AIM OF THE WORK

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The aim of the present work was to assess and compare the possible protective effects of Rebamipide (antioxidant), Tianeptine (antidepressant) and *Oleum Cinnamomi* (herbal extract) on indomethacine- and ethanol-induced gastric ulcer in rats.

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental animals

The present study was carried out on seventy two male albino rats weighing 160-180 grams. Animals were purchased from the Medical Research Institute, Alexandria University. They were housed in cages with wide mesh wire bottoms and were fed on diet consisting of wheat and bread soaked in milk, and had free excess to water ad libitum.

Experimental design

Rats were divided into three main groups:

1. 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.

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

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

2a: 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 7th day gastric lesion was induced by oral administration of a single dose 70% ethanol, 1ml for each rat.[294]

2b: Rebamipide-pretreated, ethanol-induced gastric ulcer group

Animals pretreated with rebamipide 60 mg/kg body weight (suspended in gum acacia) orally daily for 7 days before induction of ethanol ulcer.[295]

2c: Tianeptine-pretreated, ethanol-induced gastric ulcer group

Animals pretreated with tianeptine 12 mg/kg body weight (suspended in gum acacia) orally daily for 7 days before induction of ethanol ulcer.[229]

2d: *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.[285]

3. Group 3: Indomethacin induced gastric ulcer (32 rats)

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

3a: Untreated indomethacin-induced gastric ulcer group

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

We started 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.

3b: Rebamipide-pretreated, indomethacin-induced gastric ulcer group

Animals pretreated with rebamipide 60 mg/kg body weight (suspended in gum acacia) orally daily for 7 days before induction of indomethacin ulcer.

3c: Tianeptine-pretreated, indomethacin-induced gastric ulcer group

Animal pretreated with tianeptine 12 mg/kg body weight (suspended in gum acacia) orally daily for 7 days before indomethacin ulcer.

3d: *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.

By the end of experimentation period, four hours after drug treatment, animals were sacrificed under ether anesthesia. The whole stomach was gently separated from the surrounding organs and tissues. 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.[296] Then the mucosa was scrapped by using a glass slide and homogenized in 5 ml cold buffer [50 mM (Tris base: hydroxymethyl, aminomethane {C4H11No3}), 20 mM EDTA, 0.2 mM sucrose supplemented with protease inhibitor cocktail] per gram tissue. Then it was centrifuged at 100,000 $_{X}$g for 15 minutes at 4 ^{o}C. The supernatant was removed and frozen at -20 ^{o}C for assessment of the following parameters

- Superoxide dismutase.[297]
- Malondialdehyde.[298]
- Glutathione perioxidase.[299]
- Nuclear factor erythroid related factor (Nrf2).[300]

1. Assessment of ulcer index and protective ratio

The degree of ulceration was assessed according to the scale of Abouzeit-Har, shown in table (I).[296] The lesion score of each rat stomach was calculated by summing the scores of all lesions.

Table I: Degree of ulceration according to the scales of Abouzeit-Har.[296]

Score	Type of lesion
0	Normal stomach.
1	Petechial hemorrhage or hyperemia.
2	(1 mm long).
3	(2 mm long).
4	(3 mm long).
5	(4 mm long).
6	(more than 4 mm long).

Determination of the level of superoxide dismutase in the gastric mucosa[301]

Gastric SOD activity was measured by Colorimetric Method (Bio –diagnostic; Dokki, Giza, Egypt, www.bio-diagnostic.com).

Principle

This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

Reagents

R1.	Phosphate Buffer pH 8.5	50 mM/L
R2.	Nitroblue tetrazolium (NBT)	1 mM/L
R3.	NADH	1 mM/L
R4.	Phenazine methosulphate (PMS)	0.1 mM/L
R5.	Extraction Reagent	

Preparation of solution

- Reagent 1(R1) was ready for use.
- Reagent 2 (R2) was reconstituted in 5 ml d. Water.
- Reagent 3 (R3) was reconstituted in 5 ml phosphate buffer.
- Reagent 4 (R4) was reconstituted in 5 ml distilled water and diluted 1000 times immediatly before use (10 µL +10 ml distilled Water).

Procedure

- Working Reagent: R1 + R2 + R3 were mixed in ratio of (10+1+1 ml) immediately before use.
- In the control test tube, 1 ml of working reagent was added to 0.1 of distilled water, mixed well. Then the reaction was imitated by the addition of 0.1 ml of PMS.
- In the sample test tube, 1ml of working reagent was added to 0.1 ml of the sample, mixed well. Then the reaction was imitated by addition of 0.1 ml of the PMS.
- The increase in absorbance at 560 nm for 5 min for the control ($\Delta_{Acontrol}$) and for the sample ($\Delta_{Asample}$) at 25˚C was determined using SPEKOL 11 spectrophotometer.

Calculation

$$\textbf{Percent inhibition} = \frac{\triangle \mathbf{A_{control}} - \triangle \mathbf{A_{sample}}}{\triangle\ \mathbf{A_{control}}} \times \mathbf{100}$$

$\Delta A_{control}$ =

The change in absorbance at 560 nm over 5 min following the addition of PMS to the reaction mixture in the absence of the sample.

ΔA_{sample} =

The change in absorbance at 560 nm over 5 min following the addition of PMS to the reaction mixture in the presence of the sample.

SOD activity was expressed as a function of gm tissue used as follows:

SOD Activity

$$\text{U/gm tissue} = \%\ \text{inhibition} \times 3.75 \times \frac{1}{gm\ tissue\ used}$$

Determination of the level of malondialdehyde in the gastric mucosa[302]

Gastric MDA level was measured by Colorimetric Method (Bio –diagnostic; Dokki, Giza, Egypt, www.bio-diagnostic.com).

Principle

Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm.

Reagents

Reagent-1: Standard 10 nmol/mL.

Reagent-2: Chromogen: thiobarbituric acid 25 mmol/L, detergent, stabilizer.

Procedure

1. From the mucosal stomach homogenate, 0.2 ml was added to 1 ml of the chromogen.
2. Blank solution was prepared by using 1 ml of the chromogen in a test tube.
3. A volume of 0.2 ml of standard solution was mixed with 1 ml of the chromogen.
4. Test tubes were mixed well, covered with glass bead, heated in boiling water bath for 30 min, and then they were cooled.
5. Then 0.2 of the sample was added to the blank tube and all were mixed well.

The absorbance of the sample (A_{sample}) was read against blank, and the absorbance of the standard ($A_{standard}$) was read against the distilled water at 534 nm using SPEKOL 11 spectrophotometer.

Calculation

$$\textbf{Tissue MDA concentration} = \frac{^{A}Sample}{^{A}S\,\text{tan}\,dard} \times \frac{10}{g.tissue\,used}\ nmol/g.tissue$$

Determination of the level of glutathione perioxidase in the gastric mucosa[303]

Gastric GPx activity was measured by Colorimetric Method (Bio – diagnostic; Dokki, Giza, Egypt, www.bio-diagnostic.com).

Principle

The assay is an indirect measure of the activity of cellular glutathione perioxidase (c-GPx). Oxidized glutathione (GSSG), produced upon reduction of organic peroxide by c-GPx is recycled to its reduced state by the enzyme glutathione reductase (GR)

$$\textbf{R-O-O-H + 2 GSH} \xrightarrow{\text{c - GPx}} \textbf{R-O-H +GSSG + } H_2O$$

$$\textbf{GSSG + NADPH + } H^+ \xrightarrow{\text{GR}} \textbf{2 GSH + } NADP^+$$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm (A_{340}) providing a spectrophotometric means for monitoring GPx enzyme activity. The molar extinction coefficient for NADPH is 6220 M^{-1} cm^{-1} at 340 nm. To assay c-GPx, a cell or tissue homogenate is added to a solution containing glutathione, glutathione reductase, and NADPH. The enzyme reaction is initiated by adding the substrate, hydrogen peroxide and the A_{340} is recorded. The rate of decrease in the A_{340} is directly proportional to the GPx activity in the sample.

