Year:**2013**, Volume: **1**, Issue:**2**
First page: (**112**) Last page: (**116**)
ISSN: **2321-2187**
Online Available at **www.florajournal.com**

International Journal of Herbal Medicine

Gastroprotective Effect of the Aerial Parts of *Tribulusterrestris* L. against Indomethacin-Induced Gastric Ulceration in Rats

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The gastroprotective effect of the different fractions of *Tribulusterrestris* L. was investigated in the indomethacin-induced gastric ulcer in rats at dose of 30 mg/kg body weight. The petroleum ether and aqueous fractions hada significant gastroprotective activity, while the chloroform and ethyl acetate fractions hada moderate gastroprotective activity.

Keyword: *Tribulusterrestris, Zygophyllaceae,* Gastroprotective Effect.

1. Introduction

Gastric ulcer is an illness that affects aconsiderable number of people worldwide. Theetiological factors of this disorder include: stress,smoking, nutritional deficiencies, frequent andindiscriminate use of non steroidal anti-inflammatorydrugs (NSAIDs)[1].Thepathophysiology of gastric ulcer has generallyfocused on imbalance between aggressive andprotective factors in the stomach[2].The gastric ulcerogenic action of NSAIDs is believedto occur mainly due to their local inhibitory effect ongastric prostaglandin E2 (PGE2) and prostaglandin I2(PGI2) that are the main inhibitors of gastric acidsecretion[3,4].

*Tribulusterrestris*L.(Zygophyllaceae)is a herbaceous, annual, prostrate or semierect, diffusely branched herb[5];native in dry and sandy districts in South Europe to Central Asia and in tropical and South Africa[6], growing in India, other warm countries such as Ceylon[7], desert plains, waste ground, weed of cultivation and Mediterranean region[8]. It is used in folk medicine to increase spermatogenesis, for treatment of eye troubles, edema, abdominal distension, leucorrhea and impotence, as aphrodisiac, galactagogue, anti-inflammatory, antidiarrheal and diuretic[5].

2. Materials and Methods

2.1. Plant material: The aerial parts of *T.terrestris*were collected during the flowering and fruiting stage in October 2004 from the green areas of Minia University Campus, Minia, Egypt.

2.2. Preparation of Plant Fractions: The air dried entire plant was extracted by maceration with a 95 % methanol till exhaustion. The extracts were combined and

then concentrated under reduced pressure at 40°C to semisolid residue.The concentrated methanolic extract was digested in a least amount of distilled water, transferred to a separating funnel and partitioned with successive portions of petroleum ether, chloroform and ethyl acetate separately. 30 mg/kg of each fraction was dissolved in 0.5 % carboxymethylcelluose and separately injected i.p. to rats one hour prior to administration of indomethacin[9].

2.3. Drugs and Chemicals: Indomethacin was obtained from El Nile ChemicalCo. (Egypt), and was suspended in 0.5% carboxymethylcelluose(El Nasr Chemicals Company, Egypt). Ranitidine was kindlyprovided by GlaxoSmithKline, Egypt. Thiobarbituricacid and 1,1,3,3-tetramethoxy-propanewere obtained fromSigma-Aldrich (USA).Trichloroacetic acid was purchased from Nice, India.Ethanol absolute and diethyl ether were obtained fromMerck, Germany.All drug solutions andsuspensions were freshly prepared

2.4. Animals: The present study was conducted on adult male albino rats (Othman Animal House, Giza) weighing 150-250 grams. This species of animals was selected because induction of gastric ulcer was readily done and the results were consistent and reproducible.
Rats were fed a standard diet of commercial rat chow and tap water and left to acclimatize to the environment for at least one week prior to inclusion in the experiments. Rats were fasted for 24 hours prior to the experiment in mesh-bottomed cages to minimize coprophagia with free access to water[10,11]. All experiments were performed during the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions.

