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# Aqueous suspension of anise exerted an additive gastroprotective effect to misoprostol against indomethacin-induced gastric ulcers in rats

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## ABSTRACT

This work aimed to study the anti-ulcer activity of the combination of the natural product; aqueous anise suspension (Aniseed) with the standard drug misoprostol, against gastric ulcer induced by a non-steroidal anti-inflammatory drug (NSAID); indomethacin, in addition to the possible related anti-oxidant and anti-inflammatory mechanisms. Single oral pretreatment of Aniseed and its combination with misoprostol were given 30 min. before indomethacin induced acute gastric ulceration. The experiment ended four hours later and rat stomachs were tested for the presence of ulcers, then lipid peroxidation, the antioxidant and anti-inflammatory effects were assessed: gastric malondialdehyde (MDA), gastric superoxide dismutase (SOD) activity, and plasma catalase activity, gastric prostaglandin E2 (PGE2), and serum C-reactive protein (CRP) levels. All pretreatments significantly reversed the indomethacin induced increase in ulcer scores, the highest was aniseed combination, followed by misoprostol, then aniseed. Indomethacin produced a significant decrease in gastric SOD activity and PGE2 level, while produced a significant rise in plasma catalase activity and serum CRP level. All pretreatments produced a significant decrease in plasma catalase activity. Pretreatment with the standard drug; misoprostol reversed all the indomethacin induced changes in the biochemical markers. However, misoprostol had no effect on serum CRP level, even after it is combined with aniseed. All pretreatments produced a significant increase in gastric PGE2 level, except for the combination group. The combination was superior to other treatments in its ulcer healing effect, and only ameliorated gastric SOD activity, compared to indomethacin. We concluded that, aniseed combination with misoprostol has a remarkable ulcer healing effect and almost produced a complete protection from gastric ulcers induced by indomethacin, in addition to some additive anti-oxidant effect to misoprostol. Therefore, it is recommended to take anise, while receiving a treatment with misoprostol

***Keywords:*** *Anise; Aniseed; Indomethacin; misoprostol; gastric ulcer; PGE2*

## 1. Introduction

Gastric ulcer (GU) is the most prevalent gastrointestinal disorder induced by various factors and non-steroidal anti-inflammatory drugs (NSAIDs) as one of the most common reasons (Yekta, Amiri-Dashatan, Koushki,

Dadpay, & Goshadrou, 2019) and (Sabiu et al., 2015). The continuous generation of prostaglandins by cyclooxygenase isoenzymes in the gastric mucosa helps to maintain an adequate mucosal blood flow and also stimulates the generation of mucus (Takeuchi & Amagase, 2018).

NSAIDs inhibit cyclooxygenase and thereby reduce the intrinsic ability of the mucosa to resist injury induced by endogenous and exogenous aggressors. They can induce gastric ulcers through various processes, including generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis (Antonisamy et al., 2014). Decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretions which in turn, aggravate the ulcer (Wallace, 2012).

The dominant factor in the treatment of ulcers induced by anti-inflammatory drugs is the mucosal resistance against oxygen-derived free radicals, which play an important role in the pathogenesis of acute experimental gastric lesions. Thus, much attention has been recently focused on ROS contents, such as superoxide, hydroxyl radicals (OH) and singlet oxygen (Nasuti, Gabbianelli, Falcioni, & Cantalamessa, 2006).

Antioxidant defense systems, including antioxidant enzymes, foods and drugs are important in the prevention of the toxic ROS effects (Pedersen, Cribb, Read, French, & Banse, 2018). Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers. Thus, in recent years, a widespread search has been launched to identify new anti-ulcer drugs from natural sources (Cheng, Lu, & Yen, 2017). Unfortunately, current evidence of the scientific validity of many of these traditional and commercial compounds is severely limited (Fernandes et al., 2012).

Pimpinella anisum L. Umbelliferae is an annual herb their fruits are locally known as aniseed and yansoon. Anise spice is added to foods in several forms as whole spice, as ground spice or as isolates from its extracts and volatile oils (Al Mofleh, Alhaider, Mossa, Al-Soohaibani, & Rafatullah, 2007). Its extract is a source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. Anise and its compounds have been identified as free radicals or active oxygen scavengers (Gülçın, Oktay, Kıreççı, & Küfrevıoğlu, 2003; Vázquez-Fresno et al., 2019). However, the components responsible for the antioxidant activity of the extract are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and identification of the antioxidant components in the seed.

Misoprostol (cytotec), a synthetic prostaglandin E1 analogue that was designed for the prevention and treatment of peptic ulcer associated with the use of nonsteroidal anti-inflammatory drugs (Drini, 2017). The most commonly reported adverse effect of long term taking misoprostol to reduce the risk of NSAID-induced gastric ulcers is diarrhea and abdominal pain (Wallace, 2012). It showed a rapid increase in healing ulcers through enhancement of endogenous PGE2 and elevation in COX-2 expression, so we used it as a reference anti-ulcer drug (Poonam, Vinay, & Gautam, 2005).

