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Gastroprotective Effect of Garlic in Indomethacin Induced Gastric Ulcer in Rats

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# Title page :

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## Gastroprotective Effect of Garlic in Indomethacin Induced Gastric Ulcer in Rats

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## Abstract

Garlic, which is a natural plant, was documented to have a great history in the ancient medicine as a remedy for many diseases. **Objectives:** In our study, the gastroprotective effect of aged garlic extract (AGE) and the possible underlying mechanisms were investigated in an experimental model of indomethacin-induced gastric ulcer. **Methods**: Male Wistar rats were divided into **four groups**: (normal control, n=20), ulcer control (indomethacin group, n= 20), (omeprazole group, n=30) and (garlic group, n=20). Each of garlic and omeprazole was given to rats orally daily for 10 consecutive days before induction of ulcer by indomethacin. Indomethacin was given as single oral dose (100mg/kg). Four hours later after indomethacin treatment, the rats were sacrificed and gastric tissue was obtained for histopathological examination, calculation of ulcer index and measurement of oxidative stress markers as well as gastroprotective mediators. **Results:** The results showed that indomethacin induced gastric ulcer (ulcer index = 2900), was associated with significant increase of tumor necrosis factor-alpha (TNF-α) and malondialdehyde (MDA), and significant decrease of the gastroprotective mediators prostaglandin E2 (PGE2), glutathione (GSH) and nitric oxide (NO) compared to normal control. Pretreatment with AGE produced comparable results with those obtained in omeprazole group; the preventive index in AGE group was 83.4% compared to 94.5% in omeprazole group. The prophylactic role of AGE in indomethacin-induced ulcer was, in part, mediated by decreasing oxidative stress and increasing gastric level of PGE2, GSH and NO. **Conclusion:** AGE corrected the histopathological abnormalities in gastric tissue and proved a promising gastroprotective role in gastric ulcer.

**Key words**: aged garlic extract, gastroprotective mediators, glutathione, oxidative stress, malondialdehyde, nitric oxide, omeprazole, prophylactic role.

## INTRODUCTION

Complementary and alternative medicine (CAM) comprise medicinal products that aren't used as part of conventional medicine. Safety and easy intake are the most attractive advantages regarding CAM. Interestingly, natural products are gaining a worldwide attention especially in the developing populations **[1].**

Garlic, known as *Allium Sativum*, is a natural plant that belongs to family *liliaceace* **[2].** History of garlic in the ancient medicine would classify it as folk medicine or CAM **[3].** Garlic is cultivated and used in different cultures for centuries, as food and spice **[1].** It has been reported that garlic was used in the treatment of many diseases, like hypertension, and has antihypercholesterolemia, antiinflammatory and antioxidant activity **[4, 5].**

Aged garlic extract (AGE) is considered an important source of phytochemicals that possess antioxidant activity. These include lipid soluble organosulfur compounds, water soluble organosulfur compounds (e.g S-allylcysteine (SAC) and S-allylmercaptocysteine), flavonoids and phenolic compounds, which play an important role in scavenging free radicals **[5].**

Non-steroidal anti-inflammatory drugs (NSAIDS) are the most commonly used drugs throughout the world. The gastric damage caused by these drugs is known to be their most common and dangerous side effect **[6].** Indomethacin is a well known NSAID, with a high potential to cause gastric ulcer **[7].** Gastric ulcer is induced by the disturbance of the normal balance between defensive and aggressive factors in favor of the aggressive ones **[8].** Defensive factors involve mucus secretion, blood flow, cells renewal, prostaglandins (PGs) and nitric oxide (NO), while aggressive factors include NSAIDs, acid, oxidative stress, ethanol **[8, 9].**

In the current study, the gastroprotective effect of aged garlic extract and the possible underlying mechanisms were investigated in an experimental model of indomethacin induced gastric ulcer.

## MATERIALS AND METHODS

**Drugs:**

Indomethacin was obtained as a gift from Kahira Pharmaceuticals & Chemical Industries Co. (Egypt). Omeprazole was purchased from Carbosynth LLC. (USA). Aged garlic extract (AGE) was purchased from Hunan 3W Botanical Extract Inc. (China).

**Animal groups:**

The study was performed in accordance with the guidelines for the care and use of laboratory animals approved by Research Ethical Committee, Faculty of Pharmacy, Tanta University, Egypt. Male Wistar rats weighing 180-200 g (12-week-of-age) were obtained from the National Research Center, Giza (Egypt). The rats were housed in wire cages for 2 weeks for acclimatization and allowed free access to water and standard pellet diet. The starting weight of animals (230-250g).

