Gastroprotective effect of simvastatin against experimentally induced gastric ulcers in rats: Role of ATP-sensitive K^+ channels

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Abstract: Simvastatin appeared to have additional benefits beyond their lipid lowering effects, which has led to interest in the use of this class of drugs outside the field of cardiovascular disease. **Aim:** This study aimed to investigate the possible gastroprotective effect of simvastatin against both indomethacin and cold restraint stress (CRS) induced gastric ulcers in rats and to study its effect on gastric mucosal malonaldehyde (MDA) ,nitric oxide (NO)and prostaglandin E2(PGE_2) levels in both ulcer models. Exploration of the possible contribution of ATP-sensitive K^+ channels in this action. **Design:** 72 healthy, adult male albino rats were used. The rats were randomly assigned to vehicle (distilled water or carboxymethylcellulose (0.5%), simvastatin, simvastatin +glibenclamide (ATP-sensitive K^+ channels blocker), pretreated groups for 7 days then ulcers were induced using oral indomethacin or cold restraint stress. Assessment of gastric lesions was done, gastric juice parameters (total acid output, pepsin activity and mucin content) were determined for each group using pyloric ligation method .Rats from simvastatin pretreated groups in both ulcer models were used for determination of gastric mucosal level of MDA (as indicator of lipid peroxidation) , nitrite (as indicator of NO) and PGE_2 levels. **Results:** Simvastatin displayed significant ($P< 0.05$) protection against gastric lesions induced by either indomethacin or exposure to cold restraint stress by correction of both ulcer score and the measured gastric juice parameters. This effect was partially blocked by coadministration of glibenclamide. Simvastatin significantly ($P< 0.05$) reduced gastric mucosal MDA; significantly ($P< 0.05$) increased in PGE_2 levels and corrected nitrite to near normal levels in both ulcer models. **Conclusion:** This study confirmed the gastroprotective effect of simvastatin in indomethacin induced ulcer in rats and proved it in CRS induced ulcer .The gastroprotective effect of simvastatin is mediated through opening of ATP sensitive K^+ channels, free radical scavenging, increase in gastric mucosal PGE_2 and normalization of gastric mucosal NO in both ulcer models.

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Keywords: indomethacin; cold restraint stress; NO; simvastatin; ATP-sensitive K^+ channels and ulcer.

1. Introduction

Gastric ulcer is an illness that affects a considerable number of people worldwide. The etiological factors of this disorder include: stress, smoking, nutritional deficiencies, frequent and indiscriminate use of non steroidal anti-inflammatory drugs (NSAIDs) (Khazaei and Salehi 2006).The pathophysiology of gastric ulcer has generally focused on imbalance between aggressive and protective factors in the stomach (Lima, et al., 2006). The gastric ulcerogenic action of NSAIDs is believed to occur mainly due to their local inhibitory effect on gastric prostaglandin E2 (PGE_2) and prostaglandin I2 (PGI_2) that are the main inhibitors of gastric acid secretion (Bhargava, et al., 1973; Ribeiro-Rama., et al., 2009). Hypothermic -restraint stress ulcers (CRS) have been used as an experimental model in the evaluation of antiulcer activity in rats (Murakami et al., 1985).It induces ulcer through disturbance in the gastric mucosal microcirculation, enhancement of acid secretion, reduction in mucus production and abnormal gastric motility (Garrick, et al., 1986), free radicals formation (Bagchi, et al 1999) and decreased in prostaglandin synthesis (Bandyopadhyay, et al., 1999).

Statins (3- hydroxyl -3-methyl glutaryl-CoA reductase inhibitors) are a group of drugs that are originally designed to lower serum cholesterol level and have been recognized as the most efficient drugs for the treatment of hyperlipidemia(Heeba et al., 2009). Previous clinical trials have demonstrated that the therapeutic benefits of statins could not solely be explained by their inhibitory actions on cholesterol synthesis (Werner, et al., 2002; Wainwright, 2005).Clinical trials proved that they exhibit other effects unrelated to their lipid lowering effect (pleotropic effect) (Tamargo, et al., 2007).Simvastatin which has tested in this study is a commonly prescribed statin with antioxidant and anti-inflammatory properties (Ungureanu, et al., 2003 ; Franzoni, et al., 2003). Accordingly, this study aimed to investigate the possible gastroprotective

effect of simvastatin against indomethacin and CRS induced gastric ulcers in rats with exploration of the possible mechanisms underlying this effect.

2. Materials and methods

Drugs and chemicals

Simvastatin was obtained from (Pharco Egypt) as powder. Indomethacin was obtained from Sigma chemical Co. (St. Louis, MO. USA) and was suspended in carboxymethylcellulose 0.5 %(Na salts, Merck, Darmstadt, Germany). Glibenclamide powder, EIPICO, Egypt). Ether, from SD Fine chemicals (India). All drug solutions and suspensions were freshly prepared

Animals

Seventy two adult male Albino rats weighing 180-200 grams were purchased from Abu-Rawash animal house (Giza- Egypt). Animals were given tap water ad libitum , fed with standard commercial rat chow and were left to accommodate for one week before dosing , kept 12 hours light ⁄ 12 hours dark regular cycle in partially humid and well aerated room. Protocol was approved by the local animal care committee at Zagazig University (Egypt) .All experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Experimental design