Therefore, the aim of the present work is to study the gastroprotective activity of aqueous anise suspension, and its combinations with the anti-ulcer drug misoprostol, and the possible additive gastroprotective effect and the related antioxidant and anti-inflammatory mechanisms, when both drugs are administered with each other.

## 2. Material and methods

### *2.1. Drugs and chemicals*

Gum acacia powder, liometacen (Nile Co., Cairo, Egypt), cytotec (Pfizer, USA), and heparin (Nile Co., Cairo, Egypt).

Antioxidants assay kits (Biodiagnostic, Egypt), immunoassay kits: PGE2 ELISA kit (Neogen Co., Lexington KY, USA) and hs-CRP Accubind ELISA kit (Monobind Inc., Lake Forest, CA, USA). The chemicals used were all of analytical reagent grade. All drugs and reagents were prepared immediately before use.

### *2.2. Preparation of plant material*

Seeds of anise “Pimpinella anisum L” (family, Apiaceae), were purchased from (Sekem Co., Egypt) and identified under expert guidance and preserved for future reference The seeds were ground to very fine powders (75 micron), and used as an aqueous suspension for treatment in different experiments (10).

*2.3. Animals*

Adult male rats weighing about (180–220 gm), were raised in the animal house of the Faculty of Pharmacy, Zagazig University, were used. The animal room was well ventilated with a 12 h light/dark cycle throughout the experimental period. They were maintained in clean, sterile, polypropylene cages and fed with regular rat chow and water ad libitum and were left to accommodate for one week. All rats were deprived of food for 18 h before subjecting to ulcerogens, but allowed free access to water and were allocated to different experimental groups. In the day of the experiment; their weights had been measured. The study was approved by the institutional ethical committee, which follows the guidelines of CPSCEA (Committee for the Purpose of Control and Supervision of Experimental on Animals), which complies with international norms of INSA.

*2.4. Experimental protocol*

Rats were randomly classified into seven groups (eight rats per each);

(1) Control group; no treatment is administered.

(2) Indomethacin group; in which acute gastric ulcers were induced by administration of indo (62.5 mg /kg, p.o.) in water vehicle (Bhattacharya, Banerjee, Bauri, Chattopadhyay, & Bandyopadhyay, 2007).

(3) Misoprostol + indo group; in which rats were pretreated with misoprostol (100µg/kg, p.o.) suspended in 2% gum acacia (Cavallini, Andreollo, Metze, & Araújo, 2006).

(4) Aqueous anise suspension + indo group; in which rats were pretreated with Aniseed (250mg/kg, p.o.).

(5) Aniseed + Misoprostol + indo group, receiving the same treatments as above

All pretreatments are administered orally 1hr prior to induction of gastric ulcers by indomethacin. Four hours later, rats were anaesthetized with diethylether (de Barros, Sousa, Bastos, & de Andrade, 2007). Blood is collected in heparinized and non heparinized tubes, centrifuged and the supernatants were obtained for biochemical analysis. Animals were sacrificed by cervical dislocation, the stomachs were removed, and opened along the greater curvature, then washed with saline and gastric mucosa was examined for ulcers with the help of magnifying lens and expressed as ulcer index, then stomach lesions were immediately frozen in liquid nitrogen and stored at −70 °C until further investigation.

*2.5. Determination of ulcer index (UI)*

Digital pictures of the mucosal surface of each stomach, were taken for macroscopical examination of ulcers, Ulcers were scored according to the method of Valcavi et. al. (Valcavi et al., 1982), and assessed on the basis of their dimensions : Deep circular ulcers more than 8 mm = 10 ; 7–8 mm = 8 ; 6–7 mm = 7 ; 5–6 mm = 6 ; 4–5 mm = 5 ; 3–4 mm = 4 ; 2–3 mm = 3 ; 1–2 mm = 2 and 0–1 mm = 1. The deep linear ulcers more than 10 mm in length = 6 and linear ulcer less than 10 mm in length = 3. The score for each single lesion were then summed up for the determination of ulcer index.

The protective ratio (%) was calculated according to the following formula (Chen et al., 2005; Saremi et al., 2019):

Preventive ratio (%) = (a-b)/a X 100

a: the ulcer index of the ulcerated group

b: the ulcer index of the experimental group

Protective effects of aniseed, and its combination with misoprostol were compared with the results obtained from indomethacin and misoprostol groups.

*2.6. Biochemical analysis*

Glandular segments of stomach were removed, and a 10% homogenate was prepared, and further subjected to biochemical analysis & immunoassays.