Reagents

R1	Assay Buffer, pH 7.0 Phosphate Buffer Triton X-100	 50 mM 0.1 %
R2	NADPH Reagent (lyopholized) Glutathione (GSH) Glutathione Reductase β-nicotinamide-adenine dinucleotide phosphate reduced (NADPH)	 24 µmol ≥ 12 U 4.8 µmol
R3	Substrate: Hydrogen peroxide	

Procedure

- Reconstitute the content in the vial NADPH (R2) with 5 ml Buffer (R1).
- Dilute H_2O_2 (R3) 100 times immediately before use, (0.1 ml + 10 ml deionized water).
- In the sample test tube, 1ml of Buffer (R1), 0.1 ml of NADPH(R2), 0.1 ml H_2O_2(R3) were added to 0.01 ml sample.

After mixing, the decrease of absorbance at 340 nm/ min (A_{340} / min.) was recorded over a period of 3 min against deionized water using SPEKOL 11 spectrophotometer. Convenient sample dilution was done so that the starting absorbance at 340 nm not exceeds 1.5 and the A_{340}/min not exceeds 0.05.

Calculation

1- The change in absorbance per min (ΔA 340 nm / min) was obtained.
2- The net A340/min for the sample was converted to NADPH consumed (nmol/min/mL) and enzyme activity was calculated as follows:

$$\text{Enzyme Activity (U/gT)} = \frac{A_{340} \text{ / min.}}{0.00622} \times 121$$

Where, 121 represent the sample dilution factor.

Determination of nuclear factor erythroid related factor (Nrf2) in gastric mucosa[304]

Rat Nrf2 Immunoassay Kit was used (Uscnk biodiagnostic, www.uscnk.com)

Principle

This assay is a quantitative sandwich enzyme immunoassay technique, using purified rat NFE2L2 antibody to coat microtiter plate wells. Standards and samples are pipetted into wells and any insulin present is bound by the immobilized antibody. Then NFE2L2-specific enzyme linked monoclonal antibody is added, followed by a substrate. A color develops in proportion to the concentration of NFE2L2. Then color development is stopped and its intensity is measured spectrophotometrically.

Reagents

- Pre-coated, ready to use 96-well strip plate**.**
- Standard (lyophilized)**.**
- Detection Reagent A (green)**.**
- Detection Reagent B (red)**.**
- TMB Substrate**.**
- Wash Buffer (30× concentrate)**.**
- Plate sealer for 96 wells**.**
- Standard Diluent**.**
- Assay Diluent A(2×concentrate)**.**
- Assay Diluent B(2×concentrate)**.**
- Stop Solution**.**

Reagent preparation

1. Standard was reconstituted with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution was 10ng/mL.
   Seven tubes were prepared containing 0.5mL Standard Diluent and a double dilution series was prepared. Each tube was mixed thoroughly before the next transfer. The resulting standard dilutions were 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last tube with Standard Diluent was the blank having a concentration of 0 ng/mL.
2. A volume of 6mL of Assay Diluent A or B Concentrate(2×) were diluted with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B.
3. Stock Detection A and Detection B reagents were briefly centrifuged before use. The working concentration was diluted with working Assay Diluent A or B, respectively (1:100).
4. A volume of 20mL of Wash Solution concentrate (30×) was diluted with 580mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×).

Procedure

A volume of 100μL of each dilutions of standard, blank and samples was added into the appropriate wells. Covered with the plate sealer and incubated for 2 hours at 37^{o}C.

1. The liquid was removed from each well.

2. A volume of 100µL of Detection Reagent A working solution was added to each well and incubated for 1 hour at 37°C after covering it with the plate sealer.
3. The solution was aspirated and wash with 350µL of 1× Wash Solution to each well was done for 3 times. The remaining liquid was removed from all wells completely by snapping the plate onto absorbent paper. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting.
4. A volume of 100µL of Detection Reagent B working solution was added to each well and incubated for 30 minutes at 37°C after covering it with the plate sealer.
5. The aspiration/wash process was repeated for total 5 times as conducted in step 4.
6. A volume of 90µL of Substrate Solution was added to each well, covered with a new plate sealer and incubated for 15 - 25 minutes at 37°C (protected from light). The liquid turned blue by the addition of Substrate Solution.
7. A volume of 50µL of Stop Solution was added to each well. The liquid turned yellow by the addition of Stop solution. The liquid was mixed by tapping the side of the plate. Then microplate reader measurement was done at 450nm immediately.

Calculation of results

- A standard curve was drawn by the microplate reader. The OD value for each standard was plotted on the vertical axis against the corresponding standard concentration (ng/ml) on the horizontal axis of the standard curve. Samples concentrations were read as ng/ml from the standard curve by the microplate reader using the OD value for each sample.
- As samples have been diluted, insulin concentration was multiplied by the dilution factor 2. Moreover, the Nrf2 protein concentrations were normalized to total proteins in each sample (ng/ml protein).

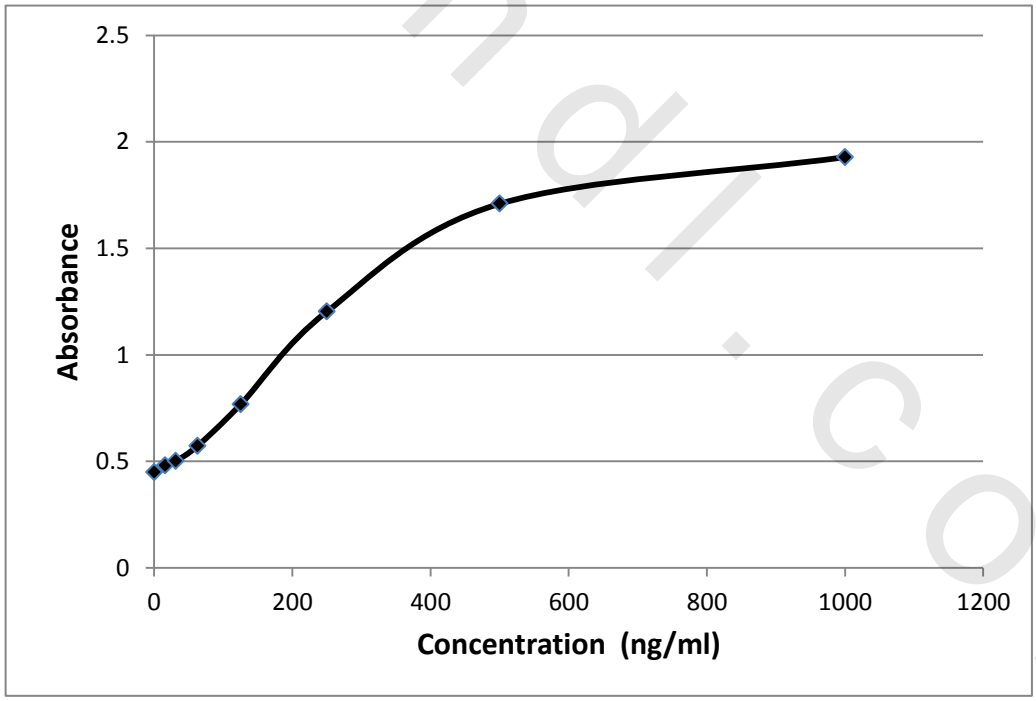

Figure (9): Standard Curve for Rat Nrf2 by ELISA.

