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## Mechanism of action of antiulcer activity of bark extracts of *Manilkara hexandra* against experimentally induced gastric ulcers in rats.

**Shah J. S.[1*], Shah M. B.[2], Goswami S. S.[2] and Santani D. D. [2]**

*[1] Shri Sarvajanik Pharmacy College, Mehsana,*
*[2]L. M. College of Pharmacy, Ahmedabad, Gujarat, India*
*Phone: (02762) 247711 ; E mail: jigashah@rediffmail.com*

**ABSTRACT :** The present study was designed to explore the mechanism of action of bark extracts of *Manilkara hexandra* Roxb. against experimentally-induced gastric ulcers. The bark extracts were tested against aspirin- and ethanol-induced gastric ulcer models and histamine infusion-induced gastric acid secretion model in rats. The acetone aqueous extract (AE) of the drug (500-800 mg/kg) and ether insoluble extract ($E_2$) (500-800 mg/kg) were administered orally against all the above mentioned models. The bark extracts have been found to be very effective in inhibiting gastric ulceration induced by aspirin and ethanol. This is evident from reduction ($p< 0.001$) in ulcer index parameter. In addition, extract $E_2$ significantly reduced stomach thiobarbituric acid reactive substance (TBARs) in above models indicating free radical scavenging property of the drug. Extract $E_2$ showed significant gastro protective effect against histamine infusion-induced gastric ulceration as evident from reduction in ulcer index. Besides, significant reduction in acid secretory parameters such as total acidity, total acid output and volume of gastric secretion were also observed at every 30 min. interval and at the end of 2 hours of experiment. Along with this parameters, mucin activity (TC: PR ratio) was also found to be significantly increased leading to gastric mucosal protection. It is concluded from this study that the drug possesses antiulcer activity in different gastric ulcer models. The antiulcer activity of the drug can be attributed to free radical scavenging property, inhibition of acid secretory parameters and strengthening of gastric mucosal barrier.

**KEY WORDS:** Antiulcer herbal drugs, aspirin, ethanol, histamine, free radical scavenging property.

### INTRODUTION

Peptic ulceration is one of the common disease affecting millions of people. It is now considered to be one of the modern age epidemics affecting nearly 10% of world population. Research advances during last decade have offered new insights in the therapy and prevention of peptic ulceration. Plants provide an alternative strategy in search for new drugs. There is a rich abundance of plants reputed in traditional medicine known to possess antiulcer properties. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved antiulcer drugs.

Chatterjee (1996) has reported antiulcer activity of number of herbal drugs known to contain a variety of active principles such as triterpenoid glycyrrhizinic acid from Glycyrrhiza glabra, nimbidin (diterpenoid) from Neem, tannins from Cinnamomum cassia and Embelica officinalis (1). Amongst these, procyanidin type of tannins are considered to be one of the most powerful antioxidant and their activity is considered to be as good as endogenous antioxidant like vitamin C and vitamin E (2). Lutomski and co-workers (1991) have shown that oxygen derived free radicals are directly implicated in ulcerogenesis and use of antioxidants may lead to gastro protective effects (3).

Ethno medicinal survey in Panchmahal region of Gujarat revealed that one of such herbal drug *Manilkara hexandra* (Roxb), commonly known as Rayan, growing throughout the region, possess antipyretic, antiulcer and analgesic properties.

In the light of various reports of herbal drugs and preliminary findings of our study, it was considered worthwhile to evaluate the effects of the bark extracts of *Manilkara hexandra* against experimentally induced gastric ulcers and elucidate its possible mechanism of action.

## MATERIALS AND METHODS

### *Animals*

Wistar albino rats of either sex weighing between 150-250 gm were used.

### *Treatment*

Extract-AE (acetone aqueous extract) was administered in two different dose regimes- 800 mg/Kg, orally, once daily for 6 days and 500 mg/Kg, orally, 5 doses, twice daily to study the effect of drug. The Extract-AE was further extracted and the resultant Extract-$E_2$ (Ether insoluble extract) was administered in the dose of 500 mg/Kg, orally.