The rats were fasted for 24 hours prior to the experiment in meshbottomed cages to minimize corporophagia .The animals had free access to water except the last hour before the experiments .All experiments were performed during the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions .The animals were randomly classified into nine groups (8 rats per each): **(1) control group**: in which the animals received distilled water orally for 7 days.**(2) control indomethacin group(indom) :** the rats received oral indomethacin in a dose of 20 mg⁄ kg in 2.5ml/kg of 0.5% of carboxymethylcellulose after daily administration of distilled water for 7 days. **(3) Indomethacin +carboxymethylcellulose:** in which ulcers were induced by indomethacin after 7 days of oral administration of carboxymethylcellulose 0.5% (the vehicle of both indomethacin and glibenclamide). **(4) indomethacin +simvastatin (indom+ sim.):** in which animals were pretreated with 40 mg ⁄kg simvastatin orally for 7 days then, gastric ulceration was induced by indomethacin administration.**(5)indomethacin +simvastatin+ glibenclamide (indom+sim+gliben) :**in which animals were concurrently pretreated with 40 mg⁄ kg simvastatin and 5 mg⁄ kg glibenclamide(K_{ATP} channels blocker) orally for 7 days then gastric ulcer was induced by indomethacin.**(6) CRS:** cold-restrained stress to induce gastric ulcer , the rats were immobilized in individual restraint boxes without possibility of visual contact (popovic' et al.1997) and subjected to cold (4± 1◦)stress for 3.5hours .This regimen of cold-restraint stress has been reported to produce gastric ulcers reliability in food-deprived rats(Senay and Levine 1967) **(7) CRS+ carboxymethylcellulose :** the rats received carboxymethylcellulose 0.5% orally for 7 days then ulcer was induced by CRS.(8) CRS+ simvastatin(CRS+sim):in which the ulcer was induced by CRS after 7 days of daily oral administration of 40 mg⁄ kg simvastatin.(9) CRS+ simvastatin+ glibenclamide (CRS+ sim+ gliben): in which the animals concurrently pretreated with simvastatin 40 mg⁄ kg and 5 mg⁄ kg of glibenclamide orally for 7 days then gastric ulcer was induced by CRS. It should be noted that all drugs, vehicle, distilled water were administered in a volume of 2.5 ml ⁄kg via gavage. Doses of drugs were in homogeny with previous reports (Tariq, et al., 2007; Aziz, 2009)

Pyloric ligation

Pyloric ligation was carried out before either indomethacin administration or exposure to CRS in order to collect gastric secretion (Alumets et al 1982). This was done under ether anesthesia , a midline abdominal incision was performed , the pyloric portion of the stomach was gently mobilized and carefully ligated with a silk ligature around the pyloric sphincter taking care not to interfere with gastric blood supply .Abdominal incision was sutured and the animals were allowed to recover from anesthesia .

Assessment of gastric mucosal lesions

Three hours later after administration of indomethacin or exposure of rats to CRS, rats of all groups were killed with an overdose of ether , their stomachs were rapidly removed , opened by an incision along the greater curvature and the gastric juice were collected , the stomachs rinsed with saline .Gastric tissues were pinned out flat on a cork board , the number and the severity of discrete areas of damage in the mucosa were scored by two trained independent observers who were unaware of the drug treatment , the ulcer score was determined according to the 1 to 5 scoring system devised by Wilhelmi and Menasse-Gdynia 1972 .(1)1 or 2 minute, sporadic, punctuate lesion (2) several small lesion (3) one extensive lesion or multiple moderate sized lesions(4) several large lesions (5)several large lesions with stomach perforation.

Analysis of gastric juice

Gastric juice from each animal was centrifuged at 1000 g for 10 minutes to remove any solid debris and the volume of the supernatant was

measured. The supernatant was then assayed for total acid concentration(Hara et al 1991) , the total acid output was calculated by multiplying the volume of gastric juice by the total acid concentration also pepsin activity (Sanyal et al 1971)and mucin content (Winzler, 1955) were determined in the obtained supernatant

Biochemical analysis of gastric mucosa

The stomach of each animal was divided into two parts. Gastric mucosa of one part was scrapped and was immersed in indomethacin (10µg⁄ml) and was immediately stored at -80 °C., afterwards, homogenized in 2 ml normal saline containing 0.1 M dithiothreitol and centrifuged at 2000g for 10 minutes at room temperature. The supernatant was used for determination of prostaglandin E2 (PGE_2) level by ELISA using PGE_2 immunoassay kit (R&D systems, USA).

The mucosa of the other part of the stomach was also scrapped, homogenized in cold potassium phosphate buffer (0.05 M, PH7.4) and centrifuged at 2000g for 10 minutes at 4°C; the supernatant was then kept at -80°C for measurement of malonaldehyde (MDA) (Mihara and Uchiyama 1978). Also the total nitrate⁄ nitrite in the gastric mucosal homogenate was assayed after reduction of nitrate to nitrite using the cadmium reduction method (Sastry et al., 2002)

Statistical analysis:

Analyzed by One-way analysis of variance (ANOVA) followed by LSD test using SPSS for windows version 11.5. Differences were considered to be significant at $P< 0.05$.

3. Results

It is noted that carboxymethylcellulose 0.5% pretreated groups did not show any significant difference as compared to indomethacin group or CRS group in any of the parameters investigated in this study.

