

# **DISCUSSION**

## DISCUSSION

Peptic ulcer disease is a multifactorial health problem affecting almost all populations worldwide, and is still a problem not completely controlled by available drugs.

The need for new or adjuvant drugs was behind undergoing the present study, which studied the effect of rebamipide, tianeptine and *oleum cinnamomi* in two models of PUD in rats, namely indomethacin- and ethanol- induced ulcer.

The study included estimation of mucosal ulcer score and protective ratio, and biochemical estimation of MDA, SOD, GPx, Nrf 2 in the gastric mucosal tissue. Ethanol intake is noxious to the stomach. Ethanol causes disruption of gastric mucosal barrier and provokes microvascular changes within a few minutes after its application. Gastric lesion caused by ethanol is associated with ROS generation.<sup>(306)</sup> Reactive oxygen species (ROS) are generated through normal metabolic process and are needed for normal functioning of the cell, any increase in them leads to tissue damage.<sup>(307)</sup>

In the present study ethanol-induced mucosal injury was associated with elevation of gastric mucosal MDA as compared to control group. Lipid peroxidation is a major outcome of free-radical-mediated injury, and MDA is a final product of lipid peroxidation. The main target of oxidative stress is the polyunsaturated fatty acids in cell membranes causing lipid peroxidation and excess formation of MDA.<sup>(308)</sup> Many studies coincide with this result.<sup>(309,310)</sup>

Walker et al (2013) found that ethanol toxicity in the liver was associated also with lipid peroxidation and elevation of MDA.<sup>(310)</sup> Another model in the brain had shown the same results in the brain tissue and serum by the same mechanism, lipid peroxidation.<sup>(311)</sup>

In the present study ethanol-induced gastric injury was associated with reduction of SOD activity in gastric mucosa. Superoxide dismutase (SOD) is a superoxide scavenger (helps conversion of harmful superoxide to hydrogen peroxide) and plays key roles in the enzymatic defense of cells against oxidative stress injury. The low level of SOD activity indicates high risks of cell injury. Tandon et al (2004) showed that ethanol decreases the activity of SOD.<sup>(307)</sup> In agreement with other study suggested that reduction is likely to be due to the high utilization of SOD in the decomposition of superoxide anion generated by lipid peroxidation.<sup>(312)</sup>

Another biomarker of oxidative stress was GPx activity; in the present study no significant changes were observed as compared to control group. GPx is an enzyme that plays a fundamental role in the elimination of hydrogen peroxide and lipid hydroperoxides in the gastric mucosa cells.<sup>(313)</sup>

The effect of ROS generation on GPx activity is multifactorial. While they can upregulate this antioxidant enzyme through nrf2, overproduction of ROS can directly decrease the activity of this antioxidant enzyme.<sup>(314)</sup>

In the present study gastric mucosal Nrf2 was significantly increased in association with ethanol-induced gastric injury.

Mild oxidative stress induces antioxidant enzymes, thereby strengthens cellular defense mechanisms against ensuing more potent oxidative stress.<sup>(315)</sup> Therefore, Nrf2 could be considered as an indicator of oxidative stress. A previous study indicated that ROS generated from mitochondria by oxidative stress dissociate binding of Nrf2 protein from Keap 1 in cytoplasm; then Nrf2 activates a variety of antioxidant enzymes. Up regulation of these antioxidant enzymes contributes to protection of cells against more potent oxidative injuries.<sup>(316)</sup>

Recently it has been demonstrated that Nrf2 effectively prevents hepatotoxicity in mice induced by acetaminophen.<sup>(317)</sup>

Taken together, the biochemical changes found in the present study with ethanol administration indicate significant increase in mucosal aggressive factors (oxidative stress-induced lipid peroxidation) associated with inhibition of some mucosal defense mechanisms. This imbalance leads to weakness in the gastric mucosal barrier, and destruction of the mucosal epithelial cells initiating erosions and ulceration.