Rats were randomly divided into 4 groups: Group 1 (control group, n=20), group 2 (ulcer group, n=20), group 3 (omeprazole group, n=30) and group 4 (garlic group, n=20)**. Note: the number of rats indicated here was larger than that used in statistics (i.e., n = 8) as the tissue from a single stomach was not enough for chemical measures and so pooling was required. In addition, the number of rats in Group 3 was higher (relative to that in other groups) as omeprazole affected gastric secretion to some extent and so the number of rats was increased to accommodate this effect.**

For rats in the **pre-treatment** groups, omeprazole (5 mg/kg) **[10]** or garlic (AGE) 200 mg/kg **[5]** was given orally (gavage) daily for 10 consecutive days; on the final day, these rats were given indomethacin, by single gavage of 100 mg/kg **[11]** for ulcer induction, 1 hr after the omeprazole or garlic dosing. Rats in Groups 1 and 2 (that had been dosed with vehicle in place of either drug for the 10 days) were dosed with, respectively, vehicle or indomethacin in parallel (in the final day).

All rats were fasted 24 hr prior to indomethacin oral treatment; over this period, the rats were kept in wide wire mesh-bottom cages to avoid coprophagia; in addition, water access was prevented for 2 hr prior to the indomethacin dosing. Four hours after the indomethacin/vehicle gavage, all rats were euthanized by ether and their stomachs excised.

**Methods:**

**measurement of gastric pH and ulcer index**

The stomach obtained was opened along the greater curvature then the gastric content was drained into a centrifuge tube. The gastric juice was centrifuged at 1000 rpm for 10 min (4°C), the clear supernatant was recovered and the pH measured **[12]** using a pH 211 meter.

For pH measurement, each stomach was washed by saline after drainage of the juice and examined by a magnifying lens. Ulcer score was calculated as the mean of ulcers in each group (total number of ulcers divided by rats number, n=8), ulcer index (U.I) was then calculated by multiplying ulcer score x100 **[13].** The preventive index was calculated according to the method of **Hano et al. [14]** Preventive index: (UI of ulcerated group - UI of treated group x 100) / UI of ulcerated group .

**Determination of gastric glutathione (GSH) and malondialdehyde (MDA)**

To prepare tissue for assaying stomach levels of glutathione (GSH) and malondialdehyde (MDA) assays, 250 mg tissue was homogenized in 2.5 ml potassium phosphate buffer (pH 7.5) using a Polytron homogenizer (PT 3100, Zurich, Switzerland) and then centrifuged at 4000 rpm for 15 min at 4°C.

The concentration of reduced GSH in the stomach tissue homogenate was determined colorimetrically with method described by **Beutler et al. [15]**, using a kit from Biodiagnostics, Egypt, according to manufacturer instructions. Reduction of 5,5`-dithiobis 2-nitrobenzoic acid (DTNB) by reduced GSH to give yellow product that was measured at 412 nm in a Unico 2100 spectrophotometer. Levels were then calculated using a kit-provided formula and presented as mg /g tissue.

The concentration of MDA in stomach tissue homogenate was determined colorimetrically using a kit from Biodiagnostics, Egypt, and following a method described by **Satoh [16]** and **Ohkawa et al. [17].** In the protocol, thiobarbituric acid (TBA) reacts with MDA present in the sample [in acidic medium, at 95$^{o}$C for 30 min] to form TBA-reactive products (TBARS). The absorbance of these pink products was then measured at 532 nm in the spectrophotometer. Levels were then calculated using a kit-provided formula and presented as nmol /g tissue.

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**Determination of stomach nitric oxide (NO)**

For tissues used for assaying stomach nitric oxide (NO) levels, 250 mg tissue was homogenized in 2.5 ml ice-cold normal (0.9 %) saline**.** Thereafter, 1 ml absolute ethanol was added to 0.5 ml homogenate to precipitate the proteins and the samples were then centrifuged at 3000 rpm for 10 min at 4°C.

The gastric nitric oxide was determined by measuring its nitrite (an indicator of original NO present). This method depends on reduction of nitrate to nitrite by vanadium trichloride (VCl3) followed by addition of Griess reagent (Miranda et al., 2001). In brief, a sample of homogenate supernatant (500 µl) was mixed with an equal volume of $VCl_3$ and of Griess reagent (0.2% naphthylethylenediamine and 2% sulphanilamide in 5% hydrochloric acid). After incubation at 37°C for 30 min, the absorbance of the mixture was measured at 540 nm in the spectrophotometer **[18].** Sodium nitrite standards assessed in parallel, values were compared to it, and the nitrite concentration in each sample was calculated and presented as nmol NO/g tissue.

**Determination of prostaglandin $E_2$ ($PGE_2$) and tumor necrosis factor (TNF)-**

Enzyme-linked immunosorbent assay (ELISA) kits were utilized for measurement of gastric (stomach tissue homogenate supernatant) content of PGE2 and TNF-α following the protocol provided by the manufacturer. The kits were obtained from CUSABIO Biotech Co., Ltd (China) and Glory Science Co., Ltd (China), respectively.

