice volume while yohimbine significantly reduced both basal and clarithromycin stimulated gastric mucous secretion.

This observation suggests that the blockade of the protective effect of intragastric clairithromycin by yohimbine was related to an effect on gastric mucous volume.

Previous studies[28,44] showed that different agents protected against ethanol and acetyl salicylic acid induced gastric damage in rats and this protection was associated with a significant increase in gastric juice and gastric mucous volumes. Endoh *et al*.[44] have also reported that gastro-protective effect of intragastric nicotin and the significant increase in gastric mucous volume were significantly attenuated by as it has been shown in the present study that the mechanism of gastroprotection offered by intragasteric clarithromycin is also mediated by a similar factor. This supports the hypothesis that α_2-adrenoceptors modulate mucosal protection[44]. Thus gastric mucous volume and α_2 adrenoceptors may be instrumental in the protective effect of a variety of agents administered intragastrically.

β- adrenoceptors agonists a nonspecific agonist of β_1 and β_2 adrenoceptors[45] and selective agonist of β_2 adrenoceptors[45] have been reported to inhibit gastric mucosal lesion induced by noxious agents and stress.[44]

The results of the current study showed that blockade of β-adrenoceptors did not enhance ethanol-induced gastric damage,pretreatment also did not modify the protective effect of intragastric clarithromycin. This indicates that β adrenoceptors do not play a role in the formation of ethanol-induced gastric mucosal lesions, or the protective effect of intragastric clarithromycin against such lesion. These data agree in part with the results of Endoh *et al*.[46] who showed that (non selective β-adrenoceptors antagonist in a dose that provided blockade of β-adrenoceptors) did not abolish the protective effect of intragastric nicotine against ethanol induced gastric mucosal injury, suggesting that β-adrenoceptors do not play a role in

the formation of ethanol induced gastric mucosal lesion.

The results of the present study have revealed that the protective effect of clarithromycin was not significantly reduced by cyclo-oxygenase inhibitor (in a dose that is adequate to inhibit the cylo-oxygenase activities)[16] given before clarithromycin. Therefore, clarithromycin protection dose not appear to involve stimulation of endogenous prostaglandin synthesis. This is in agreement with the finding obtained by Candido *et al*.[15]

Subcutaneous administration of (100mg/kg b. wt.) a specific sulfhydryl blocker, significantly decreased non protein sulfhydryls of the gastric mucosa assessed by the spectrophotometric methods.[27] It is well known that the action of sulfhydryls of the gastric mucosa is an important factor in modulating mucosal integrity in the presence of noxious agents.[8,27] The present finding showed that clairthromycin maintained its gastroprotective effect in cyclo-oxygenase inhibitor treated animals. This strongly suggests that non-protein sulfhydryls and other iodoacetamide sensitive mechanisms are not involved in clairthromycin protection against ethanol injury.

Gastric mucous has a protective role against acid peptic damage by forming a stable unstirred layer that supports surface neutralization by bicarbonate, providing a diffusion barrier.[10] Also, gastric mucin can act as an antioxidant.[47] The possibility that interagastric clarithromycin may protect the gastric mucosa by enhancing gastric mucous volume was examined and it was found that intragastric administration of clarithtomycin (100-400 mg/kg b. wt.) was associated with a significant increase in gastric mucous volume half an hour after its administration and this may account for the protection of the underlying epithelium against the damage induced by ethanol.

It is probable that clairthromycin protects rats against ethanol-induced damage as a result of a dilution of the ethanol in solution. The results of the present study have shown that intragastric clairthromycin in a dose dependent manner has increased the fluid volume retained in the gastric lumen after half an hour. The higher gastric volume at the time of ethanol administration may have dilute the challenger solution, reducing the severity of the damage in clairthromycin treated rats. The importance of higher gastric juice fluid volume was studied. It has been found that intragastric clairthromycin produced an increase in the gastric fluid in a dose dependent manner accordingly three incremental amount of vehicle (60, 400, 900 µl of distilled water) were administrated into the rat stomach immediately before ethanol. The present findings showed that the lesion score in the rats treated with 60µl of vehicle was significantly higher than those treated with 400µl or 900µl of vehicle. This indicates that the higher fluid volume retained in the gastric lumen of clarithromycin treated rats may accounts for the reduction in the lesion score.