### *Preparation of Bark Extracts*

Rayan bark collected from mature tree was sun dried for several days until it was completely dry. The bark was ground to coarse powder using a blender. The powder was stored in polythene bags at room temperature until needed.

In order to extract active components, selection of solvent was done on the basis of the result of preliminary phytochemical analysis, which indicated presence of large amount of procyanidins in the bark. Preferred solvents for the study included acetone, methanol and diethyl ether.

Defatted bark powder was extracted with aqueous acetone (80%) by maceration for 12 hours. followed by percolation. The wet powder was repeatedly extracted several times using the same solvent. The combined clear extract was stripped off acetone and further dried under nitrogen to get red-brown solid (AE), The solid was then dissolved in minimum quantity of methanol and excess of diethyl ether was added to get precipitate ($E_2$).

### *Methodology*

Animals were fed with standard chow diet and were divided into groups of eight each. Fasted animals were used as described in each of the experiment. Fasting the animals in cages with grating on the floor prevented coprophagy. Throughout the experiment, the animal house was maintained under identical conditions.

Antiulcer activity of the drug was evaluated against experimentally induced gastric ulcers. Aspirin-induced gastric ulcers in rats (4), Ethanol-induced gastric ulcers in rats (5) and Histamine infusion-induced continuous gastric acid secretion in anaesthetised rats (6) were used for various experimental investigations.

### *The following parameters investigated*

1) Physical parameters- (Ulcer index (7) and Volume of gastric acid secretion (8)),

2) Acid secretory parameters- (Total acidity (8), Total acid output (8), Pepsin output (9)),

3) Dissolved mucosubstances- (Total carbohydrates (TC) (10), Protein content (PR) (11), TC/ PR ratio)

4) Thiobarbituric acid reactive substances assay (MDA content) (12)

## RESULTS

### *1. Aspirin-induced gastric ulcer model*

Two different extracts, acetone aqueous extract and ether soluble extract were given in doses of 500 and 800 mg/Kg. Acetone aqueous extract of drug was administered in a dose of 500mg/Kg twice a day (5 doses) and 800 mg/Kg once daily for 6 days. Last dose was administered 30 minutes prior to aspirin administration. Hemorrhagic lesions were observed in the glandular region of the stomach. The parameters studied include ulcer index and stomach thiobarbituric acid reactive substances (stomach TBARs).

Acetone aqueous extract showed significant reduction in ulcer index at 500 mg/Kg ($0.004 \pm 0.00$, $p < 0.001$) and 800 mg/Kg ($0.003 \pm 0.002$, $p < 0.001$) when compared with control group ($5.37 \pm 0.61$). These results were comparable with that of cimetidine treated group. (Table: 1)

Ether insoluble extract at 500 mg/Kg (single dose) showed significant reduction in ulcer index ($0.27 \pm 0.16$, $p < 0.001$) when compared with control group ($4.56 \pm 0.47$) (Table: 2). Also stomach TBARS was reduced significantly ($p < 0.001$) when compared with treated group (Table: 2). $ED_{50}$ of acetone aqueous extract in the model was derived on the basis of the results of ulcer index (13). It is found to be 734.7 mg/Kg. In addition, $LD_{50}$ of acetone aqueous extract was determined using mice. $LD_{50}$ of this extract found to be $1940 \pm 0.57$ mg/Kg (14).

### *2. Ethanol-induced gastric ulcer model*

Ether insoluble ($E_2$) extract was studied at the dose of 500 mg/Kg (single dose). $E_2$ treatment has shown significant decrease in ulcer index ($0.57 \pm 0.24$, $p < 0.001$) when compared with ethanol treated control group ($4.25 \pm 0.39$) (Table: 3). As ethanol is known to increase free radical formation along with aspirin (Garg et al., 1991), $E_2$ extract was also studied for TBARS assay in addition to effect on gastric lesions. Extract $E_2$ showed significant decrease in stomach

TBARS (34.88 ± 3.17, p< 0.05) when compared with control group (44.70 ± 2.15) (Table: 3)

***3. Histamine infusion-induced continuous gastric acid secretion model:***

Extract $E_2$ was administered (500 mg/Kg, orally), one hour before urethane anesthesia. Hemorrhagic lesions were seen in antral region of the stomach mucosa in histamine treated control group. Along with gastric folds were also observed. The parameters under study included ulcer index, acid secretory parameters and biochemical parameters.