**Histopathology**

Stomach was fixed in formalin (10%) for 24 h, embedded in paraffin to form blocks, which were serially sectioned (5µm thick) using a Leica RM2135 microtome, mounted on glass slides, then stained by hematoxylin and eosin solution then evaluated under light microscope by certified pathologist (in blinded manner). The pathologist is Prof. Dr. Mona A. Yehia, Medical Research Center, Alexandria, Egypt.

**Statistical analysis:**

Analysis of data was performed by Statistical Package for Social Science (SPSS) software version 17.0. Data are presented as mean ± SD. Statistical comparison among groups was performed by one-way analysis of variance (ANOVA). Statistical significance was set at $p<0.05$.

## RESULTS:

**Effect on gastric acidity, ulcer index and preventive index:**

Administration of indomethacin showed a significant decrease in gastric pH (↓41.06%) when compared to normal control group. Pretreatment with AGE significantly raised the gastric pH to 3.38±0.09, whereas pretreatment with omeprazole significantly increased the gastric pH to 5.13±0.14, which was above the control value **(Table 1).**

The ulcerated indomethacin group showed ulcer score of 29±1.7 and UI of 2900. Rat groups treated with AGE or omeprazole prior to indomethacin showed a significant decrease of ulcer score, which was 4.8±1.4 and 1.6±0.51, respectively. Omeprazole showed a greater gastroprotective effect with UI of 160 and preventive index 94.5%, while AGE showed UI of 480 and a preventive index of 83.4% **(Table 1).**

**Effect on gastric TNF-α:**

Gastric TNF-α was significantly increased in indomethacin group when compared to normal control (3.26 fold increase). Pretreatment with either omeprazole

or AGE decreased gastric TNF-α level significantly when compared to ulcer control group (↓64.72% and ↓53.1%, respectively) **(Figure 1).**

**Effect on gastric oxidative stress markers:**

Indomethacin group showed a significant increase (2.2 fold increase) in gastric MDA but a significant decrease (↓62.63%) in gastric GSH when compared to normal control. Pretreatment with either AGE or omeprazole showed a significant decrease in gastric MDA when compared to ulcer control group (↓43.12% and ↓50.54%, respectively) **(Table 2).** A significant increase in gastric GSH was observed in both omeprazole and AGE groups when compared to ulcer control group (2.48 fold and 2.04 fold increase, respectively) **(Table 2).**

**Effect on cytoprotective mediators:**

Gastric NO and PGE2 decreased significantly in indomethacin group when compared to normal control group (↓57.9% and ↓44.1%, respectively). Compared to indomethacin group, the rats pretreated with omeprazole or AGE exhibited a significant elevation of gastric NO (2.35 fold and 2.3 fold increase, respectively) and gastric PGE2 (1.71 fold and 1.59 fold increase, respectively) **(Table 3).**

**Histopathological results:**

Stomach sections from indomethacin group showed gastric erosions, and infiltration of inflammatory cells **( Figure 3)** compared to normal control group (**Figure 2).** On the other hand, stomach sections from omeprazole group showed

intact gastric mucosa and slight dilatation in mucosal glands **(Figure 4),** while garlic group showed focal erosion with mild inflammatory cells infiltration **(Figure 5)**.

**Table (1): Gastric pH, ulcer index and preventive index in the studied groups**

| Groups | Gastric pH | Ulcer score | Ulcer index | Preventive index* |
|---|---|---|---|---|
| **Normal control** | 3.58±0.1 | - | - | - |
| **Indomethacin (ulcer control)** | 2.11±0.14† | 29 ±4.8 | 2900 | - |
| **Omeprazole** | 5.13±0.14¶ | 1.6 ±0.51‖ | 160 | 94.5% |
| **Garlic (AGE)** | 3.38±0.09§ | 4.8± 1.4‖ | 480 | 83.4% |

Each value expressed as mean±SD (n=8 rats/group).
* Preventive index expressed as percentage.
† p<0.05 when compared ulcer control with normal control.
¶ p<0.05 when compared omeprazole with normal and ulcer control.
§ p<0.05 when compared garlic with normal, ulcer control and omeprazole.
‖ p<0.05 when compared omeprazole and garlic with ulcer control.