(1)Investigation of the involvement of ATP sensitive K^+ channels in the gastroprotective effect of simvastatin on indomethacin and CRS induced gastric lesions

(A) Fig (1): histogram shows that either administration of indomethacin or exposure of rats to CRS induced high ulcer score 4.1±0.3 &3.1±0.3 respectively when compared to control group .Pretreatment with simvastatin significantly reduced the ulcer scores in both group to 0.5±0.2 &0.3±0.2 respectively. Coadministration of simvastatin and glibenclamide increased the ulcer score to 2.5±0.2 &2.1±0.2 in both ulcer models respectively. **Fig (2, 3):** gross appearance of mucosal lesions on exposure to indomethacin or CRS (a), pretreatment with simvastatin (b) and coadministration of simvastatin and glibenclamide (c) respectively.

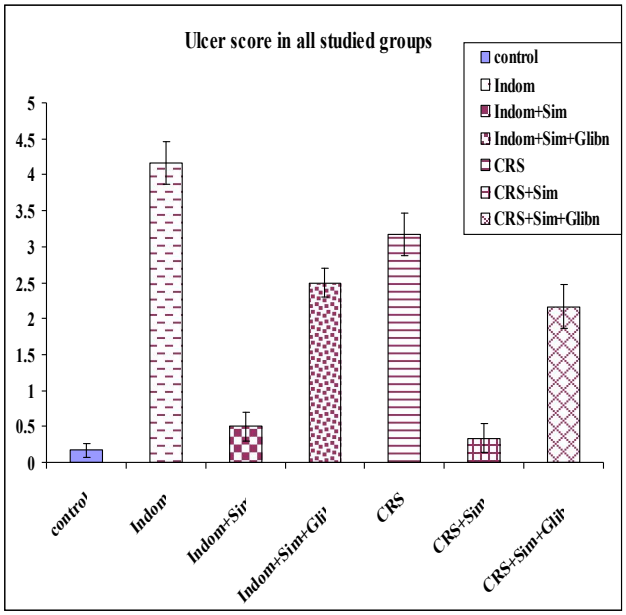
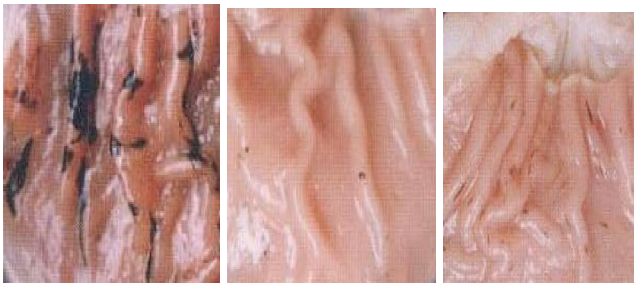
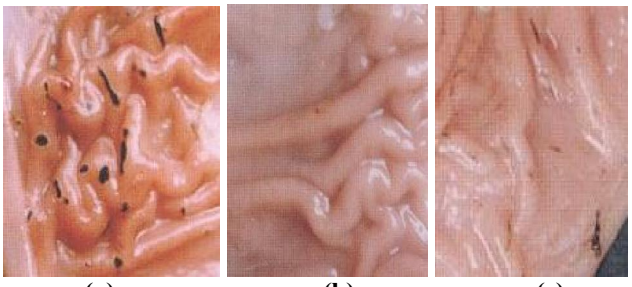


Figure (1)



(a) **INDO** **(b)** **INDO+SIM** **(c)** **INDO+SIM+GLIB**

Figure (2)



(a) **CRS** **(b)** **CRS+SIM** **(c)** **CRS+GLIB+SIM**

Figure (3)

(B) Effect of simvastatin on changes in gastric juice contents induced by indomethacin:

Table (1) shows that Indomethacin significantly increased gastric juice total acid output as compared to control group 92.5±4.2mEq/3h versus 56.1±3.2mEq /3h. Pretreatment with simvastatin significantly reduced total acid output to 61.2±4.5 mEq /3h while concurrent use of simvastatin and glibenclamide raised the above mentioned value to 74.2±2.7mEq/3h

Pepsin activity was significantly increased by indomethacin 208.4±10.7 µg/ml tyrosine versus 122±3.2 µg/ml tyrosine for the control group. Rats pretreated with simvastatin showed significant reduction in pepsin activity with mean ±S.E. 144.9±4.3µg/ml tyrosine, this reduction was attenuated by coadministration of glibenclamide with simvastatin with value 174.9±4.5µg/ml tyrosine

Indomethacin significantly reduced gastric juice mucin content from 89.3±2.8mg% hexose for the control group to 46.2± 2.3 mg% hexose. In simvastatin pretreated rats, this value significantly raised to 83.3±1.8mg% hexose while concurrent administration of simvastatin and glibenclamide significantly decreased this value to 61.8± 2.1 mg % hexose.

Table (1): Effect of indomethacin, simvastatin, simvastatin + glibenclamide on the total acid output, pepsin activity and mucin content of gastric juice in rats.