*2.6.1. Lipid peroxidation biomarker: gastric malondialdehyde (MDA) level*

Malondialdehyde (MDA), a marker for lipid peroxidation, was estimated in gastric tissues by the thiobarbituric acid (TBA) method (Uchiyama & Mihara, 1978), and results are expressed as nanomol MDA per gram wet tissue (nmol g tissue−1).

*2.6.2. Antioxidant biomarkers: plasma catalase activity and gastric superoxide dismutase (SOD) activity*

The superoxide dismutase (SOD) activity, a marker for oxidative stress, was estimated in gastric tissues by the riboflavin photoreduction method (Nishikimi, Rao, & Yagi, 1972), and results are expressed as unit per gram wt tissue (U g tissue−1).

The catalase activity, a marker for oxidative stress, was estimated in plasma by measuring the rate of decomposition of H2O2 at 240 nm (Aebi, 1984), and results are expressed as micromole per litre of plasma (μmol L−1).

*2.6.3. Inflammatory biomarkers: gastric Prostaglandin E2 (PGE2) level and serum C-reactive protein (CRP) level*

Prostaglandin E2 (PGE2) level, a marker for inflammation, was estimated in gastric tissues by enzyme-linked immunosorbent assay (ELISA), and results are expressed as nanogram per gram wet tissue (ng g tissue−1)

C-reactive protein (CRP) level, a marker for inflammation, was estimated in serum by enzyme-linked immunosorbent assay (ELISA), and results are expressed as microgram per milliliter of serum (μg ml−1)

*2.7. Statistical analysis*

All data are expressed as mean ± standard error of the mean (S.E.M.) for eight rats per experimental group. Statistical analysis was performed with SPSS statistical software program the version 16.0. One-way analysis of variance (ANOVA) followed by post hoc test (LSD) was used to compare the mean values of quantitative variables among the groups. The p value less than or equal to 0.05 were considered statistically significant.

## 3. Results

Macroscopical examination of the stomach mucosa showed that a single oral dose of indomethacin (62.5 mg/kg), caused a massive production of acute gastric ulcers. All pretreatments misoprostol (100μg/kg), aqueous anise extract (Aniseed, 250mg/kg) and their combination, significantly reduced the ulcer index, in comparison with the indomethacin group.

The combination group demonstrated the highest significant inhibition of gastric ulcers and almost completely caused the disappearance of ulcers (98.49 %), followed by misoprostol (79.69 %), then aniseed (34.54 %), as shown in Fig 1, Fig 2 and Table 1. These results indicated that addition of aniseed produced an additive anti-ulcer effect to misoprostol.

Indomethacin group produced a significant increase in gastric MDA levels compared to the control group. In addition, indomethacin caused a severe oxidative stress with a significant increase in serum catalase activity and a significant decrease in gastric SOD activity. Moreover, Indomethacin caused severe inflammation accompanied by a significant decrease in gastric PGE2 level and a significant increase in plasma catalase activity, compared to the control group, as shown in Fig 3, Fig 4 and Fig 5. All pretreatments had no significant effect on gastric MDA level, compared to indomethacin group, as shown in Fig 3

Pretreatment with misoprostol had a remarkable antioxidant effect as misoprotol produced a significant increase in gastric SOD activity and a significant decrease in plasma catalase activity. In addition, misoprostol had an anti-inflammatory effect through producing a significant increase in gastric PGE2 level, and a moderate decrease in serum CRP level, compared to indomethacin, as shown in Fig 4 and Fig 5

Pretreatment with aniseed had an antioxidant effect through producing a significant decrease in plasma catalase activity and significant increase in gastric SOD activity. In addition, aniseed had an anti-inflammatory effect, through producing a significant decrease in serum CRP level, without affecting gastric PGE2 level, compared to indomethacin group, as shown in Fig 4 and Fig 5.

Pretreatment with the combination had an antioxidant effect through producing a significant decrease in plasma catalase activity without affecting gastric SOD activity. Additionally, the combination had an anti-inflammatory effect through producing a significant increase in gastric PGE2 level and a mild decrease serum CRP level, compared to indomethacin group, as shown in Fig 4 and Fig 5

