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ANTIOXIDANT EFFECT OF *PHYLLANTHUS EMBLICA* FRUITS ON HEALING OF INDOMETHACIN INDUCED GASTRIC ULCER IN RATS

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Running title : **Fruits of *Phyllanthus emblica* as antioxidant and antiulcerogenic agent**

ABSTRACT

Post-treatment of the indomethacin induced ulcerated rats at the optimal dose of 100 mg/kg body-wt. orally for 7 consecutive days with the lyophilized aqueous extract of the fruits of *Phyllanthus emblica* L. syn. *Emblica officinalis* Gaertn. (Euphorbiaceae) exhibited highly significant ($p<0.001$) enhancement of secretion of catalase, reduced glutathione and decrease in malonyldialdehyde (MDA). Furthermore, the gross morphological observation and highly significant ($p<0.001$) decrease of ulcer index (81.43%) indicated healing effect of the extract on gastric ulcer.

KEY WORDS

Antioxidant, antiulcer, healing, *Phyllanthus emblica*.

INTRODUCTION

Phyllanthus emblica L. or *Emblica officinalis* Gaertn. (Euphorbiaceae), known as *Amla* or gooseberry, is widely distributed in India. The fruits have been extensively used in Ayurveda through centuries as a potent *Rasayana* (1,2), i.e. rejuvenating and many other therapeutic uses (3). The dried fruits are considered particularly useful in haemorrhage and in gastric ulcer (4). It has also been indicated for the treatment of *Amlapita*, a syndrome akin to peptic ulcer disease (5).

An extract of the fruits of *P. emblica* was earlier found to be much more efficient an antioxidant than vitamin-E (6,7). We also reported the preventive action of the butanol extract of the water soluble fraction of *P. emblica* fruit on indomethacin-induced gastric ulcer in animal model predominantly by its antioxidative action (8).

We now report the healing effect of the aqueous extract of the fruits of *P. emblica* on gastric ulcer in rats.

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MATERIALS AND METHODS

Extraction of plant materials – The fruits of *P. emblica* were procured from market during October to December (Voucher No. MID 154 deposited in the herbarium collection of the Department of Science and Technology, Govt. of West Bengal). Fruits without seeds (1 Kg.) cut into small pieces were homogenized with distilled water (1.5 l) and filtered through a Buchner funnel using white cheese-cloth. The filtrate was centrifuged at 2000 r.p.m. for 15 min at 40°C and the supernatant lyophilized to yield 15.7 g of residue which was kept in a conical flask sealed with parafilm.

Analysis of trace elements – The elements present in microquantities were analyzed by atomic absorption spectrophotometry using air/acetylene flame. Measurement was made against appropriate standards and blanks prepared under identical condition (9).

Preparation of solution – An aliquot part of the dried plant material was weighed and macerated with 2% gum acacia in a mortar and pestle. It was then transferred to a small tube and the volume

make-up as required, hereinafter referred to as 'drug'.

Animal experiments – Sprague-Dawley male rats (180 to 210 g) were maintained under proper lighting schedule (12:12) in a temperature controlled (24 ± 2^0) animal house. Food pellets (Hindusthan Lever, India) and water were supplied *ad libitum,* taking care to minimize stress to the animals during handling. The chemicals used are of analytical grade from either Sigma or E. Merck.

Rats were randomly divided into three groups of six animals in each in two sets. They were fasted overnight. The animals were then individually administered with indomethacin suspended in 2%-gum acacia in water as vehicle through feeding canula at a single dose of 30 mg/kg body-wt (10). for induction of gastric ulcer. All the groups received treatment orally 6 hours after induction of ulcer.

Group – I : Control receiving vehicle only
Group – II : Drug-treated (100 mg/kg body-wt.)
Group – III : Drug-treated (120 mg/kg body-wt.)

At the end of treatment for pre determined intervals of days i.e., 5, 7 and 10, the animals were sacrificed under ether anesthesia. The antral portion of stomach was opened along the greater curvature and the specimen was collected for morphological observation and biochemical estimations.

Calculation of ulcer index – Evaluated according to Shay's method (11), the ulcer index of the treated animals of groups II and III were compared with that of the control, both in terms of dosage and duration (5, 7 and 10 days).

Tissue homogenate – Stomach tissue (antrum) were cut into small pieces with a scalpel blade and transferred to glass-teflon homogenizing tube to prepare a 10% homogenate (1 g, w/v) in phosphate buffer (pH 7.2; 2 mM) under cold condition. It was centrifuged at 2500 g for 10 min. The supernatant was collected for *in vitro* experiments.