Determination of total tissue proteins by Lowry's method

A modification of the method of Lowry was used. [305]

Principle

The color produced is due to a complex between the alkaline copper-phenol reagent and the tyrosine and tryptophan residues of the protein sample.

Reagents

- 2% Sodium carbonate (anhydrous) in 0.1 N sodium hydroxide.
- 2% Sodium tartarate.
- 1% Copper sulfate.
- Lowry C reagent: prepared immediately before use by mixing volumes of sodium carbonate, Sodium tartarate and copper sulfate reagents in a ratio 100:1:1.
- 2 N Folin phenol reagent.
- 0.1% Bovine serum albumin (standard).

Folin reagent was prepared by diluting the stock reagent 1:1 (v\v) with distilled water immediately before use to give 1 N solution-dilute. Serial dilutions of bovine serum albumin standard were prepared for generation of standard curve.

Procedure

1- A suitable dilution of each sample in distilled water was prepared.
2- Aliquots of 50 µl of the diluted samples or standard were mixed with 1 ml of Lowry C reagent then vortexed.
3- After incubation for 10 minutes at room temperature, 0.1 ml of working 1 N Folin phenol reagent was added.
4- The tubes were then vortexed and incubated in the dark for 30 min at room temperature.
5- Absorbance was read at 630 nm on the spectrophotometer.
6- A blank tube was prepared; sample was replaced by distilled water.

Calculation

1- The protein concentration in each sample was determined by referring to the standard curve, which was constructed using bovine serum albumin.
2- The protein concentration of each sample was expressed as mg\ml after correction by the dilution factor.
3- The determined proteins were used for normalization of the ELISA results of the tissue samples.

Drugs and chemicals

1- Indomethacin powder (Euro OTC Pharma).
2- Ethanol (Sigma Aldrich-Chemical Co.).
3- Tianeptine (Stablon) tablet (Servier Eygpt Industries).
4- Rebamipide (Mucosta) tablet (Egypt Otuska Pharmaceuticals Co).
5- *Oleum cinnamomi* (Sigma Aldrich-Chemical Co).
6- Gum acacia (Arabic Laboratory Equipment Co).
7- Colorimetric kit for superoxide dismutase assay (Bio –diagnostic; Dokki, Giza, Egypt).
8- Colorimetric kit for malondialdehyde assay (Bio –diagnostic; Dokki, Giza, Egypt).
9- Colorimetric kit for glutathione perioxidase (Bio –diagnostic; Dokki, Giza, Egypt).
10- Nuclear factor erythroid related factor(Nrf2) elisa kit (Bio –diagnostic; Dokki, Giza, Egypt)
11- Protease inhibitor cocktail (Bio –diagnostic; Dokki, Giza, Egypt).

Drug administration

Drugs (indomethacin, ethanol, rebamipide, tianeptine, *Oleum cinnamomi*) were administered orally by an oral gavage syringe as a single daily dose in the morning.

Statistical methods

Data analysis was performed using the Statistical Package of Social Science, SPSS version 20 software package. For the SOD, and MDA, the test was ANOVA. GP, Nrf2, ulcer score the test was Kruskal-Wallis, and comparison for multiple groups; SOD, MDA the test was t-test and for GP, Nrf2, and ulcer score was the Mann-Whitney. All results were expressed as mean ± standard error (SE).

RESULTS

RESULTS

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

1. Effect on ulcer score

Indomethacin administration (100 mg/kg orally) produced gastric mucosal lesions in comparison to the plain control group. Ulcer score mean±SEvalue were 13±1.02.

Oral administration of rebamipide (60 mg/Kg), tianeptine (12mg/ Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before indomethacin administration significantly decreased ulcer score.

The mean±SE of ulcer score of rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were 3.5±0.71, 9.88±0.64, 7±0.93 and protective ratios 74%, 25% and 46% respectively.

The reduction in ulcer score was significantly more prominent in *oleum cinnamomi* and in rebamipide pretreated groups than in tianeptine pretreated group.

Furthermore, the ulcer score was significantly more decreased in rebamipide than other pretreated groups.

Table II: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on ulcer score

Ulcer Score	Indomethacin group	Indomethacin with rebamipide	Indomethacin with tianeptine	Indomethacin with *oleum cinnamomi*
Min.	10	1	7	4
Max.	17	7	12	12
Mean	13	$3.5^{\#\blacklozenge}$	$9.88^{\#@}$	$7^{\#@\blacklozenge}$
S.E Mean	1.02	0.71	0.64	0.93
Protective ratio		74%	25%	46%
P Value	P<0.001*			

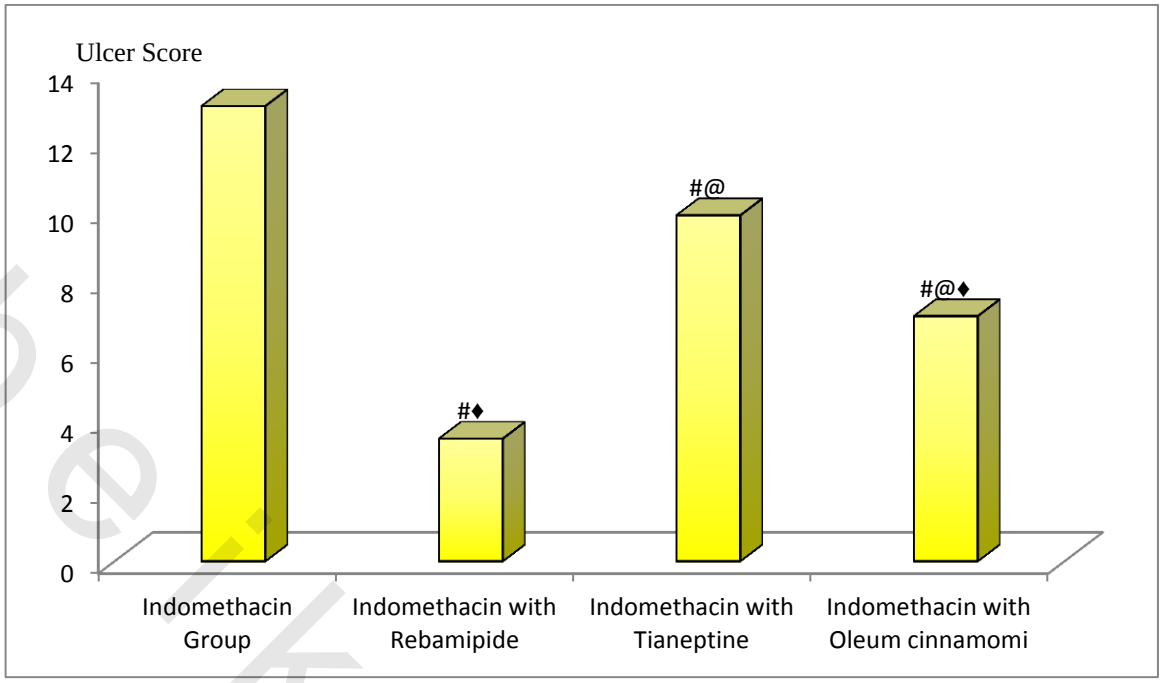

Figure10: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on ulcer score.