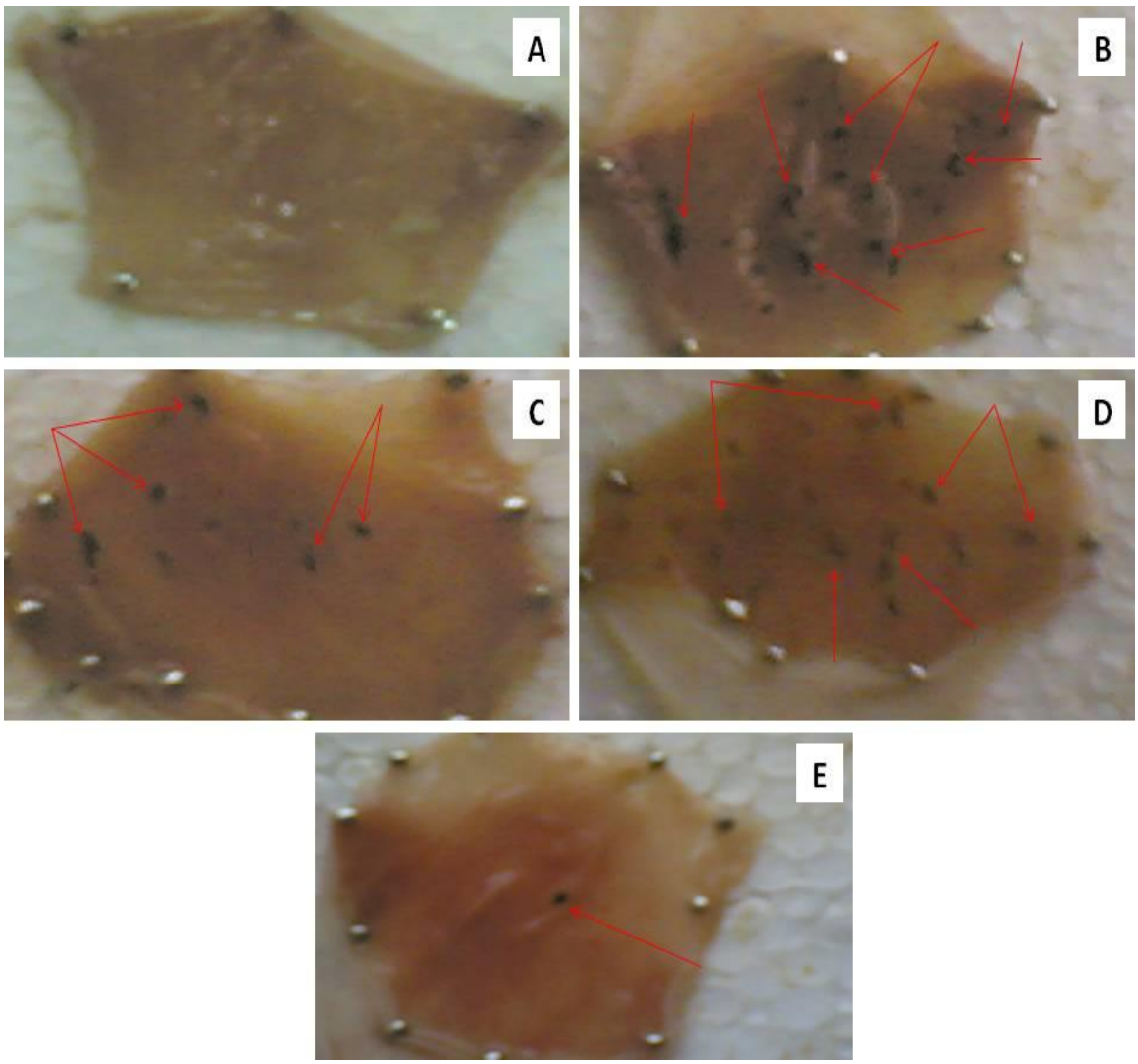


**Fig. 1. Pictures of rat stomachs cut along the greater curvature**

**A**: control group; **B**: indomethacin group; **C**: misoprostol + indo group; **D**: Aniseed + indo group; **E**: Aniseed + misoprostol + indo. The combination group demonstrated the highest significant inhibition of gastric ulcers, followed by misoprostol, then aniseed

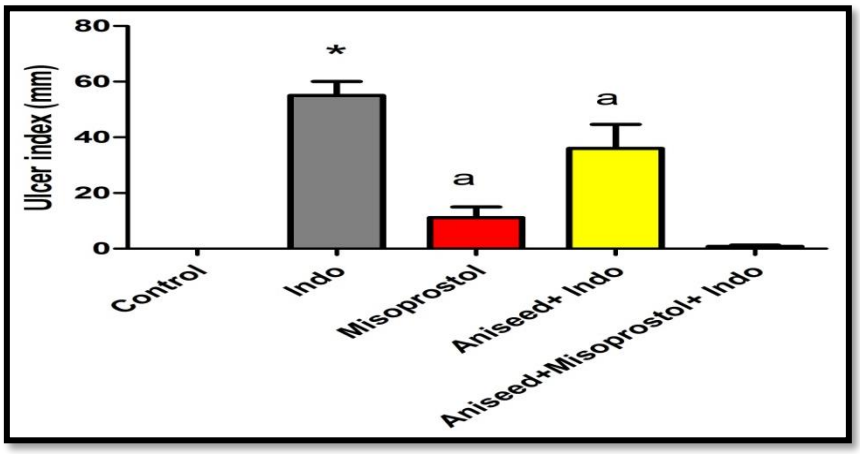


**Fig 2. The anti-ulcerogenic effect of single oral pretreatments of misoprostol (100µg/kg), aniseed (250mg/kg) and their combination against indomethacin-induced gastric ulcers in rats**
Values are presented as mean ± S.E
***** Significantly different from control group at $p \leq 0.05$
**a** Significantly different from indomethacin group at $p \leq 0.05$