**Table (2): Oxidative stress markers in the studied groups**

| Groups | MDA* | GSH** |
|---|---|---|
| **Normal control** | 143.72±13.3 | 405.59 ±46.9 |
| **Indomethacin (ulcer control)** | 315.94±28.5† | 151.58±23.5† |
| **Omeprazole** | 156.25±10‡ | 376.56±40.1‡ |
| **Garlic (AGE)** | 179.7±22.9§ | 308.6±29.7§ |

MDA: malondialdehyde; GSH: reduced glutathione.
Each value expressed as mean±SD (n=8 rats/group).
*****MDA levels were expressed as (nmol/g tissue).
******GSH levels were expressed as (mg/g tissue).
† p<0.05 when compared ulcer control with normal control.
‡ p<0.05 when compared omeprazole with ulcer control.
§ p<0.05 when compared garlic with normal, ulcer control and omeprazole.

**Table (3): Cytoprotective mediators in the studied groups**

| Groups | PGE2* | NO** |
|---|---|---|
| **Normal control** | 45.02± 4.5 | 819.47±20.4 |
| **Indomethacin (ulcer control)** | 25.18 ± 2.07† | 345.1 ±10.6† |
| **Omeprazole** | 43.04 ± 1.28‡ | 811.61 ± 14.99‡ |
| **Garlic (AGE)** | 39.95 ± 1.67§ | 793.04 ± 12.8§ |

PGE2: prostaglandin E2; NO: nitric oxide.
Each value expressed as mean±SD (n=8 rats/group).
*PGE2 values were expressed as Data are presented as (pg/g tissue).
**Nitric oxide values were expressed as (nmol/g tissue).
† p<0.05 when compared ulcer control with normal control.
‡ p<0.05 when compared omeprazole with ulcer control.
§ p<0.05 when compared garlic with normal, ulcer control and omeprazole.

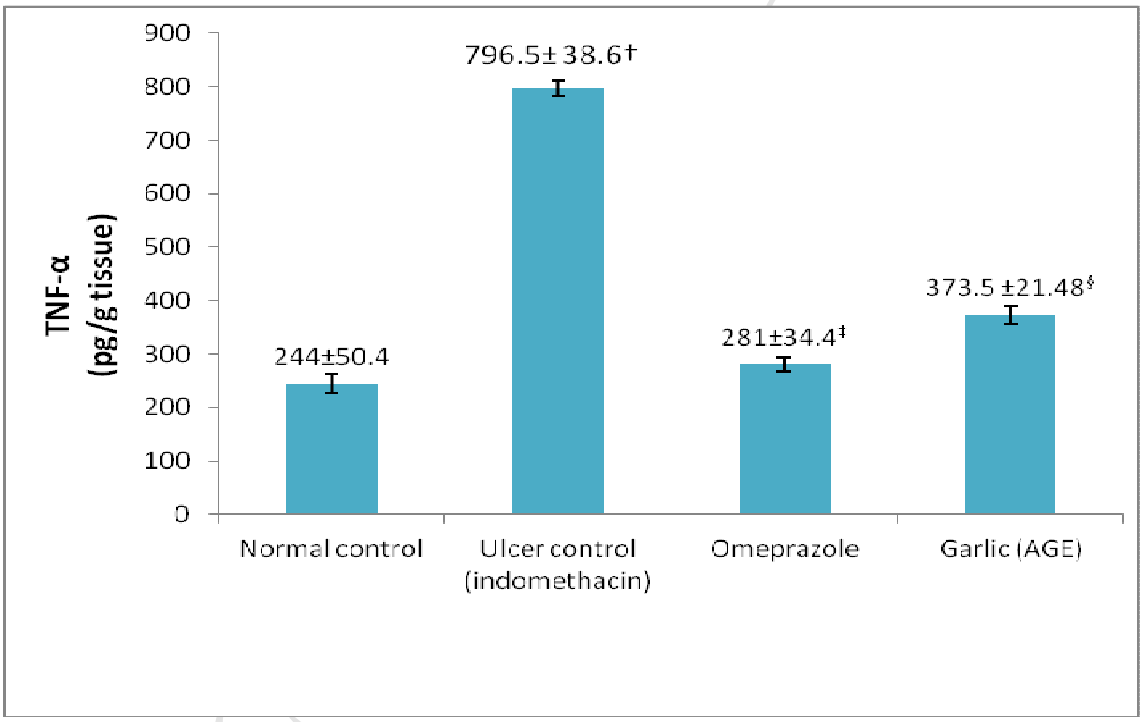


**Figure 1: Gastric TNF-α in the studied groups.**
Each value expressed as mean±SD (n=8 rats/group).
† p<0.05 when compared ulcer control with normal control.
‡ p<0.05 when compared omeprazole with ulcer control.
§ p<0.05 when compared garlic with normal, ulcer control and omeprazole.