Significant as compared to the indomethacin group.
@ Significant as compared to the indomethacin with rebamipide group.
♦ Significant as compared to the indomethacin with tianeptine group.

2. On superoxide dismutase

Indomethacin administration (100 mg/kg orally) produced a significant decrease in gastric mucosal SOD activity in comparison to the plain control group. Gastric mucosal SOD activity mean ± SE values were 5.07±0.15 and 3.06±0.62 (U/gm tissue) in the plain control and indomethacin – treated groups, respectively.

Oral administration of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before indomethacin administration significantly increased gastric mucosal SOD activity.

The mean±SE of SOD in rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were, 5.96±0.27, 5.65±0.23 and 4.72±0.43 U/gm tissue respectively.

Gastric mucosal SOD activity was significantly more increased in rebamipide pretreated group in comparison to other pretreated groups.

Table III: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg)orally for seven days before indomethacin administration (100mg/Kg) on gastric activity of superoxide dismutase (U/gm tissue).

SOD activity	Control group	Indomethacin group	Indomethacin with rebamipide	Indomethacin with tianeptine	Indomethacin with *oleum cinnamomi*
Min.	4.71	1.54	4.75	4.75	2.60
Max.	5.86	6.64	7.08	6.50	6.21
Mean	5.07	$3.06^{\$}$	$5.96^{\$\#\&}$	$5.65^{\#}$	$4.72^{\$\#}$
S.E Mean	0.15	0.62	0.27	0.23	0.43
P Value	F= 9.745 P <0.001*				

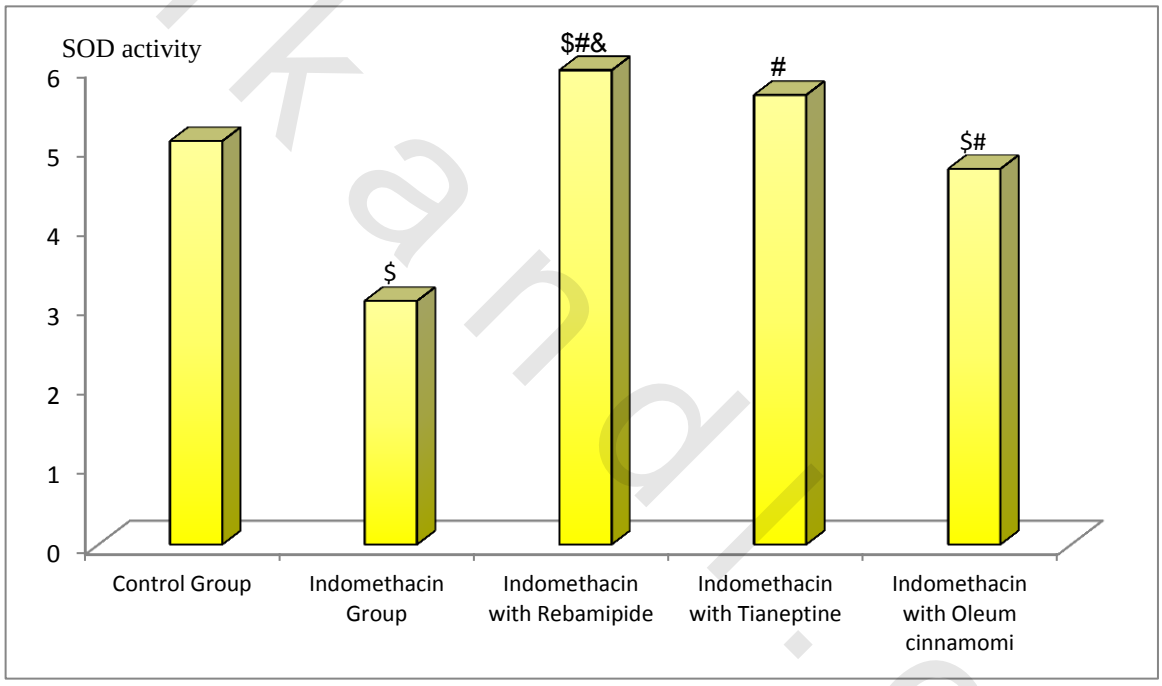

Figure 11: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin ulcer (100mg/Kg) on gastric activity of superoxide dismutase (U/gm tissue).

$ Significant as compared to the control group.
# Significant as compared to the indomethacin group.
& Significant as compared to the indomethacin with *oleum cinnamomi* group.

3. Effect on Malondialdehyde

Indomethacin administration (100 mg/kg orally) produced a significant increase in the gastric MDA level in comparison to plain control group. Gastric mucosal MDA content mean±SE values were 321.25±12.02 and 388.00±27.22 (nmol/gm tissue) in the plain control and indomethacin- treated group respectively.

Oral administration of tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before indomethacin administration significantly decreased gastric mucosal MDA content, however there was an insignificant reduction in gastric MDA content in rebamipide (60mg/kg) pretreated group as compared to indomethacin group.

The reduction in gastric mucosal MDA was significantly more prominent in *oleum cinnamomi* pretreated group in comparison to the other treated groups.

The mean±SE of MDA in rebamipide, tianeptine and *oleum cinnamomi* pretreated groups, 328.50±29.73, 283. 38±21.71 and 217.66±12.21 nmol/gm tissue respectively.

Table IV: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on gastric content of malondialdehyde (nmol/gm tissue).

MDA content	Control group	Indomethacin group	Indomethacin with rebamipide	Indomethacin with tianeptine	Indomethacin with *oleum cinnamomi*
Min.	284.00	299.00	212.00	210.00	178.50
Max.	381.00	520.00	460.00	396.00	264.00
Mean	321.25	388.00$^{\$}$	328.50$^{\&}$	283.38$^{\#\&}$	217.66$^{\$}$
S.E Mean	12.02	27.22	29.73	21.71	12.21
P Value	F=18.343			P<0.001*	

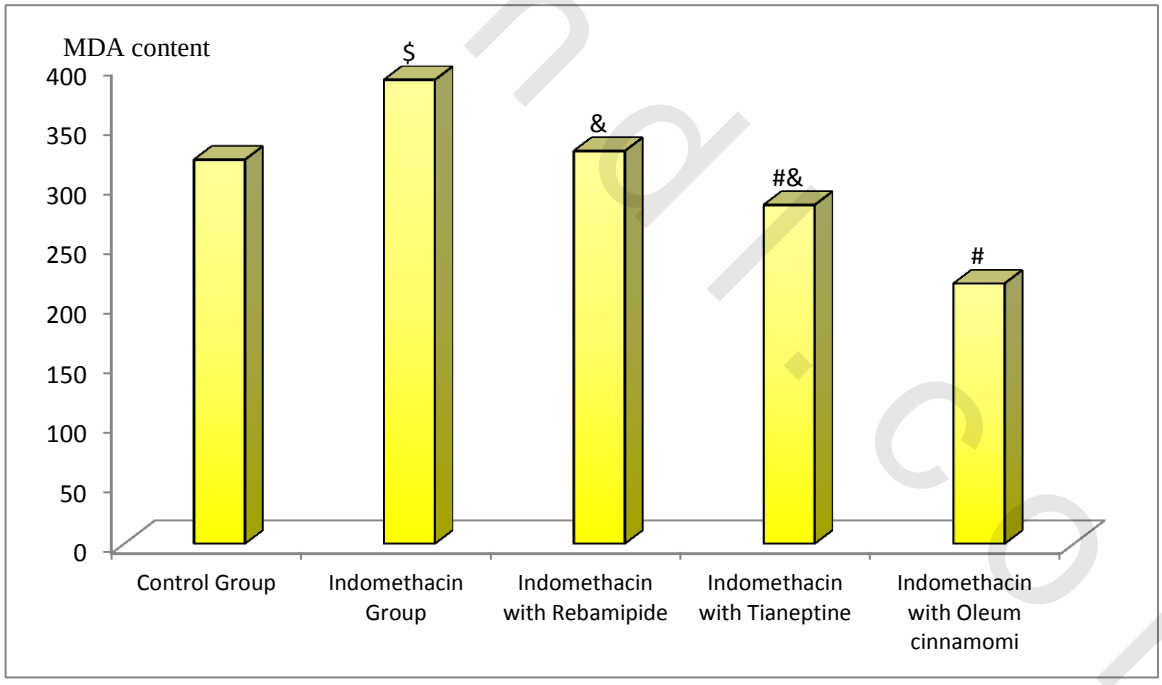

Figure12: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on gastric content of malondialdehyde (nmol/gm tissue).