2.5. Experimental design: Rats were randomly divided into the following seven groups (each group consisted of at least 6 rats each):

1-Control non-ulcer non-treated group: In which animals were left freely wandering in their cages for 3 hours.
2-Non-pretreated indomethacin group: In which rats received no further medication other than indomethacin(40 mg/kg, s.c.) .
3-Indomethacin + ranitidine group: In which ranitidine (50 mg/kg, i.p.) was administered one hour prior to administration of indomethacin.
4-Indomethacin + petroleum ether fraction group: In which petroleum ether fraction (30 mg/kg, i.p.) was administered one hour prior to administration of indomethacin.
5-Indomethacin + chloroform fraction group: In which chloroform fraction (30 mg/kg, i.p.) was administered one hour prior to administration of indomethacin.
6-Indomethacin + ethyl acetate fraction group: In which ethyl acetate fraction (30 mg/kg, i.p.) was administered one hour prior to administration of indomethacin.
7-Indomethacin + aqueous fraction group: In which aqueous fraction (30 mg/kg, i.p.) was administered one hour prior to administration of indomethacin.
After completion of the 5 hours after indomethacin administration, rats were killed by an overdose of ether. Their stomachs were removed and opened along the greater curvature. The stomach was washed with ice-cold saline and scored for macroscopic gross mucosal lesions and stored at 80°C until used for assessment of gastric mucosal lipid peroxide.

2.6. Assessment of gastric mucosal lesions: Gastric mucosal lesions were expressed in terms of the ulcer index [12] (U.I.)which depends on the calculation of a lesion index by using of a 0–5 scoring system based on the severity of each lesion. The severity factor was defined according to the length of the lesions. Severity factor 0 = no lesions; 1 = petechiae; 2 = erosions < 1mm; 3 = erosions of 1-2 mm; 4 = erosions of 2-4 mm and 5 = erosions > 4 mm. the partial scores were then summed to obtain the ulcer index of the animal examined. The

U.I. for each group was taken as the mean lesion score of all the rats in that group.
The preventive index (P.I.) of a given drug was calculated from the following equation[12]:

$$P.I. = \frac{[U.I. \text{ of ulcerated group}] - [U.I. \text{ of treated group}]}{[U.I. \text{ of ulcerated group}]} \times 100$$

2.7. Determination of Lipid Peroxides in Gastric Mucosa: Gastric mucosal content of lipid peroxides was determined using the thiobarbituric acid method[13], which measures the thiobarbituric acid reactive substances (TBARS) concentration, sometimes referred to as malondialdehyde (MDA) concentration, which are the breakdown products of lipid peroxides.

2.8. Statistical analysis: Analyzed by One-way analysis of variance(ANOVA) followed by LSD test using SPSS forwindows version 11.5. Differences were consideredto be significant at $P< 0.05$.

3. Results and Discussion

Indomethacin administrationcaused a remarkably high ulcer index (58±3) whencompared to control group. Pretreatment with rantidine offered significant protection againstindomethacin-induced gastric ulcer in the experimentalrats. Rantidine reduced ulcer index to 14.5 ±2.5 showing75 % prevention. Pretreatment ofrats with both aqueous, petroleum ether, chloroform and ethyl acetate fractionsreduced ulcer index to 16.5 ± 3.5, 22 ± 1, 29.5 ± 2.5 and 31.5 ± 6.5, respectively, showing 71, 62, 49 and 45 % prevention, , respectively.The aqueous fraction produced highergastroprotective effect as compared to rantidine, theydecreased the ulcer index to 16.5 ± 3.5providing 71 % prevention against gastric mucosal injury (**Table 1, Fig. 1**).

Table 1: Effect of indomethacin on gastric lesions development and its alteration by various pretreatments.

Group	Mean ulcer score (mm)	P.I. (%)
Control non ulcer non treated group	0	-
Non-pretreated indomethacin group (IND)	58± 3	-
Indomethacin + ranitidine group (IND+RAN)	14.5± 2.5	75
Indomethacin + petroleum ether fraction group(IND+PE)	22 ± 1	62
Indomethacin + chloroform fraction group (IND+CH)	29.5 ± 2.5	49
Indomethacin + ethyl acetate fraction group(IND+EA)	31.5 ± 6.5	45
Indomethacin + aqueous fraction group (IND+AQ)	16.5 ± 3.5	71