In indomethacin group; the injured mucosa by insult of indomethacin has also been associated with increases in MDA and Nrf2 levels, and reduction in SOD activity as compared to the control group, and no changes in GPx activity. This can be explained by the same mechanisms related to increased lipid peroxidation due to ROS generation by indomethacin.<sup>(318)</sup>

Many studies coincide with these results, indicating that lipid Peroxidation, DNA damage and neutrophil-dependent microvascular injuries that lead to cell death can explain indomethacin-induced gastric ulceration.<sup>(319-321)</sup>

An additional mechanism for indomethacin-induced mucosal injury includes a depletion of endogenous PGs by inhibiting COX activity. It is considered that deficiency of PGs plays a key role in NSAID- induced gastrointestinal side effects. The identification of COX-2, which predominates at sites of inflammation, led to suggestions that inhibition of COX-2 accounts for the therapeutic benefit of NSAIDs whereas inhibition of COX-1 underlies the NSAID-induced toxicity, particularly in the gastrointestinal tract.<sup>(322)</sup>

The reduced SOD activity observed in indomethacin group can be explained by generation of OH radicals which decrease the activity of SOD.<sup>(314)</sup>

Carrasco-Pozo et al (2011) have also shown that indomethacin induced, time-dependently, mitochondrial perturbations which led to cell losses of antioxidant enzymes.<sup>(323)</sup>

In the present study GP<sub>x</sub> activity was not significantly changed in indomethacin group as compared to the control group. This result is in accordance with another study which indicated that indomethacin-induced ulcer produced no effect on GPx activity.<sup>(324)</sup> This can be explained by multifactorial affection of GPx activity under oxidative stress as explain herein before. However; in some other studies, indomethacin administration was followed by reduction of GPx activity. This difference might be attributed to different timing of measurement of GPx activity after indomethacin administration or to measuring only the mitochondrial fraction of this enzyme.<sup>(325,320)</sup>

In the current study, indomethacin-induced ulcer was associated with increased gastric mucosal Nrf2 level, these results are also in agreement with other studies.<sup>(326)</sup> Again, this probably occurs as a defense mechanism under the effect of ROS through dissociating Nrf2 from keap1 in the cytoplasm.<sup>(316,327,328)</sup>

The present study tested the protective effect of seven days pretreatment with rebamipide (60 mg/kg b.wt./day) against indomethacin; and ethanol-induced gastric ulcer. The results showed a significant decrease in gastric mucosal lesion score with a good protective ratio was improved.

These results in are agreement with other studies in humans and experimental rat models, which showed similar findings.<sup>(329-331)</sup>

The gastroprotective effect of rebamipide can be explained by different mechanisms. It has been demonstrated that rebamipide induced prostaglandin synthesis, especially the gastroprotective PGE<sub>2</sub>, via expression of COX2 enzyme. It could also induce the expression of PG receptors.<sup>(332,333)</sup>

It was also shown that rebamipide exhibits cytoprotective and anti-inflammatory effects through inhibition of cytokines produced from leukocytes.<sup>(332)</sup>

It was demonstrated that neutrophil-mediated inflammation is involved in the development of indomethacin-induced injury. It has been found that several chemokine-related genes were upregulated 24 hours after indomethacin administration and were downregulated by rebamipide treatment. That the anti-inflammatory effect of rebamipide was attributed to downregulation of certain chemokines in gastric epithelial cells.<sup>(334)</sup>

Niwa et al (2008) proved that rebamipide dose-dependently reduced the total length of the gastric erosion, normalized the mucosal thickness and increased the number of parietal and total cells in a sodium taurocholate-induced gastritis, and tended to reduce interstitial infiltration by inflammatory cells and proliferation of collagenous fibers, and increased the PAS-positive mucus in the cell. Additional mechanisms include increased blood flow and improvement of mucosal microcirculation.<sup>(335,336)</sup>

In a clinical trial was conducted by Jimori et al (2011), rebamipide also showed other anti-inflammatory effects; it reinforced the distal colonic barrier and had a slight T helper immunomodulatory effect on mesenteric lymph node cells.<sup>(337)</sup>

It has also been determined by Kim et al (2003) that rebamipide has cytoprotective effects against indomethacin-induced cell death by inhibiting apoptosis-related genes.<sup>(338)</sup> Moreover, it was reported that rebamipide has free radical scavenging activity,<sup>(332)</sup> which also explains its antioxidant effect in the present work.