Gastric mucosal damage was prevented by pretreatment of extract $E_2$ in histamine treated rats. This is evident from the reduction in ulcer index of drug treated rats as compared to control group (Table: 6). In addition acid secretory parameters such as volume of gastric secretion, total acidity, total acid output were significantly reduced (p< 0.001) in the samples collected at different time intervals as well as in the pooled samples of gastric juice at the end of 2 hours of experiment (Table: 4, 6).

Besides, biochemical parameters were also studied such as Total Carbohydrates (Summation of total hexose, hexosamine, fucose and sialic acid) (TC), Total Protein (PR) and TC: PR ratio.

Histamine infusion caused decrease in TC and increase in PR content of gastric juice in the control group leading to decrease in mucin activity (decrease in TC: PR ratio). Thus reduction in mucin activity might be responsible for weakening of mucosa causing back diffusion of $H^+$ ion. Drug pretreatment showed increase in TC content and decrease in PR content of gastric juice at different time intervals as well as in pooled samples collected at the end of 2 hours (Table: 5, 7). Although the protein content was not altered significantly, there was a significant increase in total carbohydrates leading to improved mucin activity (Table: 5, 7). Also we found gastric mucosal folding in glandular part of the stomach mucosa of control group of animals. Prior treatment of extract $E_2$ showed reduction in these mucosal folding.

**DISCUSSION**

Bark extracts of *Manilkara hexandra* have shown significant gastro protective effect against aspirin- and ethanol-induced gastric ulcers and Histamine induced continuous gastric acid secretion model in rats. Alcoholic crude extract (AE) and ether insoluble extract ($E_2$) have shown significant reduction in ulcer index of both aspirin- and ethanol-induced gastric ulcer models. Extract $E_2$ was further studied for evaluating its effect on free radical development in the process of ulcerogenesis. In the process of lipid peroxidation, the MDA content was significantly reduced with extract $E_2$ pretreatment in both the models that indicates the scavenging effect of the drug upon oxygen derived free radicals within the stomach mucosa.

Several factors have been implicated in ethanol-induced gastric ulcers like products of arachidonate metabolism, oxygen derived radicals, mast cell secretory cells, gastric mucosal blood flow, pepsinogen (15 –17). On the other hand, depression of gastric mucosal blood flow reduces bicarbonate secretion and mucus production, thus allowing back diffusion of hydrogen ions (18). The mechanism behind cytoprotection may be mainly to maintain physicochemical properties and integrity of the gastric mucosal barrier (5). The mechanism of antiulcer activity of extract $E_2$ against aspirin model might be due to free radical scavenging property which is also related to altered prostaglandin levels within the tissue. There is an intimate relationship between lipid peroxidation and prostaglandin metabolism, in that very efficient antioxidant protection will slow down prostaglandin synthesis, at least until sufficient $PGG_2$ is formed to maximally activate cyclo-oxygenase. Besides, it may also involve acid secretory mechanism and alteration of the status of mucosal resistance (19). To confirm these mechanisms, histamine infusion-induced gastric acid secretion model was studied. Acute gastric hemorrhagic lesions in the stomach were observed which might be due to surgery, shock, and stress (6). Extract $E_2$ has shown significant protection against histamine infusion-induced gastric acid secretion model. Significant reduction in acid secretory parameters may the indicative of prevention of acid back diffusion from gastric mucosa.

Furthermore, histamine seems to be a key element in the pathogenesis of this continuous gastric acid secretion model. The sequence of events appears to involve both peripheral and central (e.g. brain) elements. The histamine infusion-induced gastric acid secretion is mainly due to $H_2$- receptor activation (20). This action is inhibited by specific $H_2$- receptor blockers (21). Along with histamine, acetyl choline and gastrin are also considered to be major endogenous stimulants of gastric acid secretion. Black et al (1986) have reported that vagal stimulation causes histamine secretion and histamine-induced gastric acid secretion has been reported to be well inhibited by truncal vagotomy (6).