Groups	Total acid output (meq/3h)	Pepsin activity µg/ml tyrosine	Mucin content mg%hexose
Control	56.1±3.2	122±3.2	89.3±2.8
Indom	92.5±4.2[a]	208.4±10.7[a]	46.2±2.3[a]
Indom+sim	61.2±4.5 [b]	144.9±4.3[b]	83.3±1.8[b]
Indom+sim+gliben	74.2±2.7[c]	174.9±4.5[c]	61.8±2.1[c]

a significantly different from control group at P < 0.05
b significantly different from CRS group at P < 0.05
c significantly different from CRS +simvastatin group at P < 0.05

(C)Effect of simvastatin on changes in gastric juice contents induced by CRS:

Table (2): shows that CRS significantly increased gastric juice total acid output as compared to control group 88.2±2.8 mEq/3h versus 56.1±3.2 mEq/3h. Pretreatment with simvastatin significantly reduced total acid output with mean ±S.E.62.2±2.9 mEq /3h while concurrent use of simvastatin and glibenclamide raised the above mentioned value to 77.7±3.2 mEq/3h.

CRS significantly raised the pepsin activity to 220.4±5.2µg/ml tyrosine versus 122±3.2 µg/ml tyrosine for the control group. Rats pretreated with simvastatin showed significant reduction in pepsin activity with mean ±S.E.152±4.3 µg/ml tyrosine. Concurrent administration of simvastatin and glibenclamide increased this value to 201±5.1 µg/ml tyrosine. Gastric juice mucin content was significantly reduced by CRS .It decreased from 89.3±2.8 mg% hexose for the control group to 48.3±1.8 mg% hexose. In CRS, simvastatin pretreated rats showed significant elevation in mucin content to 83.5±2 mg % hexose. Concurrent administration of simvastatin and glibenclamide significantly decreased this value to 59.8±2 mg% hexose.

Table (2): Effect of CRS, simvastatin, simvastatin + glibenclamide on the total acid output, pepsin activity and mucin content of gastric juice in rats.

Groups	Total acid output (meq/3h)	Pepsin activity (µg/ml tyrosine)	Mucin content (mg%hexose)
Control	56.1±3.2	122±3.2	89.3±2.8
CRS	88.2±2.8 [a]	220±5.2[a]	48.3±1.8[a]
CRS+sim	62.2±2.9 [b]	152±4.3[b]	83.5±2[b]
CRS+sim+gliben.	77.7±3.2 [c]	201±5.1[c]	59.8±2[c]

a significantly different from control group at P < 0.05
b significantly different from CRS group at P < 0.05
c significantly different from CRS +simvastatin group at P < 0.05

(2) Effect of simvastatin on gastric mucosal MDA level in both ulcer models: Figure (4): shows that both indomethacin and CRS significantly elevated the gastric mucosal MDA) to 61.05±1.9 and 62.5±1.3

(nmol/g wet tissue) respectively as compared to control group20.1±0.8 nmol/g wet tissue. Simvastatin significantly caused reduction in the gastric mucosal MDA in both ulcer models to 35.3±0.1and 30.2±1.3 nmol/g wet tissue respectively.

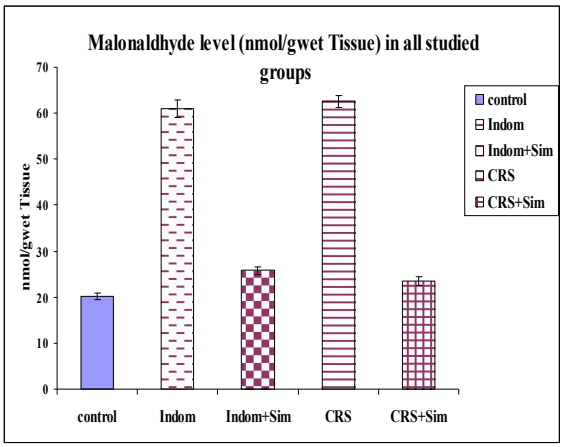


Figure (4)

(3) Effect of simvastatin on gastric mucosal nitrite level in both ulcer models: Figure (5): shows that indomethacin significantly reduced gastric mucosal nitrite level from169.9±3.7 nmol / g wet tissue for the control group to 80.3±2.6 nmol / g wet tissue .Rats pretreated with simvastatin showed significant increased in the gastric mucosal nitrite content to152.7±4 nmol / g wet tissue In contrast, CRS significantly increased it from 171.5±1.7 nmol /g tissue in control group to 371.5±4.5 nmol / g wet tissue in CRS .Rats pretreated with simvastatin showed marked reduction in gastric mucosal nitrite concentration to 219.6±4.8 nmol/g wet tissue .

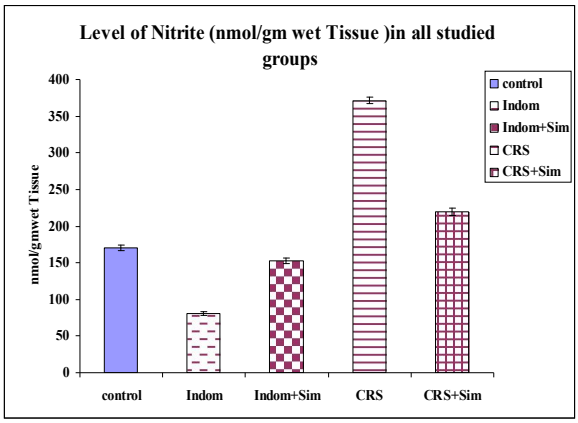


Figure (5)

(4) Effect of simvastatin on gastric mucosal PGE_2 level in both ulcer models:

Figure (6): shows that both indomethacin and CRS significantly reduced the gastric mucosal PGE_2 content from 318±4.9 ng/g wet tissue for the control group to 102±5 ng/g wet tissue and 125.4±6 ng/ g wet tissue for indomethacin group and CRS group respectively. Simvastatin significantly increased the gastric mucosal PGE_2 levels to 262.6±8.1and 256±4.7 ng/ g wet tissue for both groups respectively.