The data of the present study indicated that the protective effect of intragastric clairthromycin consists of a dilution effect and other effects such

as an increase in gastric mucous production. The importance of the protective effect of the high gastric fluid volume and the increase in the mucous volume against ethanol was examined and confirmed by other investigators[11,20,28] who concluded that the greater increase in gastric juice volume and gastric mucosa volume have a major role in the protection of gastric mucous against damage induced by ethanol.

The findings of the present study have shown that intragastric clarithromycin was not associated with a reduction in the gastric acid secretion since there was no significant difference in titratable acid in gastric juice between vehicle and clarithromycin treated rats. α_2-adrenoceptor bloker itself enhanced gastric acid secretion in vehicle and clarithromycin treated rats, suggesting that acid secretion is not significantly involved in the protective effect of intragastric clarithromycin against ethanol damage. Accordingly, acid secretion did not play a significant role in the damaging action exerted by ethanol on gastric mucosa or the protective effect of intragastric clarithromycin against such lesion.

The acute protection of clarithromycin have a limited role in chronic ulcer healing. However, it was reported that intragastric clarithromycin has a protective effect despite using different gastric lesion models, this observation suggested that the protective effect of intragastric clarithromycin in not specific for the ethanol model.[15] On the other hand, Lan *et al*.,[48] showed that treatment with antibiotics alone, without using any known ulcer healing agent, was effective in healing duodenal ulcer irrespective of whether or not the H. pylori infection had been eradicated. This indicates that antibiotics healed the duodenal ulcer through mechanisms other than their antibacterial action. This may be related to the gastroprotective properties of clarithromycin[30] metarondiazole[49] and amoxicillin.[50]

Conclusion: It could be concluded that intragastric clarithromycin has a protective action against ethanol-induced gastric damage, this could be explained by a mechanism other than its well known antibacterial action. The dose dependent increase in both the gastric mucous volume and fluid volume retained in the gastric lumen at the time when ethanol administrated may contribute to this protection.The opiate receptors, the afferent sensory nerve fibers, endogenous prostaglandins, sulfhydryl compounds of the gastric mucosa, α_1- β_1 and β_2 adrenoceptors do not seem to play a role in such protection. α_2 adrenoceptors may be involved in the mechanism of protection afforded by intragastric clarithromycin possibly by a mucous dependent mechanism.Further studies illustrating the role of α_2- adrenoceptors in the regulation of gastric mucous production may through further light on the mechanism of protection afforded by the intragastric clarithromycin.

Peptic ulcer disease although declining in prevelance,appears to be increasing in virulence,perhaps because of the overall aging of the population and improved intensive care unit care.Although helicobacter

pylori and steroidal anti-inflammatory drugs have been identified as key proulcerogenic factors,many ulcers may also result from a deficiency of other unknown host protective factors.A more detailed understanding of the host factor involved in mucosal protection will thus help identify novel therapeutic agents aimed at the prevention and treatment of upper gastrointestinal mucosal injury.

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دراسة بعض الأليات الفسيولوجية التى تقوم بتأثير مضاد لقرحة الغشاء المبطن لجدار المعدة فى الفئران

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تعد البكتريا الحلزونية هليوكوباكتر من أهم أسباب التهاب وقرحة المعدة والإثنى عشر وكذلك سرطان المعدة وتعتبر من أهم المشكلات التي تهم الصحة العامة. وبناء على خطوط الاتجاهات العالمية للإرشاد فى العلاج فإن العلاج الأول لها يتكون من استخدام أحد مضادات الجراثيم بالإضافة إلى المواد التى تقلل من الإفراز داخل المعدة ومع أن استخدام مضاد الجراثيم (الكلاريثروميسين) كان يستخدم بنجاح مع مضادات القرحة لكى يطيل فترة الهدوء فى حالات قرحة المعدة إلا أنه لم يُعرف حتى الآن إذا كان له قدرة على حماية الأنسجة المبطنة للمعدة والإثنى عشر.

ولهذا كان الغرض من هذا البحث هو دراسة ما إذا كان الكلاريثروميسين يمتلك خواص لحماية خلايا المعدة ضد الإصابة المعدية المستحدثة بالأيثانول بتركيز ٩٦% وأيضاً كان الهدف توضيح دور كل من مستقبلات الأوبيت وألياف الأعصاب الصادرة الحساسة والمستقبلات الأدرينيجية ألفا وبيتا والبروستاجلاندين والسلفاهيدريل وحجم السائل المعدى وحجم المخاط الموجود بتجويف المعدة فى حماية الخلايا المعدية بواسطة الكلاريثروميسين.