$ Significant as compared to the control group.
# Significant as compared to the indomethacin group.
& Significant as compared to the indomethacin with *oleum cinnamomi* group.

4. Effect on glutathione perioxidase

Indomethacin administration (100 mg/kg orally) produced no significant changes in GPx activity in comparison to plain control group. Gastric mucosal GPx activity mean±SE values were14.13±0.04 and 16.35±1.01 (U/gm tissue) in the plain control and indomethacin- treated group respectively.

Oral administration of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before indomethacin administration significantly increase gastric mucosal GPx activity.

Glutathione perioxidase activity further significantly increased in rebamipide pretreated group in comparison to *oleum cinnamomi* pretreated group.

The mean ± SE of GPx in rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were, 66.75±5.95, 51.63±2.89 and 45.81±3.63 U/gm tissue respectively.

Table V: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on gastric content of glutathione perioxidase activity (U/gm tissue).

GPx activity	Control group	Indomethacin group	Indomethacin with rebamipide	Indomethacin with tianeptine	Indomethacin with *oleum cinnamomi*
Min.	13.94	11.70	49.70	40.30	29.70
Max.	14.30	19.50	97.00	59.90	58.50
Mean	14.13	16.35	$66.75^{\$\#\&}$	$51.63^{\$\#}$	$45.81^{\$\#}$
S.E Mean	0.04	1.01	5.95	2.89	3.63
P Value	P<0.001*				

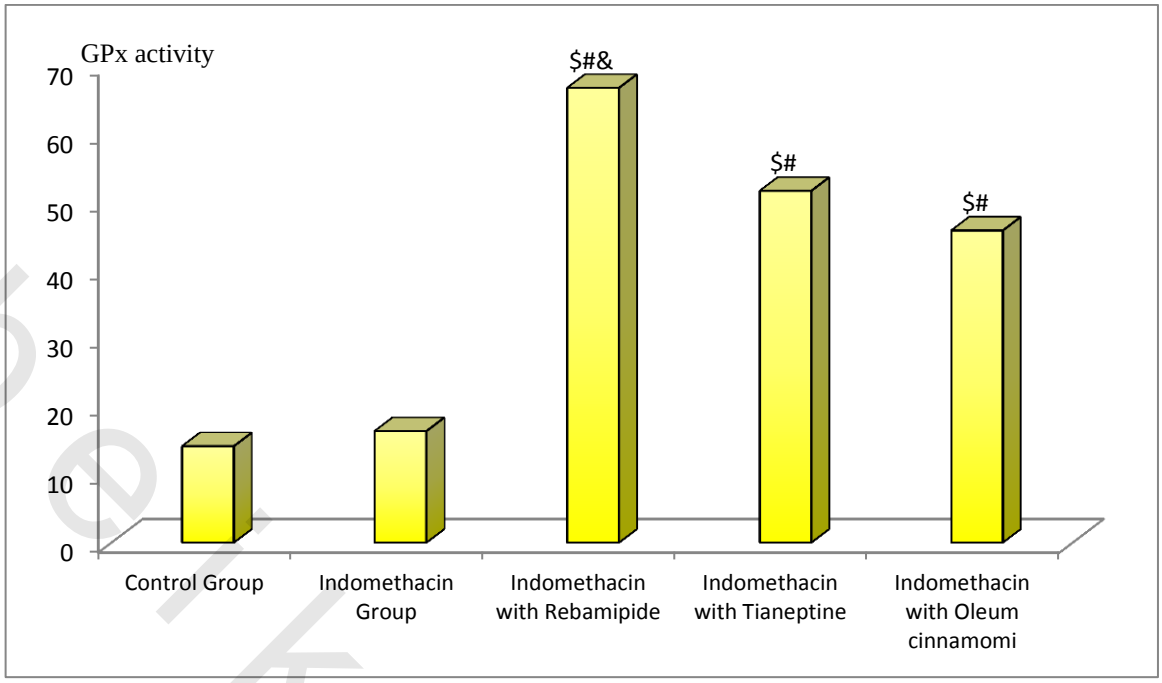

Figure 13: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on gastric mucosal glutathione perioxidase activity (U/gm tissue).

$ Significant as compared to the control group.
# Significant as compared to the indomethacin group.
& Significant as compared to the indomethacin with *oleum cinnamomi* group.

5-Effect on Nuclear factor erythroid factor (Nrf2)

Indomethacin administration (100 mg/kg orally) produced significantly increased gastric mucosal Nrf2 content in comparison to plain control group. Gastric mucosal Nrf2 content mean±SE values were 92.95±9.16and 206.13±11.49 (ng/ml) in the plain control and indomethacin treated group respectively.

Oral administration of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before indomethacin administration significantly decreased gastric mucosal Nrf2 content.

The mean±SE of Nrf 2 in rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were, 126.58±10.96, 117.50±4.82 and 112.54±11.19 (ng/ml) respectively.

Table VI: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on gastric level of nuclear factor erythroid related factor content (ng/ml).

NrF_2 content	Control group	Indomethacin group	Indomethacin with rebamipide	Indomethacin with tianeptine	Indomethacin with *oleum cinnamomi*
Min.	60.30	175.00	90.50	99.30	77.50
Max.	129.00	268.10	173.50	134.70	169.48
Mean	92.95	206.13$^{\$}$	126.58$^{\$\#}$	117.50$^{\#}$	112.54$^{\#}$
S.E Mean	9.16	11.49	10.96	4.82	11.19
P Value	P<0.001*				