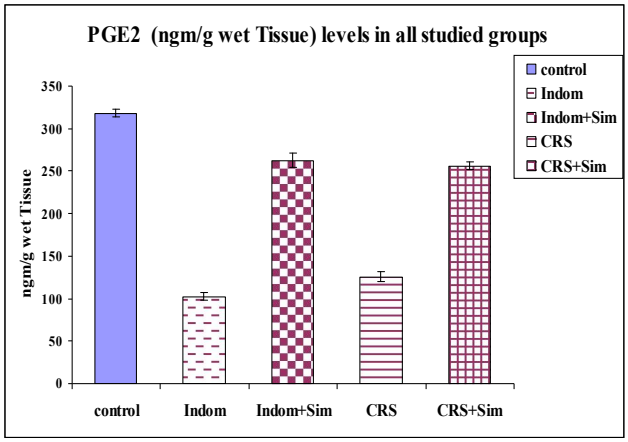


Figure (6)

4. Discussion

The diversity of etiological factors underlying gastric ulcers and the complex nature of pathways participating in healing always make peptic ulcers treatment a complicated challenge (El-Moselhy et al., 2009). Maintaining equilibrium between aggressive and defensive factors is always a critical objective in peptic ulcer management (Glavin and Szabo 1992).

The results of the present study showed either oral administration of indomethacin in rats or their exposure to cold restraint stress resulted in severe mucosal ulceration associated with significant increase in total acidity and pepsin activity and significant reduction in gastric juice mucin content.

Our work showed that simvastatin administered orally in a dose of 40 mg/ kg for 7 days reversed all the deleterious effects induced by either indomethacin or CRS on both gastric mucosa and gastric juice contents. Tariq, et al. (2007) demonstrated that oral administration of simvastatin in doses of 20, 40, 60 mg/ kg for 7 days significantly and dose dependently reduced gastric lesions induced by indomethacin and ethanol in rats, an effect accompanied by reduction in total acidity and volume of gastric juice. Heeba et al., 2009 illustrated that simvastatin in a dose of 10 mg/ kg for two weeks decrease the ulcer index induced by indomethacin in rats with concomitant elevation in gastric juice mucin content .They found that neither total acidity nor pepsin activity were affected.

The present study found that the beneficial effects mediated by simvastatin on ulcer score or on

the measured parameters of gastric juice in both ulcer models were partially blocked by co administration of glibenclamide (ATP sensitive K^+ channel blocker) which indicates that some of the gastroprotective effect of simvastatin is mediated through activation of ATP sensitive K^+ channels.

Different researches have demonstrated antiulcer activity of some K_{ATP} channel openers like cromakalim (Goswami et al., 1997), diazoxide (Toroudi et al., 1999) and nicorandil (Patel et al., 2001). Zhao et al., 2006 and Yang, et al., 2007 illustrated that simvastatin was able to reduce the myocardial no reflow after ischemia and reperfusion through activation of K_{ATP} channels.

The suggested protective effect of ATP sensitive K^+ channels on ulcerative lesions mediated by simvastatin may be attributed to increase gastric mucosal blood flow offering more resistance to ulcer (Ismail et al., 2007). Furthermore, K_{ATP} channels openers decreased the gastric hypermotility induced by activation of vagus nerve in CRS (Garrick et al., 1986).

The ability of glibenclamide to partially block the inhibitory effect of simvastatin on total acidity may be due to the fact that some of the potent gastric acid inhibitors as prostaglandins (Peskar et al., 2002), adrenomedullin and CGRP (Rossowski et al., 1997; Sakai, et al., 1999), act through K_{ATP} channels The results of the present work revealed that glibenclamide partially abolished the reduction in pepsin activity mediated by simvastatin. CGRP which acts through K_{ATP} channels was found to inhibit pepsin secretion (Kraenzlin, 1985). K_{ATP} channels openers were known to interfere with Ca^{++} ion influx and to antagonize mobilization of Ca^{++} bound to intracellular stores leading to reduction in free cytosolic Ca^{++} ion important for gastric acid and pepsin secretion (Miyamoto., et al., 1992; Bose et al., 2003). The decreased gastric acidity may be a cause as its presence is essential for cleavage of pepsinogen to active pepsin (Morsy and Fouad 2008).

The ability of glibenclamide to block the stimulatory effect of simvastatin on gastric juice mucin is compatible with a previous report which illustrated that eugenol(active ingredient of Clove oil) increased gastric mucin through ATP sensitive K^+ channels (Morsy and Fouad 2008)

The result of the present work showed significant elevation in MDA level , an indicator for lipid peroxidation which is a well established mechanism for cellular injury (Kwiecien et al., 2002),in both ulcer models. This was in accordance with Vaananen ., et al.,1991 who proved that the gastrotoxic effect of NSAID was mediated through induction of reactive oxygen metabolites which promote lipid peroxidation , Tandon et al.,(2004) who stated that there are positive correlation between the level of gastric mucosal lipid peroxidation products (marker of oxidative stress) and stomach damage in CRS in rats.