**Table 1. Effect of indomethacin (indo, 62.5mg/kg, i.g.) and gastroprotective effect of single oral pretreatments of misoprostol (100µg/kg), aqueous anise suspension (Aniseed, 250mg/kg), and its combinations with misoprostol on the gastric ulcer index in rats.**

| Treatments | Ulcer index (mm) | Preventive (%) |
|---|---|---|
| **Control** | 0 | 100 |
| **Indomethacin** | ٥٥* ± ٥,٠٤ | - |
| **Misoprostol + indo** | ١١,١٧ª ± ٣,٧٩ | ٧٩,٦٩ |
| **Aniseed + indo** | ٣٦ ª ±٨,٦٤ | ٣٤,٥٤ |
| **Aniseed + Misoprostol + indo** | ٠,٨٣ ª ± ٠,٤٧ | ٩٨,٤٩ |

Values are presented as mean ± S.E
***** Significantly different from control group at $p \leq 0.05$

**a** Significantly different from indomethacin group at $p \leq 0.05$

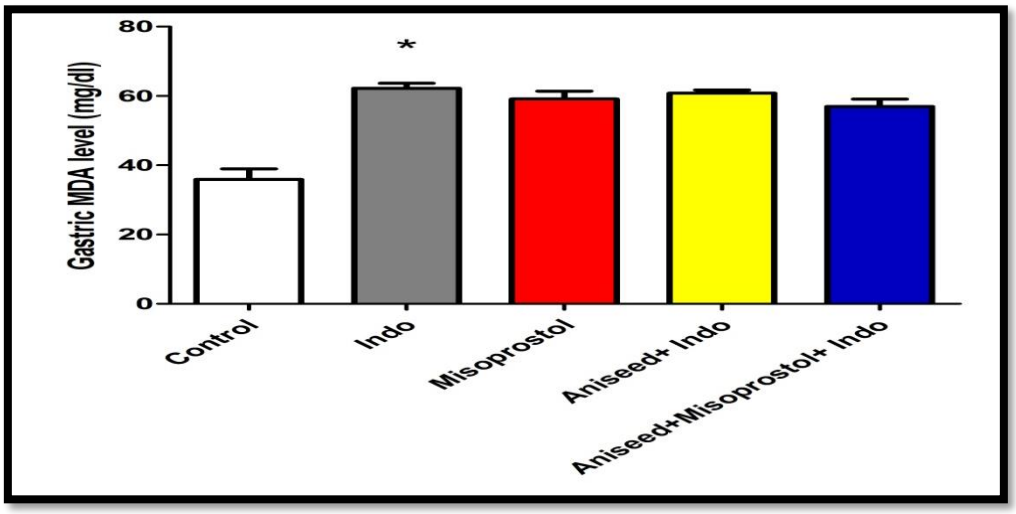


**Fig 3. Effect of single oral pretreatments with: misoprostol (100µg/kg), aniseed, (250mg/kg) and their combination on gastric MDA level**