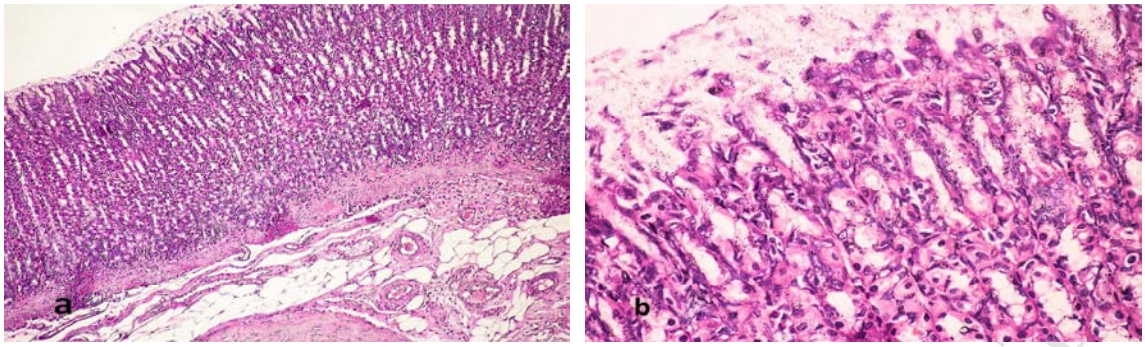


**Figure 2: Sections of gastric mucosa of normal control group (H&E).**

**(a):** Normal gastric mucosa covered with surface mucus, and more gastric glands at the bottom **(X100)**. **(b):** Normal parietal cells with central rounded nuclei are dispersed throughout the glands **(X400).**

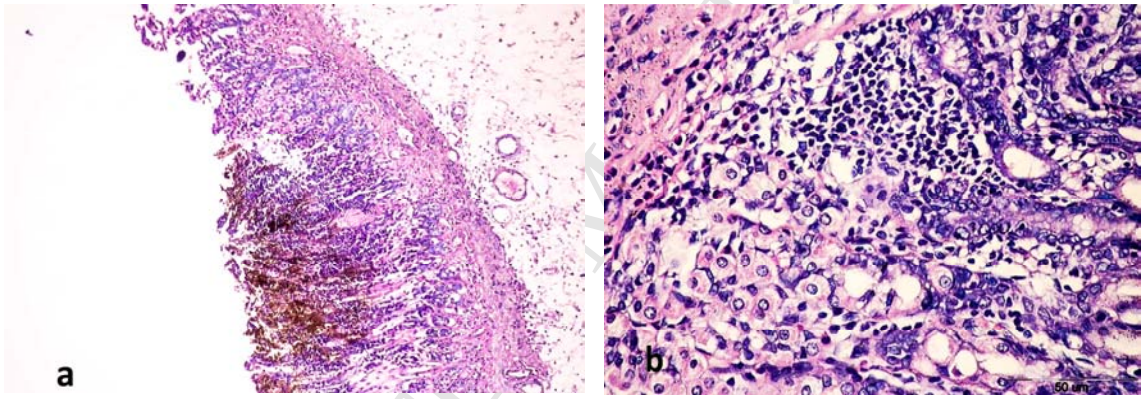


**Figure 3: Sections of gastric mucosa from ulcer control group (H&E).**

**(a):** The section indicates areas of tissue damage, loss of the epithelial layer and gastric pits and distorted arrangement of glands in addition to inflammatory cells infiltration in mucosa and submucosa **(X100)**. **(b):** The section indicates inflammatory cells infiltration of the lamina propria with a loss of normal architecture of glandular cells **(X400).**

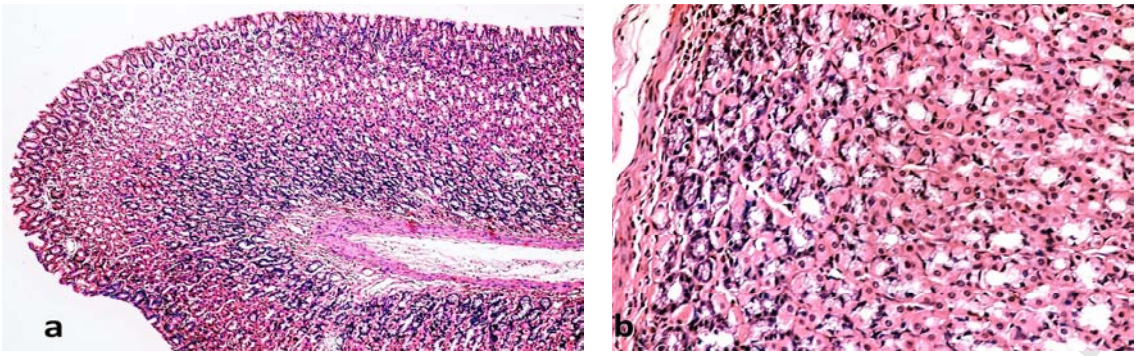


**Figure 4: Sections of gastric mucosa from omeprazole pretreated group (H&E).**

**(a):** The section indicates normal gastric mucosa and mild loss of surface mucus **(X100)**. **(b):** Gastric mucosa like normal and gastric glands with slight dilatation **(X400).**