The result of the present study showed that simvastatin pretreatment caused significant reduction in MDA level in both ulcer models as compared to non treated control groups and it was reported that simvastatin possesses free radicals scavenger activity (Haendeler et al., 2004). This suggests that simvastatin afforded part of its gastroprotective effect in both ulcer models via antioxidant activity.

NO is a double edge weapon exerting either protective or destructive effects depending on the extent on NO synthesis (Tariq et al., 2007). It has been reported that NO generated from eNOS play an important role in gastric ulcer formation and healing (Ma and Wallace 2000) whereas NO generated from iNOS participate in ulcer formation through the production of oxygen derived radical formation and their cytotoxic action (Cho 2001)

The result of the present work showed marked reduction in nitrite level (as indicator of NO) in indomethacin treated group. This is attributed to the ability of indomethacin to upregulate the endothelin-1 leading to decrease production of gastric mucosal NO (Slomiany and Slomiany 2000) while nitrite level is markedly elevated in CRS which occurred due to stimulation of iNOS which reacts with superoxide to form peroxynitrite (potent cytotoxic oxidant) causing gastric damage (Lanas 2008) .Simvastatin was able to normalize the nitrite levels in both ulcer models. It increased NO level in indomethacin treated group. Similar finding was observed by Heeba et al. (2009), while it deceased NO level in CRS group. Madonna et al., 2005 illustrated that simvastatin attenuates expression of cytokine iNOS in embryonic cardiac myoblasts.

Earlier researches demonstrated that PGE_2 influence virtually every component of the mucosal defense ; stimulate mucus, bicarbonate ; maintaining of mucosal blood flow ; enhancing the resistance of the epithelial cells to injury induced by cytokines (Miller., 1983)

In the present study , oral administration of indomethacin resulted in significant reduction in gastric mucosal PGE_2 level , through non selective inhibition of cyclooxygenase (Schmassmann et al., 1998) and the same in CRS rats .Bandyopadhyay et al. (1999) attributed this effect to accumulation of hydrogen peroxide (a potent PG biosynthesis inhibitor). Rats pretreated with simvastatin showed significant increase in PGE_2 level in both types of ulcers.

NO was reported to increase PGE_2 biosynthesis in vivo through CGMP independent mechanism and it is

possible to assume that NO might regulate the release and or the biosynthesis of PGE2 in the stomach after damage (Takeuchi et al., 1999). Chen, et al., 2004 proved that statins induce cyclooxygenase -2 gene expressions and PGE_2 release in murine macrophage. **Conclusion :** the results obtained from this study confirmed the gastroprotective effect of simvastatin in indomethacin induced ulcer and proved it in CRS induced ulcer .This effect is mediated through ATP sensitive K^+ channel opening activity, free radical scavenging , decrease in total acid output and proteolytic activity , increase in gastric juice mucin content , increase in gastric mucosal PGE_2, returning of gastric mucosal NO to near normal values in both ulcer models.

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References

1. Alumets, J. , Ekelund M. , Hakanson, R. , Hedenbro , J. , Rehfeld, J.F. , Sundler, F. , Vallgren, S.(1982): Gastric acid response to pylorus ligation in rats : is gastrin or histamine involved?J. Physiol. 323 , 145-156.
2. Aziz O.H.(2009): Effects of soybean alone or in combination with insulin or glibenclamide on srum lipid profiles in alloxan-induced diabetic rats. Iraqi J. of Vetr. Scie.23 ; 17-23.
3. Bagchi M. ; Milnes M. ; Williams C. ; Balmoori J. ; Ye X. ; Stohs S. ; Bagchi D.(1999): Acute and chronic stress-induced oxidative gastrointestinal injury in rats, and the protective ability of a novel grape seed proanthocyanidian extract. Nutr. Res. 19 ; 1189-1199
4. Bandyopadhyay U. ; Das D. ; Bandyopadhyay D. ; Bhattacharjee M. ; Benerjee R.K. (1999): Role of reactive oxygen species in mercaptomethlimidazole- induced gastric acid secretion and stress-induced gastric ulceration. Curr.Sci. 76 ; 55-63.
5. Bhargava K.P. , Gupta M.B. , Tangri K.K. (1973): Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone . Eur. J. Pharmacol. 22 (2): 191-5.
6. Bose M. ; Motghare V.M. ; Dakhale G.N. ; Turankar A.V. (2003): Antiulcer activity of levcromakalim and nicorandil in albino rats : a comparative study. Pol. J. Pharmacol. 55(1):91-5.
7. Chen J.C. ; Huang K.C. ; Wingerd B. ; Wu W.T. ; Lin W.W.(2004): HMG-CoA reductase inhibitors induce COX-2 gene expression in murine macrophage :role of MAPK cascades and promoter elements for CREB and C⁄EBPbeta . Exp Cell. Res. 301(2) ; 305-19.
8. Cho C.H. (2001): Current roles of nitric oxide in gastrointestinal disorders . J. Physiol. Paris.95(1-6) ; 253-6.
9. El-Moselhy M.A., Abdel-Hamid N.M,Abdel-Raheim S.R. (2009): Gastroprotective Effect of Nicorandil in Indomethacin and Alcohol-Induced Acute Ulcers .Appl. Biochem. Biotechnol. 152 (3) : 449-459.
10. Franzoni,F. ; Quinones-Galvan A. ;Regoli, F. ; Ferrannini, E. and Galetta, F.(2003): A comparative study of the in vitro antioxidant activity of statins. Int. J. Cardiol. , 90 (2-3)317-21.
11. Garrick T. , Leung F.W. , Buack S. , Hirabayashi K. , Guth P.H. (1986): Gastric motility is stimulated but overall blood flow is unaffected during cold restraint in the rat. Gastroenterology .91(1) ; 141-148.
12. Glavin, G.B. ; Szabo, S.(1992): Experimental gastric mucosal injury : laboratory models reveal mechanisms of pathogenesis and new therapeutic strategies. The FASEB J. 6 ; 825-831.
13. Goswami S. ; Jain S. ; Santani D. (1997): Anti-ulcer activity of cromakalim (BRL34915), a potassium-channel opener against experimentally induced gastric and duodenal ulcers in rats and guinea-pigs. J Pharm Pharmacol 49(2) : 195-199.
14. Haendeler J. , Hoffmann J. , Zeiher A.M. , Dimmeler S. (2004): Antioxidant effects of statins via S-nitrosylation and activation of thioredoxin in endothelial cells : a novel vasculoprotective function of statins. Circulation. 110 (7): 856-61
15. Hara,N. : Hara,Y.;Natsume,Y.and Goto,Y. (1991):Gastric hyperacidity and mucosal damage caused by hypothermia correlate with increase in GABA concentrations of the rat brain . Eur. J.Pharmacol. 194 (1): 77-81.
16. Heeba G.H. ; Hassan M. K ; Amin R.S. (2009): Gastroprotective effect of simvastatin against indomethacin-induced gastric ulcer in rats : Role of nitric oxide and prostaglandins. Eur. J. Pharmacol. 607 (1-3): 188-193.
17. Ismail H.A.. ; Khalifa M. M .; Hassan M.K. ; Ashour O.M.(2007): Insight in the mechanisms underlying the anti-ulcer activity of nicorandil,Pharmazie 62(1) : 60-66.
18. Khazaei M. and Salehi H. (2006): Protective effect of falcaria vulgaris extract on ethanol induced gastric ulcer in rat. Iran. J. Pharmacol .Therap .5(1) ; 43-46.