The present study showed a decrease in mucosal MDA content in rebamipide-pretreated groups as compared to indomethacin and ethanol control groups. Recent studies have revealed that NSAIDs induce lipid peroxidation in gastric epithelial cells by generating superoxide anion in the mitochondria, independently with cyclooxygenase-inhibition and the subsequent prostaglandin deficiency. Impairment of mitochondrial oxidative phosphorylation, or uncoupling, is associated with the generation of superoxide anion. The pretreatment of rebamipide significantly decreased the signal intensity of superoxide anion from the mitochondria, and attenuated lipid peroxidation by increasing the expression of MnSOD protein and decreasing superoxide anion leakage from mitochondria.<sup>(339)</sup> Rebamipide was found to scavenge the hydroxyl radicals in cell free system in both animal models and human.<sup>(340)</sup>

The present study showed an increased SOD activity in rebamipide-pretreated groups as compared to indomethacin and ethanol groups. It has been demonstrated the ROS and the generation of hydroxyl radicals cause reduction in SOD activity, thus rebamipide, by scavenging the radicals and inhibiting the production of ROS, could increase SOD activity.<sup>(341)</sup> As mentioned above a previous study revealed that pretreatment with rebamipide attenuates lipid peroxidation by increasing the expression of MnSOD protein in mitochondria and decreasing the leakage of superoxide anion.<sup>(339)</sup>

Glutathione peroxidase (GPx) activity was also increased in rebamipide pretreated groups as compared to indomethacin and ethanol groups. Previous reports have shown that rebamipide prevents the impairment of this enzyme, probably due to its antioxidant activity of rebamipide.<sup>(341,342)</sup>

The present study also showed decreased Nrf2 level in rebamipide pretreated groups as compared to indomethacin and ethanol groups. This can be explained by the antioxidant activity of rebamipide which eliminates ROS that are responsible for inducing Nrf2.

The above collected data indicated that rebamipide has mucoprotective effects through its antioxidant and anti-inflammatory effects.

The present study tested the protective effect of seven days pretreatment with tianeptine (12 mg/kg b.wt./day) against indomethacin- and ethanol-induced gastric ulcer. The study showed a significant gastric protection with reduction in gastric mucosal lesion score.

These results are in agreement with previous studies which revealed that tianeptine has antiulcer effect in a dose-dependent manner in rat stomach tissue. The rats were administered two different doses five minutes before indomethacin administration.<sup>(344)</sup>

The mechanism of tianeptine antiulcer effect could involve stimulation of  $\alpha_2$ -adrenoreceptors. The stimulation of such receptors inhibits gastric acid secretion and motility.<sup>(343)</sup> However; antioxidant effect of tianeptine has also been postulated as mechanism for gastric protection.<sup>(344)</sup>

In the current study mucosal MDA content in tianeptine-pretreated groups was decreased as compared to indomethacin and ethanol control groups. This result coincides with other studies which showed a progressive decrease in MDA content in rats with increasing the dose of tianeptine before induction of ulcer.<sup>(344,345)</sup>

The present study also showed an increase in SOD activity in tianeptine-pretreated indomethacin group as compared to indomethacin control group together with an increase in glutathione peroxidase activity in both tianeptine-pretreated groups as compared to the corresponding control groups.

The current study showed also a decrease in Nrf2 gastric mucosal content in both tianeptine-pretreated groups as compared to indomethacin and ethanol control groups. This can be also explained by the antioxidant activity of tianeptine which eliminates ROS that are responsible for inducing Nrf2.