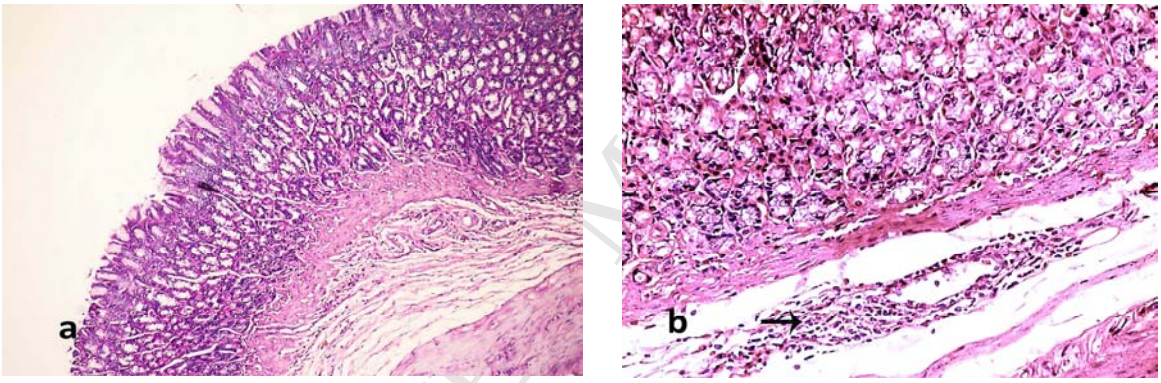


**Figure 5: Sections of gastric mucosa from garlic pretreated group (H&E).**

**(a):** The section indicates focal erosion with desquamation of the superficial layer **(X100). (b):** Submucosa shows inflammatory cells infiltration (arrow) **(X400)**.

## DISCUSSION

Indomethacin has higher ulcerogenic potential than other NSAIDs, thus it was considered as the drug of choice for gastric ulcer experimental induction **[7].** In the present study, indomethacin (100mg/kg) given orally to rats produced an increase of gastric acidity and ulcer index, compared to normal group. Our findings were in line with previous reports, which demonstrated that indomethacin induces gastric ulcer

through increasing gastric acidity **[19, 20, 21].** The ulcer manifestations, herein, were alleviated in rat groups pretreated with either omeprazole or garlic extract. The gastric pH in omeprazole group was raised above the normal control group, while garlic extract decreased the gastric acidity to relatively normal level. Omeprazole was superior to garlic extract with a higher preventive index .

The histopathological examination of gastric tissue in our ulcer model indicated obvious ulcer injury. These observed change were consistent with the significant elevation of gastric level of the inflammatory mediator TNF-α measured in our study. TNF-α was known to play an important role in pathophysiology of indomethacin-induced gastric ulcer mainly through activating neutrophil infiltration **[7]**.

The present histopathological findings supported the gastroprotective effects of omeprazole and garlic extract and revealed relatively normal mucosa in rats pretreated with omeprazole and erosion in rats pretreated with garlic extract. Moreover, aged garlic extract, herein, showed a significant decrease in gastric TNF-α. Previous reports documented the ability of the garlic constituents allicin, SAC and diallyldisulfide to inhibit activation of nuclear factor kappa B, and the ability of SAC to inhibit translocation of factor kappa B to the nucleus, and hence, inhibit TNF-alpha production**[22, 4, 23].**

In the present work, omeprazole also exhibited an antiinflammatory effect as shown by the histopathological results and by the suppression of the level of TNF-α in gastric tissue. **Abood and coworkers [24]** reported that omeprazole administration, in a model of ethanol-induced ulcer, caused a significant decrease in serum TNF-α.

The inflammatory insult produced by indomethacin could be mediated by induction of oxidative stress **[7, 5].** Our results were in line, as single dose of indomethacin caused a significant increase in gastric MDA, while gastric GSH was significantly decreased. Garlic extract showed a protective effect and alleviated the oxidative stress in gastric tissue. AGE contains many bioactive products like S-allylcysteine, S-allylmercaptocysteine, diallylsulfide, flavonoids, selenium, saponins and phenolic compounds, which act together to give AGE its antioxidant property **[5].** Garlic phenolic compounds exert free radical scavenging activity through donating its hydrogen atom from the hydroxyl group **[5].** Moreover, garlic extract was found to decrease activity of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) thus decreasing lipid peroxidation **[25].**

Omeprazole pretreatment in our study was found to correct the gastric levels of MDA and GSH, indicating antioxidant property. Omeprazole possesses antioxidant activity and decreases lipid peroxidation by scavenging hydroxyl radicals **[26]** and reacting with hypochlorous acid, which is known as the most toxic oxidant **[27].** In the present work, omeprazole pretreatment returned both MDA and GSH levels to near the normal, whereas garlic extract pretreatment partially corrected gastric MDA and GSH levels with a significant difference from the control values.