19. Kwiecien S. ; Brzozowski T. ; Konturek J. (2002): Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. J. Physiol. Pharmacol.53(1) ; 39-50.
20. Lanas A. (2008): Role of nitric oxide in the gastrointestinal tract.Arthritis Res. Ther., 10 suppl 2 :S4 .
21. Lima Z.P. , Severi J.A. , Pellizzon C.H. , Brito A.R. , Solis P.N , Ca'ceres A ., Giro'n L.M.; Vilegas W. and Hiruma-Lima C.A. (2006): Can the aqueous decoction of mango flowers be used as antiulcer agent ? J. of Ethnopharmacology ,106(1):29-37.
22. Ma, L. and Wallace,J.L. , (2000): Endothelial nitric oxide synthese modulators gastric ulcer healing in rats. Am. J. Physiol. Gastrointest. Liver Physiol , 279(2) ;G341-6.
23. Madonna R. , Di Napoli P. , Massaro M. , Grilli A. , Felaco M. , De Caterina A. , Tang D. , De Caterina R. , GengY.J. (2005): Simvastatin attenuates Expression of Cyokine-Inducible Nitric-Oxide Synthase in Embyonic Cardiac Myoblasts. J. Biol. Chem. 280(14): 13503-11.
24. Mihara, M. , and Uchiyama, M. (1978): Determination of malonaldehyde precursor in tissue by thiobarbituric acid test, Anal. Biochem. 86(1) , 271-278.
25. Miller, T.A. (1983): Protective effects of prostaglandins against mucosal damage: current knowledge and proposed mechanisms .Am. J. Physiol. 245(5 pt 1) ; G601-G623.
26. Miyamoto T. ; Itoth M. ; Noguchi Y. ; Yokochi K. (1992): Role of intracellular Ca^{2+} and the calmodulin messenger system in pepsinogen secretion from isolated rabbit gastric mucosa. Gut 33(1) ; 21-25.
27. Morsy M.A. and Fouad A.A. (2008): Mechanisms of gastroprotective effect of eugenol in indomethacin –induced ulcer in rats.Phytother. Res. 22(10) , 1361-1366.
28. Murakami M. , Lam S.K. , Inada M. , Miyake T. (1985): Pathophysiology and pathogenisis of acute gastric mucosal lesions after hypothermic restraint stress in rats. Gastroenterology. 88(3) : 660-665.
29. Patel H.M. ; Santani D.D. ; Goswami S.G. (2001): Evaluation of the effects of nicorandil on experimentally induced gastric ulcers. Pharmacology.63(3) ; 154-159.
30. Peskar B.M. , Ehrlich K. , Peskar B.A. (2002): Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in rat. J. Pharmacol. Exp. Ther. 301(3): 969-974.
31. Popovic' M. , popovic' N. , Bokonjic' D. and Dobric' S. (1997): Cold restraint induced gastric lesions in individual-and group stressed rats. Int. J.Neurosci. 91(1-2), 1-10
32. Ribeiro-Rama A.C. , Fiqueiredo I.V. , Veiga F. , Castel-Branco M.M. . Cabrita A.M. , Caramona M.M. (2009): Evaluation of gastric toxicity of indomethacin acid , salt form and complexed forms with hydroxypropyl-beta-cyclodextrin on wister rats : histopathologic analoges. Fundam Clin. Pharmacol. 23(6) , 747-55.
33. Rossowski W.J.; Jiang N.Y.; Coy D.H.(1997): Adrenomedullin , amylin , calcitonin gene related peptide and their fragments are potent inhibitors of gastric acid secretion in rats . Eur J Pharmacol 336(1) : 51- 63.
34. Sakai K. ; Akima M. ; Katsuyama I. (1999): Effects of nicorandil on experimentally induced gastric ulcers in rats : a possible role of K(ATP) channels. Jpn J Pharmacol .79(1): 51-7.
35. Sanyal,A.R.;Denath,O.K.;Bhattacharya,S.K. and Gode,K.D.(1971): The effect of cyproheptadine on gastric acidity . In Pfeiffer,C.J.(Ed.)Peptic ulcer. Scandinavian University Books, Munksgoard p312-318.
36. Sastry K.V., Moudgal R.P.;Mohan G.;Tyagi.J.S.and Rao,G.S.(2002): Spectro-photometric determination of serum nitrite and nitrate by copper-cadium alloy.Anal. Biochem. 306(1):79-82.
37. Schmassmann A. ; Peskar B.M. ; Settler C. , Netzer P. , Stroff T. , Floqerzi B. , Halter F. (1998): Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. Br. J. Pharmacol. 123(5) ; 795-804.
38. Senay,E.C.and Levine, R.J.(1967): Synergism between cold and restraint for rapid
39. Slomiany B.L. and Slomiany A.(2000): Role of endothelin-converting enzyme-1 in the suppression of constitutive nitric oxide synthese in rat gastric mucosal injury by indomethacin . Scand J. Gastroenterol 35(11) ; 1131 – 6
40. Takeuchi K ; Suzuki K. ; Araki H. ; Mizoguchi H. ; Suqamoto S. ; Umdeda M. (1999): Roles of endogenous prostaglandins and nitric oxide in gasroduodenal ulcerogenic responses induced in rats by hypothermic stress. J. Physiol. 93(5) ; 423-431.
41. Tamargo J. , Caballero R. , Go´mez R. . Nu´nez L. , Vaquero M. , Delpo´n E.(2007): Lipid lowering therapy with statins , a new approach to antiarrythmic therapy . Pharmacol. Therap. 114 (1), 107-126.
42. Tandon R. ; Khanna H.D. ; Dorababu M. ; Goel R.K.(2004): Oxidative stress and antioxidants status in peptic ulcer and gastric carcinoma. Indian J. Physiol. Pharmacol. 48(1) ; 115-118.