The antioxidant properties of tianeptine has been demonstrated in different previous experimental studies. A study has shown that pretreatment with tianeptine five minutes before ulcer induction in a rat model significantly decreased MDA content and increased SOD activity.<sup>(344)</sup>

Oreščanin-Dušić et al (2012) in a different model showed that tianeptine treatment increased glutathione peroxidase in  $Ca^{2+}$ -stimulated and spontaneously contracted uteri.<sup>(346)</sup>

Del Soldato Di Joseph et al (1986) and Suleyman et al (2009) revealed that pre-synaptic  $\alpha_2$ -adrenoreceptors have a role in the inhibition of indomethacin-, aspirin-, ethanol, stress-, and pyloric-ligation-induced ulcers. The  $\alpha_2$ - mediated gastroprotective effect is thought to be through multiple mechanisms. One of these mechanisms may be through stimulation of cyclooxygenase-1 (COX-1); previous experimental studies have shown that the stimulation of  $\alpha_2$ -adrenoreceptors produced an increase in the activity of COX-1 which is responsible for synthesis of prostaglandins which have gastro-protective effects. It has also been revealed that the stimulation of  $\alpha_2$ -adreno-receptors causes a decrease in oxidant parameters while increasing anti-oxidant parameters. It was

demonstrated that tianeptine exerted a gastro-protective effect that was accompanied by inhibiting oxidant parameters while increasing anti-oxidant parameters which had been decreased by indomethacin in the stomach tissue of rats.<sup>(347-351)</sup>

From the above collected data activation of the enzymatic antioxidant mechanism and inhibition of some toxic oxidant mechanisms play a role in tianeptine's antiulcer effect.

The present study, tested the protective effect of seven days pretreatment with *oleum cinnamomi* (2.5 ml/kg b.wt./day) against both indomethacin-induced ulcer and ethanol-induced ulcer. *Oleum cinnamomi* exerted gastric protection in both groups with significant decrease in gastric mucosal lesion score.

Tankam and Ozbayer et al (2013) found that cinnamon extract treatment significantly protected animals against gastric ulceration produced by ethanol one hour before induction in a rat model. Other studies also demonstrated gastroprotection by cinnamon extract against HCL and aspirin induced ulcers.<sup>(352,353)</sup> Another study investigated the gastroprotective effects of eugenol (a major constituent of cinnamon leaf oil 70-90%) in different animal models, and demonstrated that eugenol (100 and 250 mg/kg) reduced ethanol-induced gastric lesions. Also in indomethacin-induced ulcer model, eugenol (50 and 250 mg/kg) significantly reduced the incidence of ulcers. Furthermore, eugenol (250 mg/kg) did not affect secretion or gastric parameters; the gastroprotective effect was, thus, related to factors that increase mucus production and barrier resistance.<sup>(354,355)</sup>

An anti-inflammatory effect of eugenol and cinnamaldehyde was demonstrated in vitro, where they reduced the production tumor necrosis factor (TNF).<sup>(356)</sup> Additional mechanisms include an antiradical effect which was shown against fructose-induced oxidative stress in rat liver.<sup>(357)</sup>

Jung et al (2011) has elucidated the protection by cinnamic acid against ulcer and gastritis by cinnamic acid; it demonstrated potent antioxidant activity, acid-neutralizing capacity, and good activity against *Helicobacter pylori*. In that study cinnamic acid (100 mg/kg) significantly inhibited HCl/ethanol-induced gastric lesions and increased mucus content in rats.<sup>(358)</sup>

Kamel et al (2014) demonstrated that polyphenolic compounds; as these in cinnamon oil exhibit several biological activities in the gastroprotective area, including anti-secretory, cytoprotective, and antioxidant actions.<sup>(359)</sup> These polyphenolic compounds protect the gastrointestinal mucosa from lesions in various experimental ulcer models against different necrotic agents. Therefore, our obtained data about cinnamon oil as an antiulcer and gastroprotective agent strongly go hand in hand with other studies.<sup>(360)</sup>

The present study showed a significant decrease in mucosal MDA content and an increase in SOD activity in both pretreated cinnamon oil groups as compared to indomethacin and ethanol control groups.