Values are presented as mean ± S.E

***** Significantly different from control group at $p \leq 0.05$

**a** Significantly different from indomethacin group at $p \leq 0.05$

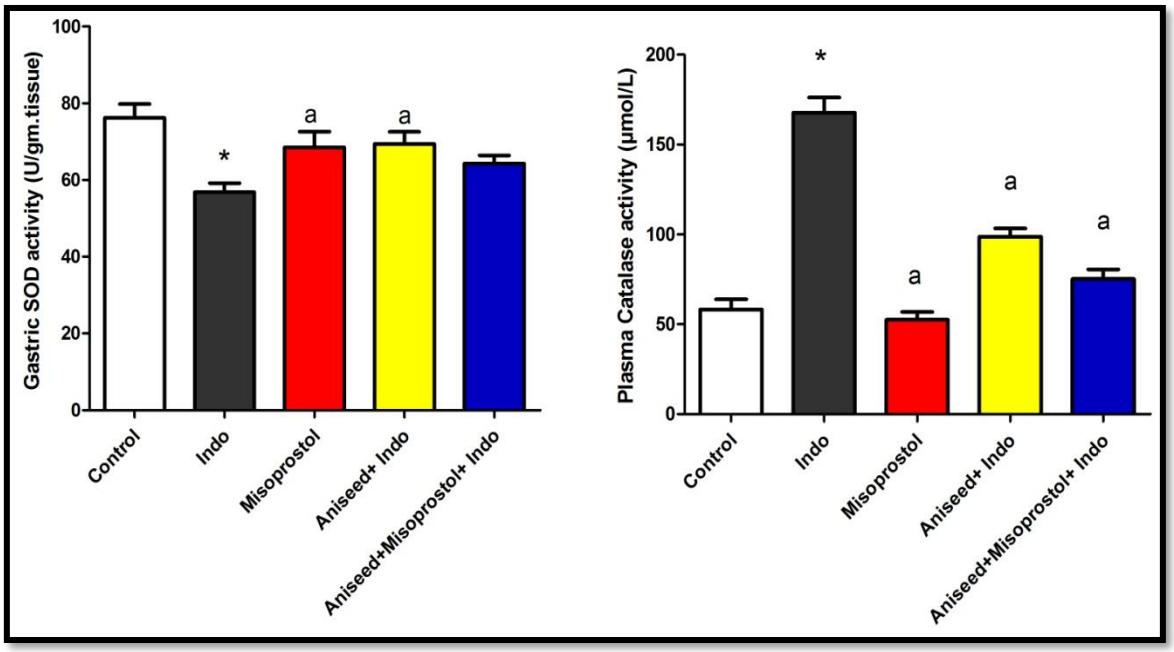


**Fig 4. Effect of single oral pretreatments with: misoprostol (100µg/kg), aniseed, (250mg/kg) and their combination on the antioxidant markers: gastric SOD activity, and plasma catalase activity**

Values are presented as mean ± S.E

***** Significantly different from control group at $p \leq 0.05$

**a** Significantly different from indomethacin group at $p \leq 0.05$

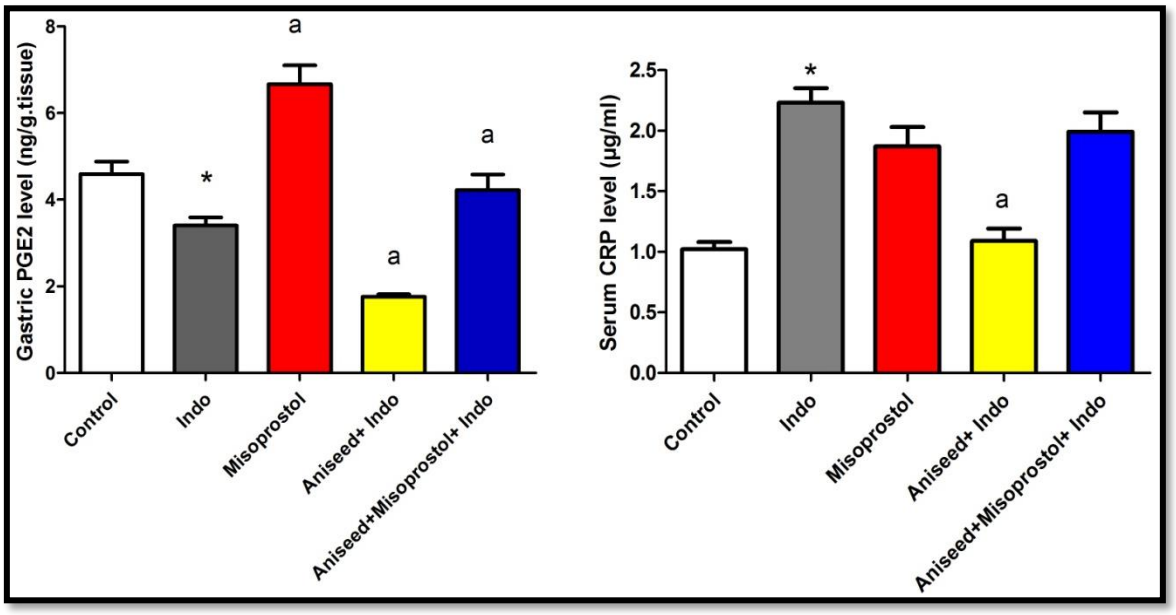


**Fig 5. Effect of single oral pretreatments with: misoprostol (100µg/kg), aniseed, (250mg/kg) and their combination on the inflammatory markers gastric PGE2 levels and serum CRP level**
Values are presented as mean ± S.E
***** Significantly different from control group at $p \leq 0.05$
**a** Significantly different from indomethacin group at $p \leq 0.05$

## 4. Discussion

The effects of combination of drugs on human health have recently become important issues because many patients take more than one drug at the same time. Naturally, drugs that reduce the side effects of NSAIDs should be selected for patients taking NSAIDs who require treatment with other drugs (Hagiwara et al., 2005).

The present work demonstrated that indomethacin administration induced a severe mucosal ulceration associated with significant increase in gastric MDA level which has no influence in our results as all pretreatments didn't show any significant change in its level, probably owing to the use of single dose and shortage of time.