BIOCHEMICAL ESTIMATIONS

Assay of Protein – Protein was estimated by the method of Lowry *et al* (12).

Assessment of lipid peroxidation – The assay of malonyldialdehyde (MDA), produced as an index of lipid peroxidation, was done by the method of Esterbaner *et al* (13). Briefly, a mixture of 5 ml each of tissue homogenate and ice cold 20% trichloroacetic acid (TCA) was incubated for 20 mins, and centrifuge at 2000 rpm for 5 mins. To 2 ml of protein free supernatant, 1 ml of freshly prepared thiobarbituric acid (TBA, 0.67%) in 0.5 M Tris-buffer adjusted to pH 3.2-3.8 with glacial acetic acid, was added and heated over a water bath (98°C) for 1 hour. The red chromophore (MDA-TBA complex) was extracted with 2 ml of n-butanol and the absorbance of butanol layer was determined at 535 nm using 1 mM 1, 1',3, 3'-tetra ethoxypropane as standard.

Catalase activity – The specific activity of catalase (CAT) was estimated according to the method described by Luck (14). The tissue homogenate (20 ml) as prepared earlier, was added to a H_2O_2 - phosphate buffer mixture (3 ml), maintaining the optical density at 240 nm to 0.500 ± 0.010 (d = 1 cm). The rate of change of optical density with time at 240 nm was recorded for the calculation of catalase activity.

Estimating Reduced glutathione (GSH) – The method of Ellmann (15) was followed for estimation of reduced sulfhydryl group in the gastric tissue. The tissue homogenate (200 µl) was taken in 10 mM phosphate buffer, pH 7.0 to which 200 µl of 10 mM DTNB - phosphate buffer pH 8.0 was added, mixed thoroughly and kept at room temperature for 20 min and the absorbance was measured thereafter in a spectrophotometer at 412 nM.

The results were analysed statistically using the unpaired student's 't' test.

RESULTS

The spectral analysis of the extract revealed the concentration of Mn, Zn and Cr as 0.594, 0.440, 0.247 ppm respectively.

Morphological observations – Group-I animals showed presence of oedema, sub-mucosal haemorrhagic lesions, deep ulcer with erosions and invasive lesions of different sizes, number and presence of acid haematin in stomach content (Fig. 1).

Presence of healed scars of different sizes and numbers were observed in group II rats. No active lesion with oozing blood was found (Fig. 2).

The morphology in group III was virtually the same as in group II, therefore, has been omitted.

Determination of ulcer index – In one set of experiments, with group II, the percent inhibition of

ulcer index was found to be 50.89, 79.39 and 79.59 respectively on days 5, 7 and 10. The maximum decrease was observed on day 7, corroborated by another set of experiments with the same duration when the percent inhibition of ulcer indices were 81.43 and 73.86% in group II and III respectively as against the control group I, the maximum decrease being observed in group II (Fig. 3).

Effect of the drug on lipid peroxidation – The decrease in the MDA (n mole/mg protein) level was highly significant ($p<0.001$ in all the experimental groups compared to group 1 (Table 1).

Effect on reduced glutathione (GSH) – Increase in the levels of GSH (n mole/mg protein) was highly significant ($p<0.001$) in all the groups against control (Table 1).

Effect on catalase – Increase in the level of catalase (U/mg of protein) was highly significant in-group II and also significant in the group III as compared with the control group I (Table 1).

DISCUSSION

The morphological observation on the drug-treated ulcerated rats of group II as compared to the ulcerated control group I (Figs. 1 & 2) indicated healing action on indomethacin-induced gastric ulcer. The rate of healing of ulcer was found to be maximum on day 7 in treated animals as compared with the control (Fig. 3). It would be understandable since bodies own defensive mechanism would be expected to play its role for the natural recovery of the animals by day 10 upto which we studied. The highly significant ($p<0.001$) decrease in the level of lipid peroxidation as well as increase in the activities of catalase and GSH on gastric mucosa of the drug-treated groups (Table 1) strongly suggested involvement of antioxidative action of catalase and GSH in the healing process.

It is well known that glutathione; a major non-protein thiol plays a crucial role in the coordination of the body's antioxidant defense mechanism. Yoshikawa *et.al* (16) have shown that indomethacin-induced gastric mucosal injury decreased the glutathione peroxidase activity and aggravated the injury due to accelerated accumulation of hydrogen peroxide and lipid peroxidation. Furthermore, excessive peroxidation could cause increased glutathione consumption (17) as the case in the control group I in our study showing depletion of the glutathione content.