Measurement of the gastroprotective mediators PGE2 and NO in our work indicated a significant decrease in indomethacin group. Meanwhile, a significant improvement in the gastric levels of PGE2 and NO was observed in rats pretreated with omeprazole or garlic extract.

Decreased synthesis of PGE2 and disruption of NO could participate in the induction of gastric ulcer induced by NSAIDs like indomethacin **[7, 20, 8].**

Prostaglandin E2 protects the gastric mucosa by increasing mucus secretion, maintaining blood flow and decreasing acid secretion **[28, 7]**. **Abood et al. [24]** reported the ability of PGE2 to inhibit TNF-α production, which was confirmed by our findings. Moreover, the gastroprotective mediator NO regulates gastric pH and increases blood flow through its vasodilatation effect **[29].** NO exerts its vasodilatation effect through activation of soluble guanylcyclase which in turn converts GTP to c-GMP that leads to vasodilatation of vascular smooth muscle of the endothelium **[30].**

The increase in gastric NO by garlic extract, herein, could be explained on the basis that AGE activates nitric oxide synthesis and restores its bioavailability **[31].** Aged garlic extract could enhance the synthesis of nitric oxide by increasing the activity of constitutive nitric oxide synthase (cNOS), which may be due to garlic extract ability to accelerate calcium influx **[32].** Previous reports confirmed the ability of garlic to maintain endothelial function through NO pathway **[33, 34].** It has been reported that NOS and COX enzymes had a positive mutual interaction, and both NO and PGE2 act side by side in the gastroprotection process **[35, 36, 37].** This interaction could explain the NO-dependent elevation of gastric PGE2, herein, exerted by pretreatment with garlic extract.

Omeprazole exhibited similar results and increased the gastric level of NO and PGE2. Earlier reports documented the ability of omeprazole to increase gastric NO level and protect the gastric tissue against ethanol induced ulcer in rats **[38],** which may be through activating cNOS enzyme thus restoring nitric oxide level **[39].** Pretreatment with omeprazole, herein, increased gastric PGE2 and this observed effect was in line with other reports which documented the ability of omeprazole to increase PGE2 level in rats with ethanol induced ulcer **[24].** Omeprazole was

documented to have anti-ulcerative activity through alpha-2 adrenergic receptor, which has a direct correlation with gastroprotective cyclooxygenase-1 (COX-1) and PGE2 [**40, 41].** In addition, omeprazole upregulates cyclooxygenase-2 (COX-2) and PGE2, which play an important role in ulcer healing through re-epithelization **[42].**

## CONCLUSION

Garlic extract proved an antioxidant and antiinflammatory activities required for maintaining the gastric mucosa homeostasis, thereby, it could keep the required balance between aggressive and defensive factors in the stomach. Garlic extract could be used as a prophylactic therapy for patients who have high tendency to gastric ulceration. Although omeprazole was superior to garlic extract and returned the gastroprotective parameters to near normal, garlic extract retains the advantage of being a natural product with no reported side effects. The gastroprotective effect of garlic extract may require further investigation in other experimental models as well as in clinical settings.



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## Conflict of Interest statement

The authors declare that there are no conflicts of interest.

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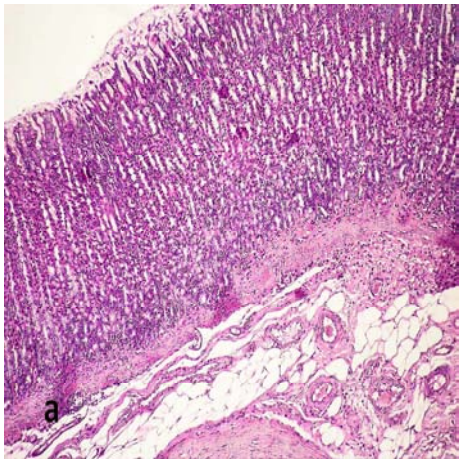