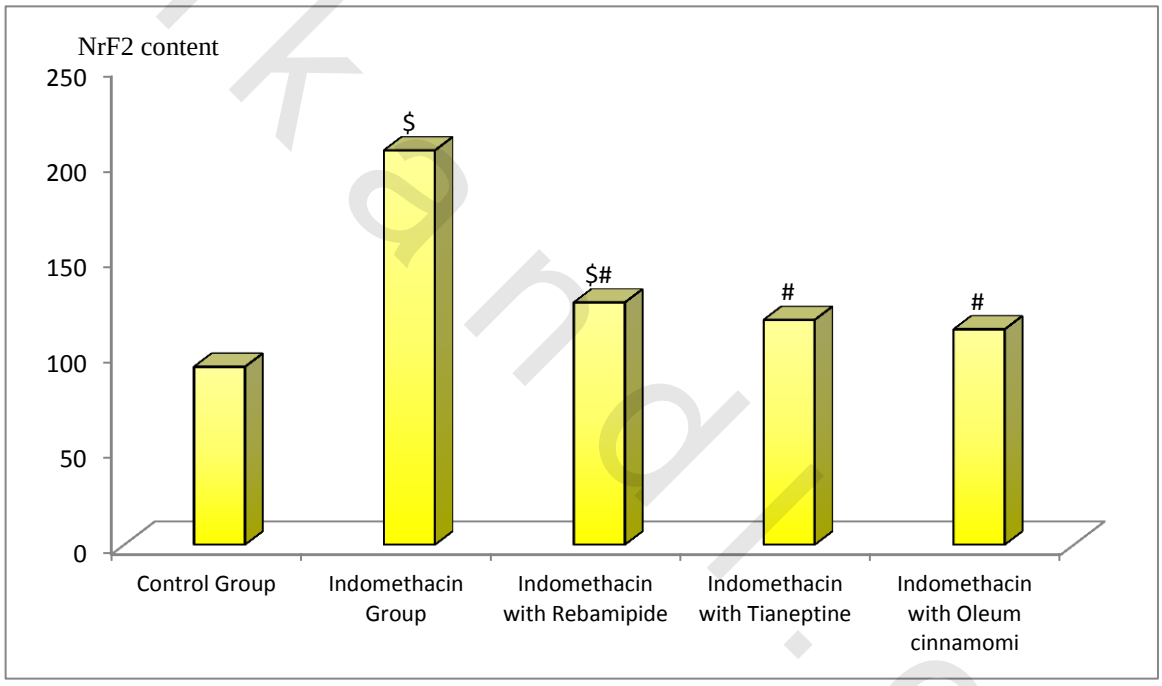

Figure 14: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before indomethacin administration (100mg/Kg) on gastric level of nuclear factor erythroid related factor content (ng/ml).

$ Significant as compared to the control group.
# Significant as compared to the indomethacin group.

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

1. On ulcer score

Ethanol administration (1ml 70% orally) produced gastric mucosal lesions in comparison to the plain control group. Ulcer score mean ± SE value were 26.88±2.29.

Oral administration of rebamipide (60 mg/Kg), tianeptine (12mg/ Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before ethanol administration significantly decrease ulcer score.

The ulcer score was significantly more decreased in rebamipide pretreated group in comparison to other treated groups.

The mean±SE of ulcer score of rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were 7.75±0.65, 13.50±0.98, 10.75±1.0 and protective ratios 72%, 48% and 60% respectively.

Table VII: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol administration (1ml 70%) on ulcer score.

Ulcer Score	Ethanol group	Ethanol with rebamipide	Ethanol with tianeptine	Ethanol with *oleum cinnamomi*
Min.	20	5	10	7
Max.	38	10	18	15
Mean	26.88	7.75$^{\#}$	13.50$^{\#@}$	10.75$^{\#@}$
S.E Mean	2.29	0.65	0.98	1.00
Protective ratio		72%	48%	60%
P Value	P<0.001*			

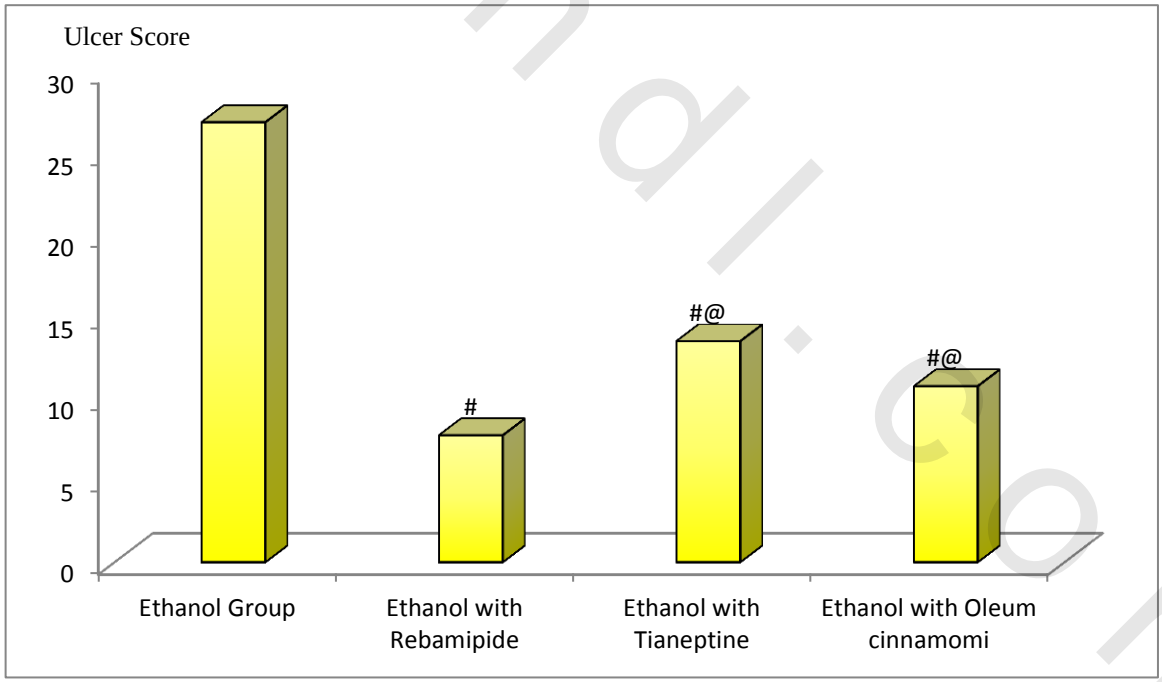

Figure 15: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol administration (1ml 70%) on ulcer score.

Significant as compared to the ethanolgroup.
@ Significant as compared to the ethanol with rebamipide group.

2-On superoxide dismutase

Ethanol administration (1ml 70% orally) produced a significant decrease in gastric mucosal SOD activity in comparison to the plain control group. Gastric mucosal SOD activity mean ± SE values were 5.07±0.15 and 2.69±0.73 U/gm tissue in the plain control and ethanol – treated groups, respectively.

Oral administration of *oleum cinnamomi* (2.5ml/Kg) daily for seven days before ethanol administration significantly increased gastric mucosal SOD activity and no significant changes were detected in SOD activity in rebamipide or tianeptine pretreated groups.

The mean±SE of SOD in rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were, 3.00±0.37, 2.49±0.0.32 and 6.63±0.94 U/gm tissue respectively.

Table VIII: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol ulcer (1ml 70%) on gastric activity of superoxide dismutase(U/gm tissue).

SOD activity	Control group	Ethanol group	Ethanol with rebamipide	Ethanol with tianeptine	Ethanol with *oleum cinnamomi*
Min.	4.71	1.00	1.77	1.10	3.54
Max.	5.86	7.30	4.44	3.97	11.46
Mean	5.07	2.69$^{\$}$	3.00$^{\$\&}$	2.49$^{\$\&}$	6.63$^{\#}$
S.E Mean	0.15	0.73	0.37	0.32	0.94
P Value	F=8.921 P<0.001*				

$ Significant as compared to the control group.
# Significant as compared to the ethanol group.
& Significant as compared to the ethanol with *oleum cinnamomi* group.