The observed increase in glutathione level of the gastric tissue along with healing of indomethacin-induced ulcer in the treated animals adduce additional support to the view that glutathione inhibits gastric mucosal injury possibly mediated through scavenging indomethacin-generated metabolites (18).

Results in Table 1 also show that drug-treatment at the dosage of 100 mg/kg and 120 mg/kg body-wt. significantly decreased the level of lipid peroxidation in gastric tissue compared to the ulcerated animals.

It may, however, be pointed out that there was no significant difference between the effect of the two doses used in all the experiments done. Therefore, 100 mg/kg body-wt. of the extract of *P. emblica* fruits appear to be the optimal dose for healing of indomethacin-induced ulcer in rats.

A growing body of evidences also suggest that a number of *plants and plant products* (eg, *Ocimum sanctum* Linn (19), *piper betle* leaves (20), rhizome of *zigiber officinale* (21), rhizome of *picrorhiza kurrooa* (22), *phyllanthus emblica*) are known as potent antiulurogenic properties as well as for healing ulcer and atleast part of the therapeutic values of these plant products may be ascribed to their antioxidant properties.

The role of Zn^{2+} and Mn^{2+} on protection/healing of gastric ulcer through their antioxidative action has been reported (24,25). Moreover, Zn^{2+} and Mn^{2+} are also a constitutent part of superoxide dismutase (SOD), a free radical scavenging enzyme. The presence of high amount of Mn (0.594 ppm) and Zn (0.440 ppm) thus adduce further support to the significant antiulcerogenic effect of the extract of *P. emblica* based on its antioxidative action. Recently, protective effect of zinc gluconate on chemically induced gastric ulcer have also been reported (26).

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TABLE I : HEALING EFFECT OF THE AQUEOUS EXTRACTIVE OF *PHYLLANTHUS EMBLICA* FRUITS ON GASTRIC ULCER IN RATS ON DAY 7.

Group	Malonyldialdehyde (n mole/mg protein)	Catalase *U/mg protein)	Glutathione (n mole/mg protein)
I	9.39 ± 3.08	11.63 ± 1.236	110.13 ± 17.22
II	2.69 ± 0.69 ***	20.01 ± 0.662***	251.16 ± 8.64***
III	2.23 ± 0.257***	15.95 ± 0.9**	232.32 ± 12.98***

[Values are mean + SEM of 6 animals in each group)

** p<0.01 ***p <0.001

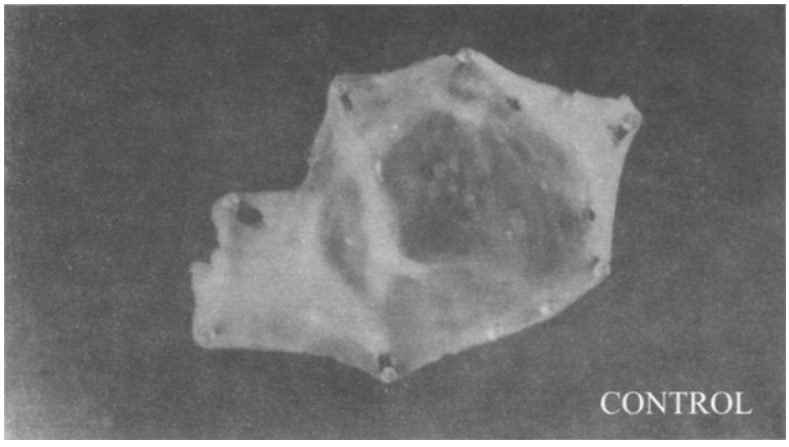

Fig. 1 Ulcerated control group showing thin mucosal layer and different size of ulcerated spots.