Again oxidative stress and ROS production cause lipid peroxidation and elevation of MDA content, and decrease SOD activity. A previous study which has shown that cinnamon oil reduces MDA production and increases the activity of SOD, these effects were attributed to the antioxidant properties of cinnamon oil.<sup>(361)</sup>

It was also reported that cinnamon essential oil was able to reduce lipid peroxidation in the  $\beta$ -carotene-linoleic acid system. It exhibited a protective capacity against irradiation-induced lipid peroxidation in liposomes, and quenched hydroxyl radicals and hydrogen peroxide.<sup>(362,363)</sup>

Lin and Wu (2003) revealed that cinnamon contained high level of phenolic groups that inhibited the chain reaction of lipid peroxidation in rat hepatocytes resulting in decreased MDA content and elevated SOD activity.<sup>(361)</sup> The active components of cinnamon could act as an electron donor, which can react with free radicals such as hydroxyl and superoxide radicals to form more stable products and, thereby, terminate the radical chain reaction.<sup>(364)</sup>

In the present study, GPx activity was increased in *oleum cinnamomi*-Pretreated group as compared to indomethacin and ethanol control groups, however; and it was statistically significant only in indomethacin group but insignificant in ethanol group.

Vernet et al (2006) and Kim SH (2004) found that *oleum cinnamomi* increase the activity of GPx and demonstrated a significant free radical scavenging activity in testicular rat model and in liver of diabetic mice.<sup>(365,366)</sup>

Antioxidant effects of cinnamon essential oil may result from its content of polyphenolic compounds. These phenolic compound increase the activity of antioxidant enzymes, which in turn detoxify hydrogen peroxide and convert lipid hydroperoxides to nontoxic substances.<sup>(367,368)</sup> Therefore; polyphenolic compounds they are now a subject of considerable scientific and therapeutic interest mainly due to their antioxidant properties and related health promoting benefits. The evidences strongly support the contribution of polyphenols in the prevention of cardiovascular diseases, cancers, osteoporosis, neurodegenerative diseases and diabetes mellitus.<sup>(359)</sup>

The present study showed a significant decrease in the Nrf2 content of gastric mucosa in both *oleum cinnamomi*- pretreated groups as compared to indomethacin and ethanol control groups. This also reflects improvement in oxidative stress in both groups in view of considering that Nrf2 as a marker of increased oxidative stress.

On comparing the results of the studied drugs in the present work, rebamipide exhibited the most prominent reduction in ulcer score in both indomethacin- and ethanol-induced ulcer groups. However, it was not the best in improving the oxidative stress and antioxidant parameters; *oleum cinnamomi* produced the most prominent reduction in mucosal MDA content in indomethacin group and the most prominent increase in SOD activity in ethanol group. Therefore, the prominent anti-ulcer activity of rebamipide, exhibiting the highest protection ratio among other drugs in the present study indicates that this agents produces such an effect through multiple mechanisms; not only via antioxidant effects.

# SUMMARY

## SUMMARY

Considering the diseases of the stomach and duodenum, peptic ulcer has been the one with a significant clinical impact. The pathophysiology of peptic ulcer centred on an imbalance between aggressive and protective factors, to which the mucosa is exposed. An effective way of tackling this problem is to detect the associated risk factors and to target treatment toward their improvement. In the present study, rat models of indomethacin and ethanol-induced gastric ulcer were used to test and compare the gastroprotective antioxidant effects of rebamipide, tianeptine and *oleum cinnamomi* on the development of gastric ulcer.

The present study was carried out on seventy two male albino rats weighing 160-180 grams. Animals were randomly divided into three groups as follows:

### **I. Group 1: Control group: 8 rats**

These animals received 5 ml/kg body weight of 2% gum acacia orally daily for 7 days as vehicle control of the studied drugs.

### **II. Group 2: Ethanol induced gastric ulcer (32 rats)**

This group was subdivided in to four groups each of eight rats.

#### **IIa: Untreated ethanol-induced gastric ulcer group**

Animals received 5 ml/Kg body weight of 2% gum acacia orally daily for 7 days as vehicle control of the studied drugs. Then on the 7<sup>th</sup> day gastric lesion was induced by oral administration of a single dose 70% ethanol, 1ml for each rat.<sup>(294)</sup>

#### **IIb: Rebamipide-pretreated, ethanol-induced gastric ulcer group**

Animals pretreated with rebamipide 60 mg/kg body weight orally daily for 7 days before induction of ethanol ulcer.