43. Tariq M. , Khan H.A. , Elfaki I., Arshaduddin M. , Al Moutaery M. , Al Rayes H., Al Swailam R. (2007): Gastric antisecretory. antiulcer effects of simvastatin in rats.J. Gastroenterology and hepatology 22(12) : 2316-2323.
44. Toroudi H.P. ; Rahgozar M. ; Bakthiarian A. ; Djahanguiri B. (1999): Potassium channel modulators and indomethacin –induced gastric ulceration in rats. Scand J. Gastroenterol. , 34 (10); 962-966.
45. Ungureanu D. , Filip C. , Artenie A. , Artenie R. (2003): Evaluation of simvastatin antioxidant effects. Res. Med. Chir. Soc. Med. Nat. Lasi. 107(1),66-71.
46. Vaananen P.M. ; Meddings J.B. ; Wallace J.L. (1991): Role of oxygen derived free radicals in indomethacin-induced gastric injury. Am. J. Physiol. 261(3 pt 1) ; G470-G475.
47. Wainwright, C.L.(2005): Statins : is there no end to their usefulness ? Cardiovasc. Res.65(2), 296-298.
48. Werner, N. , Nickenig G. , Laufs U.(2002): Pleotropic effects of HMG-CoA reductase inhibitors. Basic Res. Cardiol. 97 , 105-116.
49. Wilhelmi, G. And Menasse- Gdynia, R. (1972): Pharmacology, 8: 321-328.Quoted from Sadik, S.A.E (1984)
50. Winzler R.J. (1955): Determination of serum glycoproteins. In : Glick, D.P. (Ed), Methods of biochemical Analysis .Interscience Publishers Inc.,New Work, pp,279-311.
51. Yang Y.J. ; Zhao J.L. ; You S.J. ; Wu J.Y. ; Jing Z.C. ; Gao R.L. ; Chen Z.J.(2007): Postinfarction treatment with simvastatin reduces myocardial no-reflow by opening of the K_{ATP}.E. ; Ch'nq J.L.; Mulderry P.K. ; Ghatei M.A.; Bloom S.R. (1985): Infusion of a novel peptide , calcitonin gene-related peptide (CGRP) in man.Pharmacokinetics and effects on gastric acid secretion and on gastrointestinal hormones.Regul Pept 10(2-3) ; 189-197.
52. Zhao J.L ; Yang Y.J. ; Cui C.J. ; You S.J. ; Gao R.L.(2006): Pretreatment with simvastatin reduces myocardial no reflow by opening mitochondrial K(ATP) channels . Br. J. Pharmacol. 149 (3) ; 243-9.

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