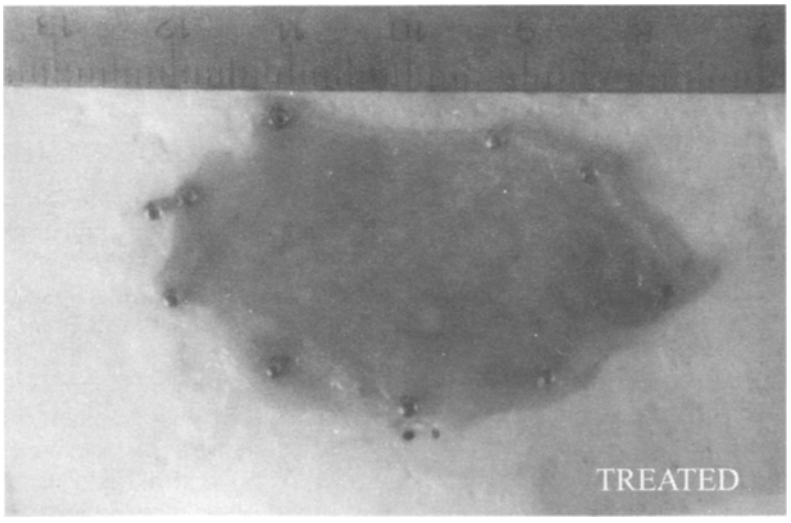

Fig. 2 Ulcerated drug-treated (100 mg/kg body-wt.) group showing healed scars at different size of numbers.

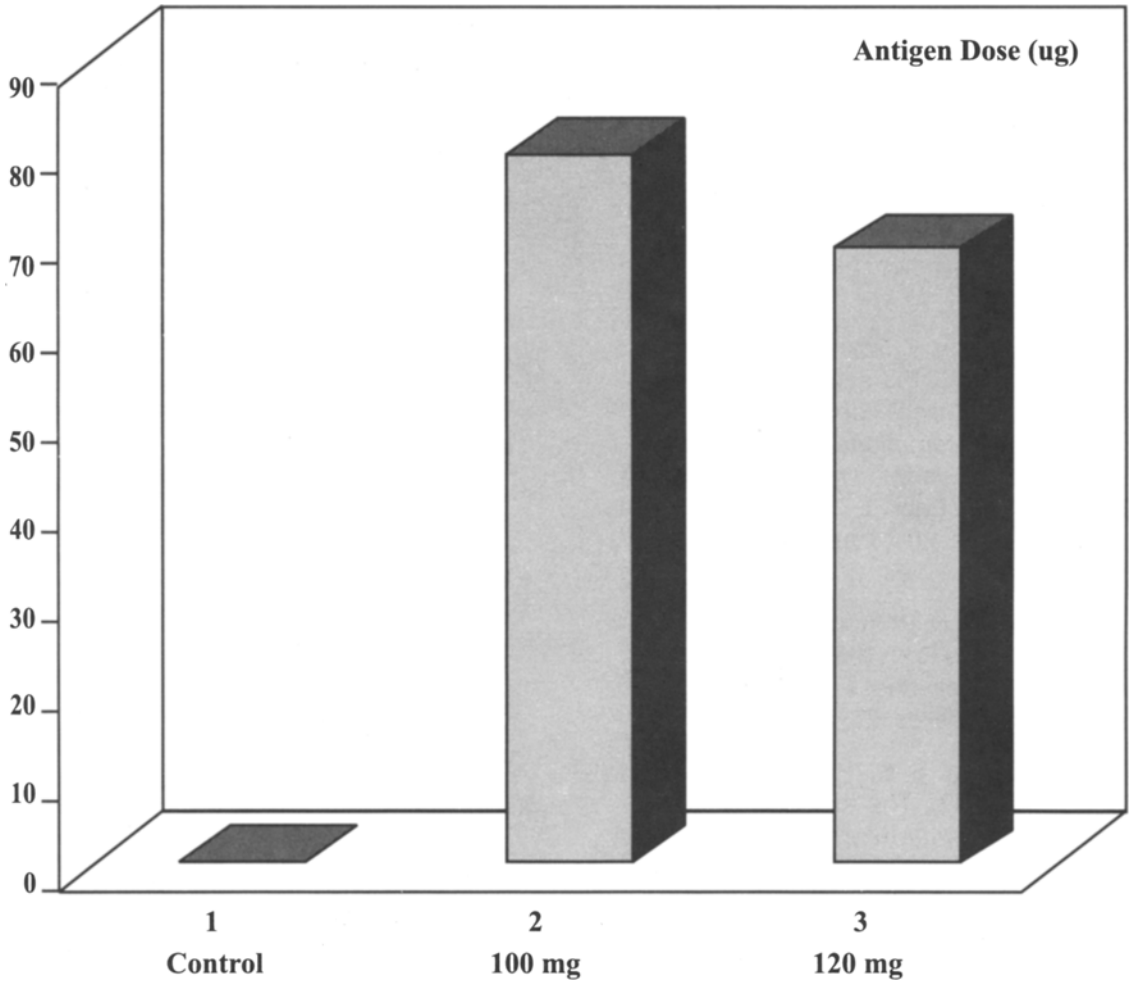

Fig. 3 Ulcer index of rats treated with different dosages of *phyllanthus emblica* (aqueous extract).

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