Superoxide dismutase is considered as the first line of defence against oxygen toxicity and the central regulators of reactive oxygen species (ROS) levels by catalyzing the decomposition of superoxide, the first but most abundant ROS, into hydrogen peroxide and water (Yao et al., 2006). In our experiment, all pretreatments increased significantly gastric SOD activity. However, the increase was mild in the combination group

Catalase is very effective in high-level oxidative stress and protects cells from hydrogen peroxide produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced glutathione peroxidase activity (Yao et al., 2006). However, according to present results, the plasma levels of CAT were found to be increased in indomethacin group as compared with control group. This increase may be due to an increase in plasma H2O2 and OH− level, occurred by inhibition of peroxidases (Odabasoglu et al., 2006).

All pretreatments produced a significant decrease in plasma catalase activity, which can be attributed to the decrease in plasma H2O2 and OH levels. Moreover, the combination exerts some additive effect on the resulted decrease in plasma catalase activity, compared to misoprostol group

In view of the present results, all pretreatments possess reducing effects against the oxidative damage with some additive antioxidant effect exerted by addition of aniseed with misoprotol.

Misoprostol and its combination with aniseed increased PGE2 level significantly. However, aniseed alone didn't affect PGE2 level. Therefore, no additive anti-inflammatory effect on gastric PGE2 level is exerted by aniseed, when combined with misoprostol.

C-reactive protein (CRP) is an acute-phase reactant that originates from the liver. CRP has many clinical and biological effects. It is a marker of inflammation and can be used for the diagnosis and follow-up of different inflammatory and traumatic processes (Saribas et al., 2004).

All pretreatments significantly decreased serum CRP level, however, this decrease was mild with the combination, indicating that aniseed had no additive anti-inflammatory effect on serum CRP level, when combined with misoprostol

In view of the present results, all pretreatments possess an anti-inflammatory effect, however, no additive anti-inflammatory effect is exerted by aniseed, when combined with misoprostol and aniseed may have exerted anti-inflammatory action possibly through another mechanism, which needs further investigation

## 5. Conclusion

This study provides evidence that aniseed afford gastroprotection against indomethacin induced ulcers through its antioxidant and anti-inflammatory properties. Combination of aniseed with misoprostol improved ulcer healing, and antioxidant effect of misoprostol, leading us to conclude that concomitant administration of misoprostol with aniseed provided an additive gastroprotective effect to misoprostol, possibly though a synergistic ulcer healing and antioxidant mechanisms, in addition to other mechanisms which needs further investigation.

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## Disclosure

The authors report no conflicts of interest in this work.

## References

Aebi, H. (1984). [13] Catalase in vitro *Methods in enzymology* (Vol. 105, pp. 121-126): Elsevier.

Al Mofleh, I. A., Alhaider, A. A., Mossa, J. S., Al-Soohaibani, M. O., & Rafatullah, S. (2007). Aqueous suspension of anise "Pimpinella anisum" protects rats against chemically induced gastric ulcers. *World journal of gastroenterology: WJG, 13*(7), 1112.

Antonisamy, P., Kannan, P., Aravinthan, A., Duraipandiyan, V., Valan Arasu, M., Ignacimuthu, S., . . . Kim, J.-H. (2014). Gastroprotective activity of violacein isolated from Chromobacterium violaceum on indomethacin-induced gastric lesions in rats: investigation of potential mechanisms of action. *The scientific world journal, 2014*.

Bhattacharya, S., Banerjee, D., Bauri, A., Chattopadhyay, S., & Bandyopadhyay, S. (2007). Healing property of the Piper betel phenol, allylpyrocatechol against indomethacin-induced stomach ulceration and mechanism of action. *World journal of gastroenterology: WJG, 13*(27), 3705.

Cavallini, M. E., Andreollo, N. A., Metze, K., & Araújo, M. R. (2006). Omeprazole and misoprostol for preventing gastric mucosa effects caused by indomethacin and celecoxib in rats. *Acta cirurgica brasileira, 21*, 168-176.

Chen, S.-H., Liang, Y.-C., Chao, J. C., Tsai, L.-H., Chang, C.-C., Wang, C.-C., & Pan, S. (2005). Protective effects of Ginkgo biloba extract on the ethanol-induced gastric ulcer in rats. *World journal of gastroenterology: WJG, 11*(24), 3746.

Cheng, Y. T., Lu, C. C., & Yen, G. C. (2017). Phytochemicals enhance antioxidant enzyme expression to protect against NSAID-induced oxidative damage of the gastrointestinal mucosa. *Molecular Nutrition & Food Research, 61*(6), 1600659.

de Barros, M. P., Sousa, J. P. B., Bastos, J. K., & de Andrade, S. F. (2007). Effect of Brazilian green propolis on experimental gastric ulcers in rats. *J Ethnopharmacol, 110*(3), 567-571.