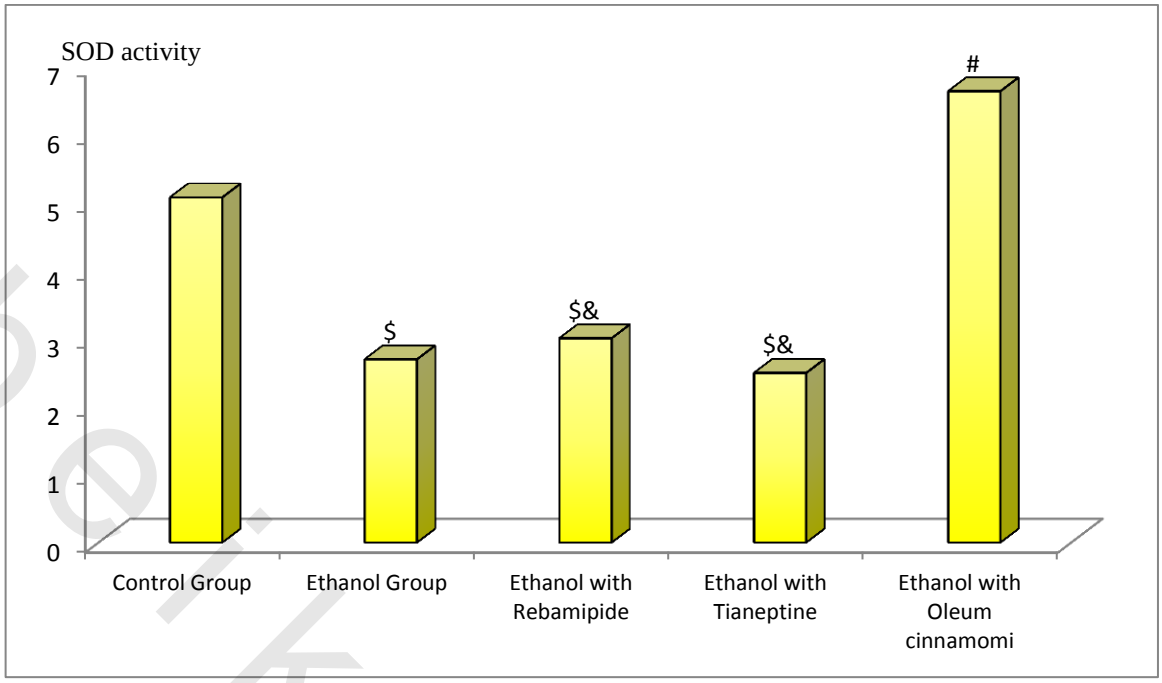

Figure 16: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol administration (1ml 70%) on gastric activity of superoxide dismutase (U/gm tissue).

$ Significant as compared to the control group.
# Significant as compared to the ethanol group.
& Significant as compared to the ethanol with *oleum cinnamomi* group.

3- On malondialdehyde

Ethanol administration (1ml 70% orally) produced increase in the gastric MDA level in comparison to plain control group. Gastric mucosal MDA content mean±SE values were 321.25±12.02 and 330.13±22.46 nmol/gm tissue in the plain control and ethanol treated group respectively.

Oral administration of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before ethanol administration significantly decreased gastric mucosal MDA content.

The mean±SE of MDA in rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were, 143.63±7.84, 163.75±9.01 and 183.91±37.56 nmol/gm tissue respectively

Table IX: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol administration (1ml 70%) on gastric content of malondiaaldehyde (nmol/gm.tissue).

MDA content	Control group	Ethanol group	Ethanol with rebamipide	Ethanol with tianeptine	Ethanol with *oleum Cinnamomi*
Min.	284.00	320.00	100.00	122.00	90.00
Max.	381.00	460.00	171.00	194.00	325.00
Mean	321.25	383.25$^{\$}$	143.63$^{\$\#}$	163.75$^{\$\#}$	183.91$^{\$\#}$
S.E Mean	12.02	18.23	7.84	9.01	37.56
P Value	F=8.246 P<0.001*				

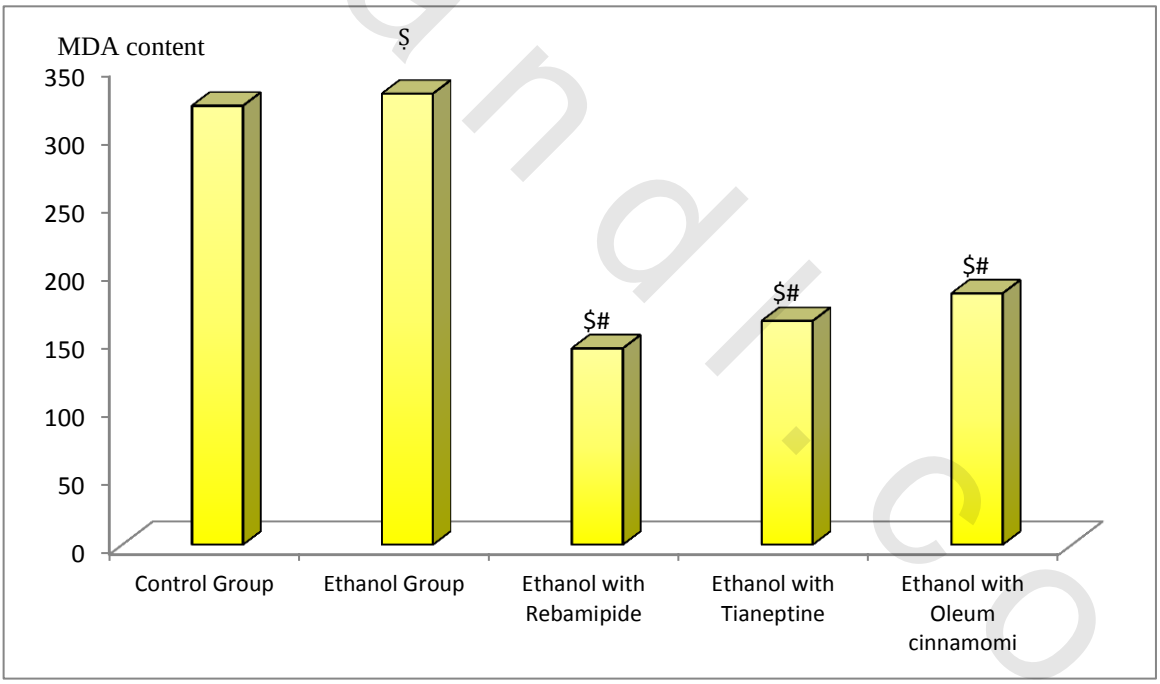

Figure 17: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol ulcer (1ml 70%) on gastric content of malondiaaldehyde.

$ Significant as compared to the control group
# Significant as compared to the ethanol group

4-On glutathione perioxidase

Ethanol administration (1ml 70% orally) produced no significant changes in the gastric mucosal GPx activity in comparison to plain control group. Gastric mucosal GPx activity mean ± SE values were14.13±0.04and18.49±2.76 (U/gm tissue) in the plain control and ethanol treated group respectively.

Oral administration of rebamipide (60mg/Kg), tianeptine (12mg/Kg) daily for seven days before ethanol administration significantly increased gastric mucosal GPx activity, while there was insignificant changes in GP activity in the *oleum cinnamomi* (2.5ml/Kg) pretreated group comparison to ethanol control group.

The mean±SE of GPx in rebamipide, tianeptin and *oleum cinnamomi* pretreated groups were, 75.69±4.48, 65.66±5.32 and 15.64±1.29 U/gm tissue respectively.