وقد استحدثت إصابة المعدة بالأيثانول بتركيز ٩٦% فى الفئران وتم بعدها دراسة تأثير إعطاء الكلاريثروميسين عن طريق المعدة بجرعات من ٥٠-٤٠٠ مجم/كجم على إصابة المعدة بالأيثانول. كما تم دراسة تأثير الحقن البريتونى بمقفلات مستقبلات الأوبيت بجرعة قدرها ٨ مجم/كجم وغلق ألياف الالتهاب الصادرة الحساسة بجرعة قدرها ١٢٥ مجم/كجم تحت الجلد ومقفلات المستقبلات الأدرينية الفا (١) (٠.٥ مجم/كجم) تحت الجلد وألفا (٢) ٥مجم/كجم تحت الجلد والحقن البريتونى لكل من مقفلات الستقبلات الأدرينية بيتا-١، بيتا-٢(٤ مجم/كجم).

وكذلك تم دراسة تأثير كل من مضادات البروستاجلانديل (٥مجم/كجم تحت الجلد) ومضادات السلفاهيدريل ١٠٠ مجم/كجم تحت الجلد على تأثير حماية الكلاريثروميسين للخلايا. وبالإضافة إلى هذا تم دراسة دور حجم محتويات المعدة على هذه الحماية وقد نفذت كل دراسة باستخدام ٦ فئران.

وقد أظهرت نتائج هذا البحث أن إعطاء الكلاريثروميسين عن طريق المعدة قد أحدث حماية للجدار المبطن للمعدة ضد الإصابة المستحدثة بواسطة الأيثانول (٩٦%). وهذه الحماية كانت معتمدة على الجرعة. وكان تثبيط الإصابة بنسبة ٣١% و٥١.٣٣% و٧٩.٧٥% و٩١.١٥% للجرعات ٥٠ و١٠٠ و٢٠٠ و٤٠٠ مجم/كجم على التوالى. وقد وجد أن الحماية المعدية بالكلاريثروميسين لم تتغير تغير ذو دلالة إحصائية بعلاجها مسبقاً بمقفلات مستقبلات الأوبيت أو مضادات الالتهاب الصادرة الحساسة. كما لم يؤثر الحقن المسبق تحت الجلد بمقفلات المستقبلات الأدرينية ألفا-٢ أو الحقن البريتونى المسبق بمقفلات المستقبلات الأدرينية ألفا-١ أو مقفلات المستقبلات الأدرينية بيتا-١ على حماية المعدة بالكلاريثروميسين ولكن حماية الكلاريثروميسين كانت قد قلت إحصائياً بالعلاج المسبق بمقفلات المستقبلات الأدرينية بيتا-٢. كما أن الحماية المعدية بالكلاريثروميسين لم تتأثر تأثير ذو دلالة إحصائية بمقفلات البروستاجلاندين أو مضاد السلفاهيدريل هذا وقد وجد زيادة ذات دلالة إحصائية فى حجم السائل المعدى وحجم المخاط تعتمد على الجرعة (٥٠-٤٠٠ مجم/كجم) وقد قلل مقفلات المستقبلات الأدرينية بيتا-٢ المخاط المعدى المثار بواسطة الكلاريثروميسين وكذلك الغير مثار.

ولهذا من الممكن أن نستخلص من هذا البحث أن إعطاء الكلاريثروميسين عن طريق المعدة قد أدى إلى حمايتها ضد الإصابة المستحدثة بالأيثانول وكانت هذه الحماية غير معتمدة على مستقبلات الأوبيت أو المقفلات الأدرينيجية ألفا-١ أو بيتا-١ أو بيتا-٢ أو البروستاجلاندين أو السلفاهيدريل ولكن زيادة حجم المخاط والسائل المعدى ممكن أن يكون لهما دور فى هذه الحماية ضد الإصابة المستحدثة بالأيثانول ٩٦% كما أن مستقبلات ألفا-٢ الأدرينيجية من المحتمل أن يكون لها دور فى هذه الحماية بطريقة معتمدة على المخاط.