*Table 1: Effect of bark extract of M. hexandra against aspirin-induced gastric ulcers*

| Treatment | No. of rats | Dose, p.o. (mg/kg) | Ulcer index |
|---|---|---|---|
| Control (aspirin) | 6 | 500, Single Dose | 5.37 ± 0.61 |
| Extract-ae | 6 | 500, b.i.d., 5 Doses | 0.004 ± 0.003* |
| Extract-ae | 6 | 800 o.d., 6 Doses | 0.0032 ± 0.001* |
| Cimetidine | 8 | 50 Single dose | 0.24 ± 0.03* |

*All values represent Mean ± SEM ; * P < 0.001: Compared with control (Aspirin) Group.*

*Table 2: Effect of bark extract of M. hexandra against aspirin-induced gastric ulcers*.

| Treatment | No. of rats | Dose, p.o. (mg/kg) | Ulcer index | Stomach TBARS (mmoles mda/g) |
|---|---|---|---|---|
| control (aspirin) | 6 | 500 | 4.56 ± 0.47 | 47.69 ± 5.51 |
| extract – e2 | 6 | 500 | 0.03 ± 0.01* | 1.84 ± 0.42 * |

*All values represent Mean ± SEM; * p < 0.001: Compared with control (Aspirin) Group.*

*Table 3: Effect of bark extract of M. hexandra against ethanol-induced gastric ulcers.*

| Treatment | No. of rats | dose, p.o. | Ulcer index | stomach TBARS (mmoles mda/g) |
|---|---|---|---|---|
| control (ethanol) | 6 | 1 ml | 4.26 ± 0.39 | 44.7 ± 2.15 |
| extract – e2 | 8 | 500 $mg.kg^{-1}$ | 0.57 ± 0.24** | 34.88 ± 3.17 * |

*All values represent Mean ± SEM ; * p < 0.05, ** p < 0.001 : Compared with control (Ethanol) Group.*

*Table 4: Effect of bark extract of M. hexandra against histamine infusion-induced continuous gastric acid secretion.*

| Parameters | | Volume of gastric sectretion | Total acidity | Total acid output |
|---|---|---|---|---|
| Time | Group | (ml/100 g.b.w.) | (meq/ l) | (µeq/100 g.b.w.) |
| 30 min | control | 2.89 ± 0.32 | 1.98 ± 0.49 | 5.81 ± 1.63 |
| | treated | 1.05 ± 0.15 *** | 1.69 ± 0.25 | 2.33 ± 0.52 |
| 60 min | control | 3.06 ± 0.39 | 2.53 ± 0.33 | 7.72 ± 1.35 |
| | treated | 1.21 ± 0.09 *** | 1.71 ± 0.19 * | 2.32 ± 0.44 ** |
| 90 min | control | 3.14 ± 0.412 | 2.86 ± 0.35 | 7.84 ± 1.25 |
| | treated | 1.09 ± 0.033 *** | 1.92 ± 0.19 *** | 2.47 ± 0.37 ** |
| 120 min | control | 3.3 ± 0.48 | 3.5 ± 0.32 | 11.303 ± 1.31 |
| | treated | 1.25 ± 0.12 ** | 1.95 ± 0.2 ** | 2.43 ± 0.42 *** |

*All values represent Mean ± SEM ; n= 6 in each group, *p < 0.05; ** p < 0.01; ***p < 0.001: Compared with control (Histamine)*