Table X: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol ulcer (1ml 70%) on gastric of glutathione perioxidase activity (U/gm tissue)

GPx activity	Control group	Ethanol group	Ethanol with rebamipide	Ethanol with tianeptine	Ethanol with *oleum cinnamomi*
Min.	13.94	9.70	60.00	49.00	9.70
Max.	14.30	29.20	97.00	88.00	19.50
Mean	14.13	18.49	75.69$^{\$\#\&}$	65.66$^{\$\#\&}$	15.64
S.E Mean	0.04	2.76	4.48	5.32	1.29
P Value	P<0.001*				

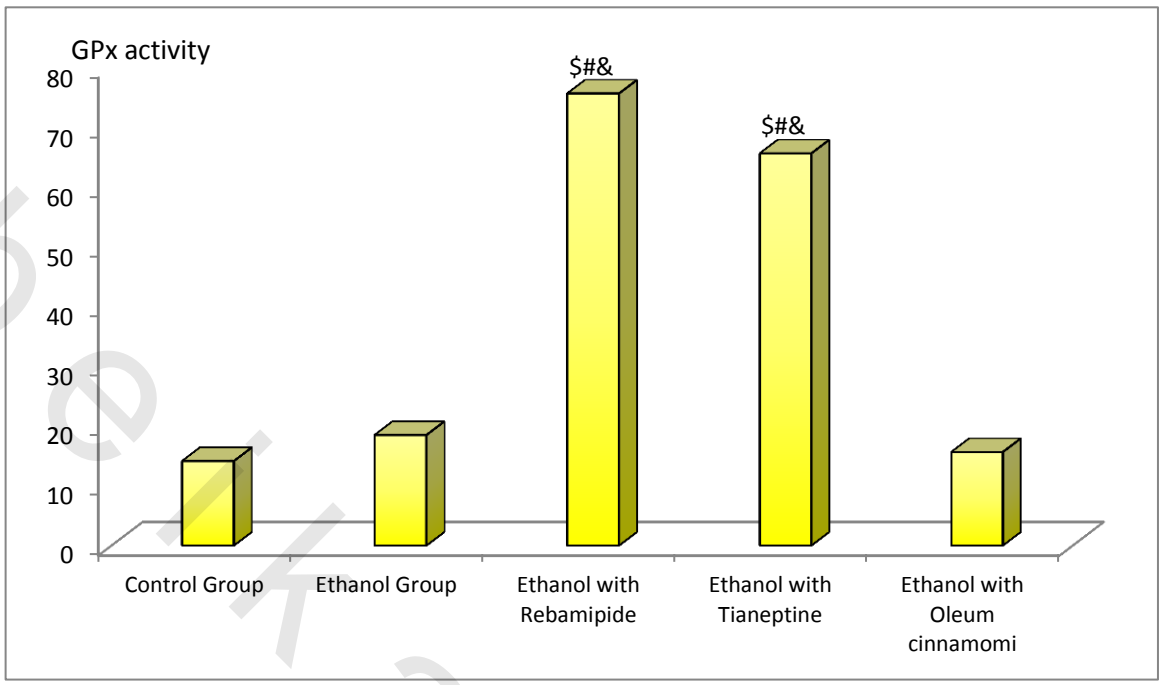

Figure 18: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol administration (1ml 70%) on gastric activity of glutathione perioxidase (U/gm tissue).

$ Significant as compared to the control group.
# Significant as compared to the ethanol group.
& Significant as compared to the ethanol with *oleum cinnamomi* group.

5-On Nuclear erythroidreleated factor (Nrf2)

Ethanol administration (1ml 70% orally) produced significantly increased gastric mucosal Nrf2 level in comparison to plain control group. Gastric mucosal Nrf2 content mean±SE values were 92.95±9.16and 165.55±11.11 (ng/ml) in the plain control and ethanol treated group respectively.

Oral administration of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) daily for seven days before ethanol administration significantly decreased gastric mucosal Nrf2 content in comparison to ethanol control group.

Further, Nrf2 content was significantly more reduced in rebamipide and tianeptine pretreated groups in comparison to *oleum cinnamomi* treated group.

The mean±SE of Nrf2 in rebamipide, tianeptine and *oleum cinnamomi* pretreated groups were, 70.13±6.43, 74.59±3.31 and 93.46±3.10 ng/ml respectively.

Table XI: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol administration (1ml 70%) on gastric content of nuclear factor erythroid related factor (ng/ml).

NrF2 content	Control group	Ethanol group	Ethanol with rebamipide	Ethanol with tianeptine	Ethanol with *oleum cinnamomi*
Min.	60.30	127.00	44.90	61.50	81.70
Max.	129.00	213.60	100.30	89.50	105.00
Mean	92.95	$165.55^{\$}$	$70.13^{\#\&}$	$74.59^{\#\&}$	$93.46^{\#}$
S.E Mean	9.16	11.11	6.43	3.31	3.10
P Value	P<0.001*				

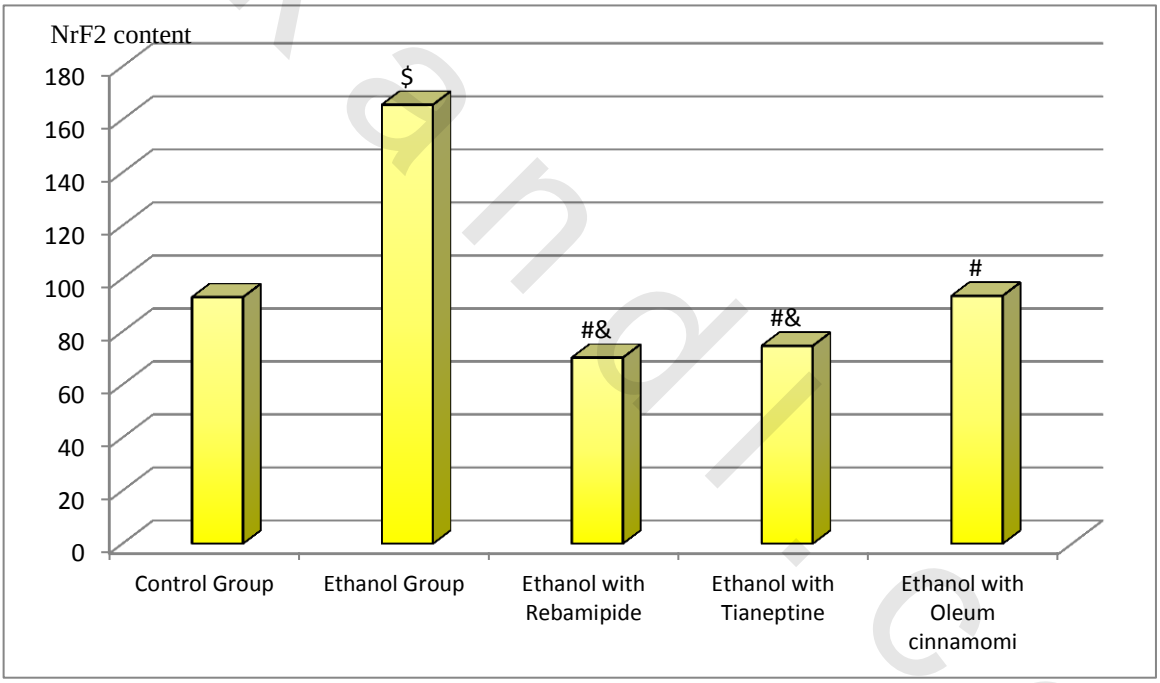

Figure19: Effect of rebamipide (60mg/Kg), tianeptine (12mg/Kg) and *oleum cinnamomi* (2.5ml/Kg) orally for seven days before ethanol administration (1ml 70%) on gastric content of Nuclear factor erythroid related factor (ng/ml).

$ Significant as compared to the control group.
# Significant as compared to the ethanol group.
& Significant as compared to the ethanol with *oleum cinnamomi* group.