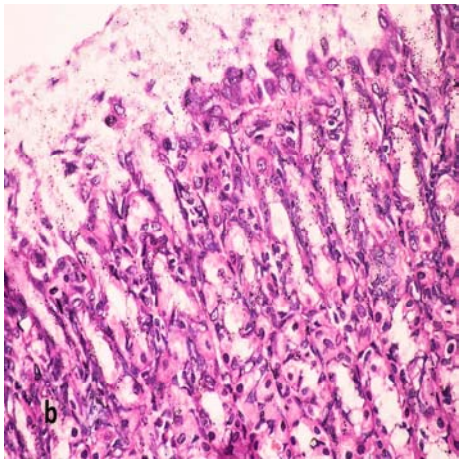

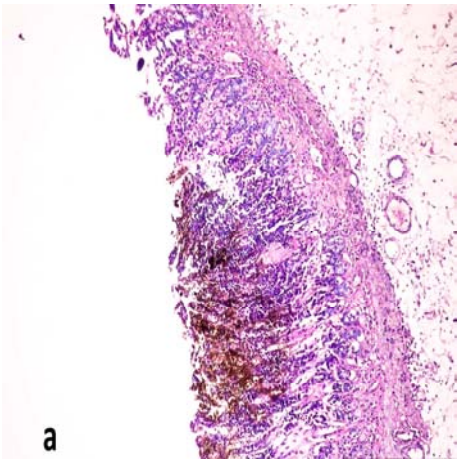

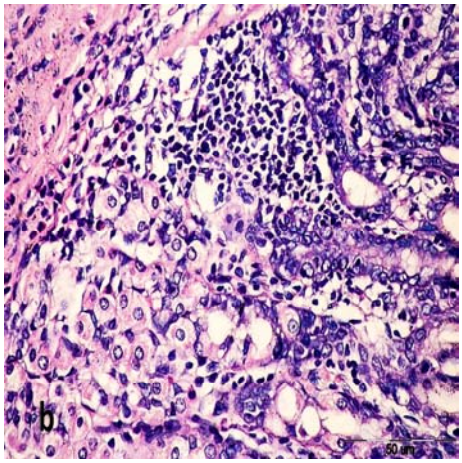

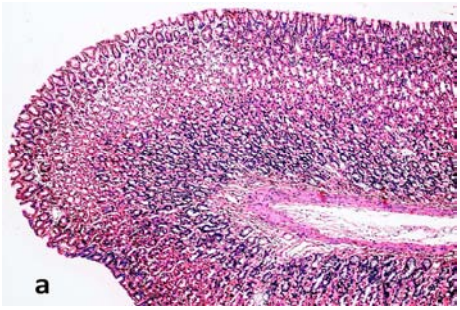

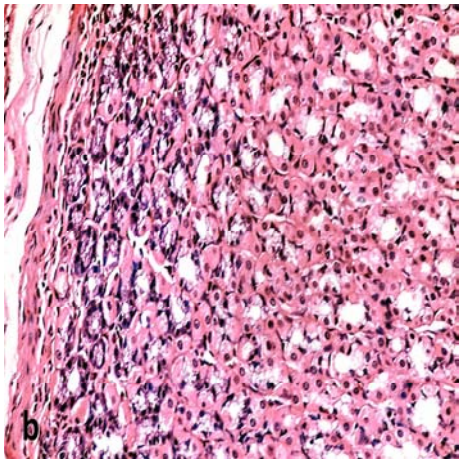

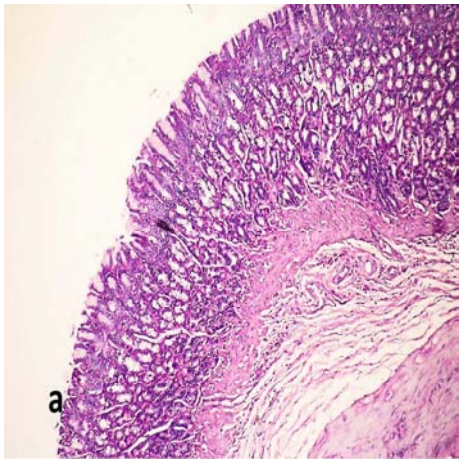

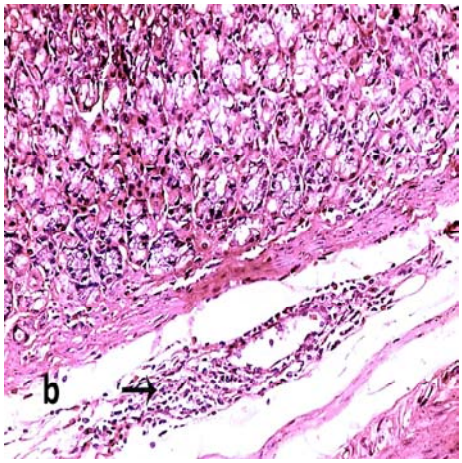

# Highlights

- Garlic is a natural plant with many beneficial effects
- Gastric ulcer is worldwide disease
- Ulcer was induced by indomethacin
- Garlic was given as aged garlic extract
- Garlic decrease inflammatory mediators and increase antioxidants
- Garlic showed antiinfalmmatory and antioxidants effect in rats pretreated with it before indomethacin
- Garlic considered a promising herbal plant for protection against ulcer