Drini, M. (2017). Peptic ulcer disease and non-steroidal anti-inflammatory drugs. *Australian prescriber, 40*(3), 91.

Fernandes, C., De Souza, H., De Oliveria, G., Costa, J., Kerntopf, M., & Campos, A. (2012). Investigation of the mechanisms underlying the gastroprotective effect of Cymbopogon citratus essential oil. *Journal of Young Pharmacists, 4*(1), 28-32.

Gülçın, İ., Oktay, M., Kıreçcı, E., & Küfrevıoğlu, Ö. İ. (2003). Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. *Food Chemistry, 83*(3), 371-382.

Hagiwara, M., Kataoka, K., Arimochi, H., Kuwahara, T., Nakayama, H., & Ohnishi, Y. (2005). Inhibitory effect of fluvastatin on ileal ulcer formation in rats induced by nonsteroidal antiinflammatory drug. *World journal of gastroenterology: WJG, 11*(7), 1040.

Nasuti, C., Gabbianelli, R., Falcioni, G., & Cantalamessa, F. (2006). Antioxidative and gastroprotective activities of anti-inflammatory formulations derived from chestnut honey in rats. *Nutrition Research, 26*(3), 130-137.

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun, 46*(2), 849-854.

Odabasoglu, F., Cakir, A., Suleyman, H., Aslan, A., Bayir, Y., Halici, M., & Kazaz, C. (2006). Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. *J Ethnopharmacol, 103*(1), 59-65.

Pedersen, S., Cribb, A., Read, E., French, D., & Banse, H. (2018). Phenylbutazone induces equine glandular gastric disease without decreasing prostaglandin E2 concentrations. *Journal of veterinary pharmacology and therapeutics, 41*(2), 239-245.

Poonam, D., Vinay, C. S., & Gautam, P. (2005). Cyclo-oxygenase-2 expression and prostaglandin E2 production in experimental chronic gastric ulcer healing. *Eur J Pharmacol, 519*(3), 277-284.

Sabiu, S., Garuba, T., Sunmonu, T., Ajani, E., Sulyman, A., Nurain, I., & Balogun, A. (2015). Indomethacin-induced gastric ulceration in rats: Protective roles of Spondias mombin and Ficus exasperata. *Toxicology reports, 2*, 261-267.

Saremi, K., Rad, S. K., Tayeby, F., Abdulla, M. A., Karimian, H., & Majid, N. A. (2019). Gastroprotective activity of a novel Schiff base derived dibromo substituted compound against ethanol-induced acute gastric lesions in rats. *BMC Pharmacology and Toxicology, 20*(1), 1-13.

Saribas, S., Kocazeybek, B., Aslan, M., Altun, S., Seyhun, Y., Öner, Y. A., & Memisoglu, N. (2004). Do procalcitonin and C-reactive protein levels have a place in the diagnosis and follow-up of Helicobacter pylori infections? *Journal of medical microbiology, 53*(7), 639-644.

Takeuchi, K., & Amagase, K. (2018). Roles of cyclooxygenase, prostaglandin E2 and EP receptors in mucosal protection and ulcer healing in the gastrointestinal tract. *Current pharmaceutical design, 24*(18), 2002-2011.

Uchiyama, M., & Mihara, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem, 86*(1), 271-278.

Valcavi, U., Caponi, R., Brambilla, A., Palmira, M., Minoja, F., Bernini, F., . . . Fumagalli, R. (1982). Gastric antisecretory, antiulcer and cytoprotective properties of 9-hydroxy-19, 20-bis-nor-prostanoic acid in experimental animals. *Arzneimittel-forschung, 32*(6), 657-663.

Vázquez-Fresno, R., Rosana, A. R. R., Sajed, T., Onookome-Okome, T., Wishart, N. A., & Wishart, D. S. (2019). Herbs and spices-biomarkers of intake based on human intervention studies–a systematic review. *Genes & nutrition, 14*(1), 1-27.

Wallace, J. L. (2012). NSAID gastropathy and enteropathy: distinct pathogenesis likely necessitates distinct prevention strategies. *Br J Pharmacol, 165*(1), 67-74.

Yao, P., Li, K., Jin, Y., Song, F., Zhou, S., Sun, X., . . . Liu, L. (2006). Oxidative damage after chronic ethanol intake in rat tissues: prophylaxis of Ginkgo biloba extract. *Food Chemistry, 99*(2), 305-314.

Yekta, R. F., Amiri-Dashatan, N., Koushki, M., Dadpay, M., & Goshadrou, F. (2019). A metabolomic study to identify potential tissue biomarkers for indomethacin-induced gastric ulcer in rats. *Avicenna journal of medical biotechnology, 11*(4), 299.