* Each value represents the mean of six observations ± S.E.M

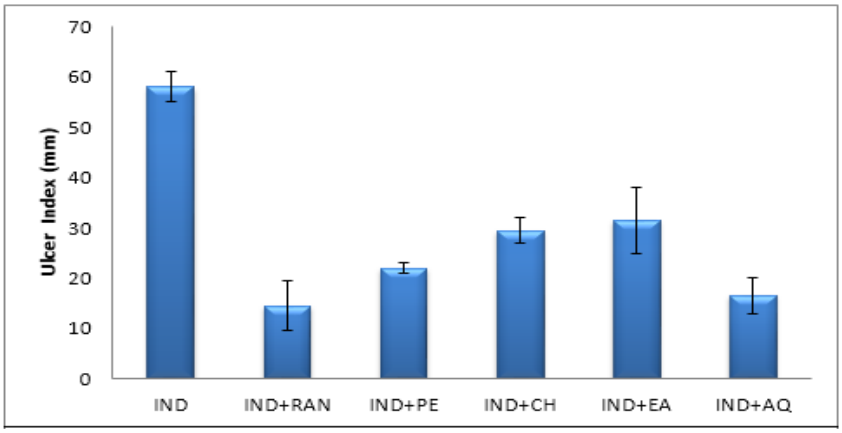

Fig 1: Effect of indomethacin on gastric lesions development and its alteration by various pretreatments.

3. 1 Effect of different plant fractions on the gastric mucosal lipid peroxides (MDA):

Administration of indomethacin significantly elevated the gastricmucosal MDA concentration reaching 32.22 ± 4.13nmol/g wet tissue as compared to 22.9 ± 2.5nmol/g wet tissue for control group (**Table 2, Fig. 2**). Interestingly, allthe pretreatments which used produced significantreduction in gastric mucosal MDA concentration ascompared to indomethacin group.

Table 2: Effect of indomethacin on the level of gastric mucosal lipid peroxides and its alteration by various pretreatments.

Group	gastric mucosal lipid peroxides (nmol/g tissue)
Control non ulcer non treated group (Control)	22.9 ± 2.5
Non-pretreated indomethacin group (IND)	32.22 ± 4.13
Indomethacin + ranitidine group (IND+RAN)	22.6 ± 5.18
Indomethacin + petroleum ether fraction group(IND+PE)	26.6 ± 2.2
Indomethacin + chloroform fraction group (IND+CH)	25.8 ± 2.14
Indomethacin + ethyl acetate fraction group(IND+EA)	26.8 ± 2.36
Indomethacin + aqueous fraction group (IND+AQ)	24.9 ± 1.45

Each value represents the mean of six ob servations □ S.E.M

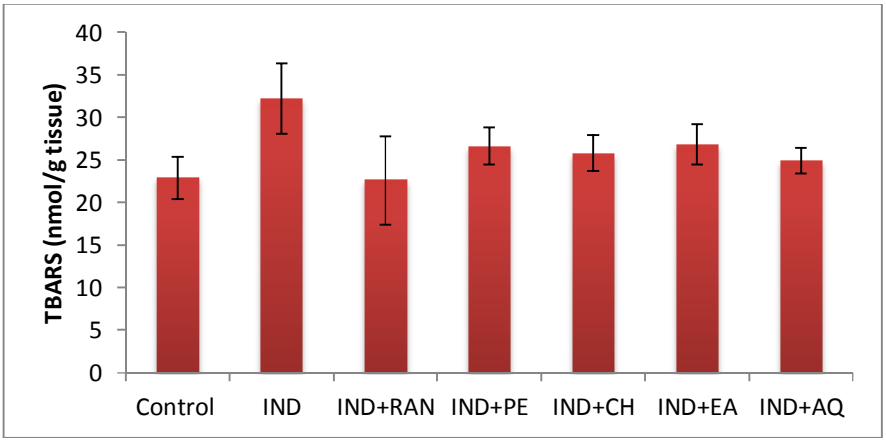

Fig. 2: Effect of indomethacin on the level of gastric mucosal lipid peroxide and its alteration by various pretreatments.

4. Conclusion

This study investigated the gastroprotective effect of the different fractions of *T.terrestris*against indomethacin-induced gastric ulcer in rats. The experimental rats were treated with 40 mg/kg body weightindomethacin after pretreatment with various fractions of *T.terrestris*(30 mg/kg body weight), and the control rats received only indomethacin. Intraperitoneal pretreatment with fractions of *T.terrestris* significantly inhibited the formation of indomethacin-induced gastric lesions and the elevation of the lipid peroxide level. These results suggest that the gastroprotective effect of *T.terrestris* removes the indomethacin-induced lipid peroxides and free radicals and that it may offer a potential remedy for the treatment of gastric lesions.

5. Acknowledgments

The authors are grateful for Mr. RemonRoshdy, Department of Pharmacology, Faculty of Medicine, Minia University, Egypt for his useful help in this work.

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