***Table 5: Effect of bark extract of M. hexandra against histamine infusion-induced continuous gastric acid secretion.***

| Parameters | | | Total carbohydrates (µg/ml) | Protein content (µg/ml) | TC/ PR ratio |
|---|---|---|---|---|---|
| Time | | Group | | | |
| 30 | min | control | 91.0 ± 10.84 | 103.4 ± 4.48 | 0.902 ± 0.17 |
| | | treated | 506.6 ± 44.7 *** | 60.0 ± 2.72 *** | 6.49 ± 0.79 *** |
| 60 | min | control | 167.0 ± 29.45 | 75.2 ± 4.91 | 2.29 ± 0.43 |
| | | treated | 491.6 ± 51.6 ** | 72.5 ± 3.79 | 7.02 ± 1.47 ** |
| 90 | min | control | 170.0 ± 26.51 | 85.4 ± 4.68 | 1.48 ± 0.29 |
| | | treated | 730.9 ± 97.7 ** | 78.75 ± 3.76 | 8.66 ± 2.58 * |
| 120 | min | control | 237.5 ± 27.64 | 99.8 ± 3.1 | 2.3 ± 0.3 |
| | | treated | 625.1 ± 56.7 * | 89.33 ± 6.83 | 7.52 ± 1.67 ** |

*All values represent Mean ± SEM ; n = 6 in each group, * p < 0.05; ** p < 0.02; ***p < 0.001 : Compared with control (Histamine)*

***Table – 6: Effect of bark extract of M. hexandra against histamine infusion-induced continuous gastric acid secretion (at the end of 120 min.)***

| Parameters | Control (histamine) (1 mg/kg, iv) | Treatment (800 mg/kg, p.o.) |
|---|---|---|
| Ulcer index | 2.20 ± 0.29 | 0.13 ± 0.1 * |
| Volume of gastric secretion (ml/100 g.b.w) | 4.38 ± 0.21 | 2.42 ± 0.16 * |
| Total acidity (meq/ l) | 5.02 ± 0.25 | 1.76 ± 0.13 * |
| Total acid output (µeq/ 100 g.b.w) | 21.75 ± 1.3 | 4.31 ± 0.49 * |
| Pepsine output (µg tyrosine/100 g.b.w) | 537.4 ± 75.34 | 59.16 ± 18.17 * |

*All values represent Mean ± SEM; n = 6 in each group, *p < 0.001 : Compared with control (Histamine) Group.*

***Table – 7: Effect of bark extract of M. hexandra against histamine infusion-induced continuous gastric acid secretion(at the end of 120 min.)***

| Parameters | Control (histamine) (1 mg/kg, iv) | Treatment (800 mg/kg, p.o.) |
|---|---|---|
| Total carbohydrates (µg/ ml) | 106.0 ± 17.11 | 703.33 ± 69.56 ** |
| Protein content (µg/ ml) | 30.0 ± 7.67 | 8.66 ± 1.94 * |
| TC/ pr ratio | 8.66 ± 4.79 | 95.76 ± 10.0 ** |

*All values represent Mean ± SEM ; n = 6 in each group, * p < 0.05; ** p < 0.001 : Compared with control (Histamine) Group*

In addition, soluble mucosubstances of gastric juice were also estimated. Pretreatment of extract $E_2$ showed rise in total carbohydrate content ($p< 0.001$) and decrease in protein content ($p< 0.05$) at different time intervals as well as in pooled samples at the end of 2 hours. The effect of an agent on mucin activity is reflected by its effect on TC/ PR ratio (22). Thus, TC/ PR ratio was significantly increased when compared with control group indicating strengthening of mucosal barrier of stomach mucosa.

The rise in glycoprotein content of gastric mucosa seems to be associated with rise in soluble mucosubstances of gastric juice. The high molecular weight glycoproteins are mainly responsible for the viscous and gel forming characteristics of the mucus (23,24). Hence, the ulcer healing effect of bark extract of *Manilkara hexandra* seems to be associated with increase in mucosubstnaces of gastric juice. Thus it is suggested that antiulcer activity of the bark extracts of *Manilkara hexandra* in histamine infusion-induced gastric acid secretion model is likely to be associated with both inhibition of acid secretory parameters and strengthening of gastric mucosal barrier.

In summary, our data suggest that the bark extract of *Manilkara hexandra* seems to be effective against gastric ulcers induced by histamine, ethanol and aspirin. The antiulcer effect could be attributed to free radical scavenging property of the drug, inhibition of gastric acidity leading to prevention of $H^+$ ion back diffusion from stomach mucosa and strengthening of the gastric mucosal barrier.

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