#### **IIc: Tianeptine-pretreated, ethanol-induced gastric ulcer group**

Animals pretreated with 12 mg/kg body weight tianeptine orally daily for 7 days before induction of ethanol ulcer.

#### **IId: *Oleum cinnamomi*-pretreated, ethanol-induced gastric ulcer group**

Animals pretreated with *Oleum cinnamomi* 2.5 ml/kg body weight orally daily for 7 days before induction of ethanol ulcer.

### **III. Group 3: Indomethacin-induced gastric ulcer (32 rats)**

This group was subdivided into 4 groups each of 8 rats.

#### **IIIa: Untreated indomethacin-induced gastric ulcer group**

Animals received 5 ml/Kg body weight of 2% gum acacia orally daily for 7 days as vehicle control of the studied drugs, then gastric lesion was induced by oral administration of a single dose of 100 mg /kg indomethacin.

We started in a separate pilot study with small doses 20, 30 and 50 mg/ kg but they did not give the effect, then we have increased the dose, we got the effect at a dose 100 mg/ kg.

### **IIIb: Rebamipide-pretreated, indomethacin-induced gastric ulcer group**

Animals pretreated with rebamipide 60 mg/kg body weight orally daily for 7 days before induction of indomethacin ulcer.

### **IIIc: Tianeptine-pretreated, indomethacin-induced gastric ulcer group**

Animal pretreated with 12 mg/kg body weight tianeptine orally daily for 7 days before indomethacin ulcer.

### **IIIId: *Oleum cinnamomi*-pretreated, indomethacin-induced gastric ulcer group**

Animals pretreated with *oleum cinnamomi* 2.5 ml/kg orally daily for 7 days before induction of indomethacin ulcer.

Drugs and vehicle were administered orally by an oral gavage syringe. By the end of experimentation period animals were sacrificed under ether anesthesia. Each stomach was dipped in a beaker filled with ice cold physiological saline, then carefully dried. The stomach was opened along the greater curvature, the ulcer index and protective ratio were assessed on the basis of lesion diameter according to Abou Zeit Har.<sup>(296)</sup> Then the mucosa was scrapped by using a glass slide and homogenized in 5 ml cold buffer [50 mM (Tris base: hydroxymethyl, aminomethane {C<sub>4</sub>H<sub>11</sub>No<sub>3</sub>}), 20 mM EDTA, 0.2 mM sucrose] per gram tissue. Then it was centrifuged at 100,000  $\times$ g for 15 minutes at 4 °C. The supernatant was removed and frozen at -20 °C for assessment of the following parameters

- Superoxide dismutase.
- Malondialdehyde.
- Glutathione peroxidase.
- Nuclear factor erythroid related factor (Nrf2).

## **C. Effect of rebamipide, tianeptine and *oleum cinnamomi* on indomethacin-induced gastric ulcer in rats**

### **1- Effect on ulcer score**

The ulcer score significantly decreased in all pretreated groups as compared to indomethacin control group.

The mean $\pm$ SE of ulcer score in indomethacin control group was 13 $\pm$ 1.02 while in rebamipide-, tianeptine- and *oleum cinnamomi*- pretreated groups it was 3.5 $\pm$ 0.71, 9.88 $\pm$ 0.64 and 7 $\pm$ 0.93; with protective ratios 74%, 25% and 46% respectively.

### **2- Effect on superoxide dismutase**

There was a significant increase in gastric mucosal SOD activity in all pretreated groups as compared to indomethacin control group. The mean $\pm$ SE of SOD in rebamipide-, tianeptine- and *oleum cinnamomi*- pretreated groups was 5.96 $\pm$ 0.27, 5.65 $\pm$ 0.23 and 4.72 $\pm$ 0.43 U/gm respectively versus 3.06 $\pm$ 0.62 U/gm.tissue in indomethacin control group.

### **3- Effect on Malondialdehyde**

There was a decreased in MDA content in tianeptine and *oleum cinnamomi* pretreated groups as compared to indomethacin control group. The mean $\pm$ SE was 283.38 $\pm$ 21.71 and 217.66 $\pm$ 12.21nmol/gm.tissue respectively versus 388.00 $\pm$ 27.22 nmol/gm.tissue in indomethacin control group, while there was insignificant changes in rebamipide-pretreated group.

#### **4- Effect on glutathione peroxidase**

There was a significant increase in gastric mucosal GPx activity in all pretreated groups as compared to indomethacin control group. The mean±SE of GPx in rebamipide-, tianeptine- and *oleum cinnamomi*- pretreated groups was: 66.75±5.95, 51.63±2.89 and 45.81±3.63 U/gm tissue respectively versus 16.35±1.01 U/gm.tissue in indomethacin control group.

#### **5- Effect on Nuclear factor erythroid factor (Nrf2)**

There was a significant decrease in gastric mucosal Nrf2 content as compared to indomethacin control group. The mean±SE of Nrf2 in rebamipide-, tianeptine- and *oleum cinnamomi*- pretreated groups was, 126.58±10.96, 117.50±4.82 and 112.54±11.19 ng/ml respectively versus 206.13±11.49 ng/ml in indomethacin control group.

### **D. Effect of rebamipide, tianeptine and *oleum cinnamomi* on ethanol-induced gastric ulcer in rats**

#### **2. Effect on ulcer score**

There was a significant decrease in ulcer score in all pretreated groups as compared to ethanol control group.

The mean±SE of ulcer score in ethanol control group was 26.88±2.29 while in rebamipide-, tianeptine- and *oleum cinnamomi*- pretreated groups it was 7.75±0.65, 13.50±0.98 and 10.75±1.0 with protective ratios of 72%, 48% and 60% respectively.

#### **2. Effect on superoxide dismutase**

There was a significant increase in the gastric mucosal SOD activity in *oleum cinnamomi*- pretreated group; the mean±SE was 6.63±0.94 U/gm.tissue versus 2.69±0.73 U/gm.tissue in ethanol control control group. No significant changes were detected in rebamipide or tianeptine- pretreated groups.

#### **3. Effect on malondialdehyde**

There was a significant decrease in the gastric mucosal MDA content in all pretreated groups as compared to ethanol control group. The mean±SE in rebamipide-, tianeptine- and *oleum cinnamomi*- pretreated groups was 143.63±7.84, 163.75±9.01 and 183.91±37.56 nmol/gm.tissue respectively versus 321.25±12.02 nmol/gm.tissue in ethanol control group.

#### **4. Effect on glutathione peroxidase**

There was significant increase in GPx activity in rebamipide- and tianeptine-pretreated groups as compared to ethanol control group. The mean±SE in rebamipide- and tianeptine- pretreated groups was 75.69±4.48 and 65.66±5.32 U/gm.tissue respectively versus 18.49±2.76 U/gm.tissue in ethanol control group. On the other hand, no significant changes was detected in *oleum cinnamomi*- pretreated group.

#### **5- Effect on Nuclear erythroid related factor (Nrf2)**

There was a significant decrease in the gastric mucosal Nrf2 content in all pretreated groups in comparison to ethanol control group. The mean±SE of Nrf2 in rebamipide-, tianeptine- and *oleum cinnamomi*- pretreated groups was 70.13±6.43, 74.59±3.31 and 93.46±3.10 ng/ml respectively versus 165.11±11.11 ng/ml in ethanol control group.

# CONCLUSIONS

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**From the results of the present study it could be concluded that**

1. Rebamipide, tianeptine and oleum cinnamomi all successfully protect the gastric mucosa against indomethacin and ethanol injury with variable protective ratio.
2. Rebamipide has the most gastroprotective effect against indomethacin- and ethanol-induced gastric ulcer by multiple mechanisms including antioxidant effects.
3. Tianeptine has an anti-ulcer effect possibly through mechanisms that involve activation of antioxidant enzymes.
4. *Oleum Cinnamomi* has antiulcer effects accompanied with a prominent antioxidant activity.