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EFFECT OF AQUEOUS EXTRACT OF NEEM *(AZADIRACHTA INDICA)* LEAVES ON OFFENSIVE AND DIFFENSIVE GASTRIC MUCOSAL FACTORS IN RATS

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Abstract : Standardized aqueous extract of *Neem (Azadirachta indica)* leaves (AIE) has been reported to show both ulcer protective and ulcer healing effects in normal as well as in diabetic rats. To study the mechanism of its ulcer protective/healing actions, effects of AIE (500 mg/kg) was studied on various parameters of offensive acid-pepsin secretion in 4 hr pylorus ligation, pentagastrin (PENTA, 5 µg/kg/hr)- stimulated acid secretion and gastric mucosal proton pump activity and defensive mucin secretion including life span of gastric mucosal cells in rats. AIE was found to inhibit acid-pepsin secretion in 4 hr pylorus ligated rats. Continuous infusion of PENTA significantly increased the acid secretion after 30 to 180 min or in the total 3 hr acid secretion in rat stomach perfusate while, AIE pretreatment significantly decreased them. AIE inhibited the rat gastric mucosal proton pump activity and the effect was comparable with that of omeprazole (OMZ). Further, AIE did not show any effect on mucin secretion though it enhanced life span of mucosal cells as evidenced by a decrease in cell shedding in the gastric juice. Thus, our present data suggest that the ulcer protective activity of AIE may be due to its anti-secretary and proton pump inhibitory activity rather than on defensive mucin secretion. Further, acute as well as sub acute toxicity studies have indicated no mortality with 2.5 g/kg dose of AIE in mice and no significant alterations in body or tissues weight, food and water intake, haematological profile and various liver and kidney function tests in rats when treated for 28 days with 1 g/kg dose of AIE.

Key words : *Azadirachta indica* acid-pepsin secretion proton pump inhibition pentagastrin mucin secretion

INTRODUCTION

Azadirachta indica (Malvacea, AI) popularly known as neem (Hindi), is a medicinal plant that grows freely all over Indian subcontinent. Neem has a role in the treatment of disorders like microbial infections, skin diseases, dental disorders,

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malaria, syphilis, leprosy and has antiseptic property (1–3). Anti-inflammatory, immunostimulant and antiulcerogenic actions have also been reported in the extracts of *A. indica* (3–6). The medicinal and industrial uses of various parts of Neem tree and the compounds isolated from it have been reviewed (7). More than 135 compounds of diverse structure have been isolated from various parts of Neem (8, 9) but few of them have been studied for their biological and pharmacological actions. Flavanoids like rutin and quercetin have been reported to possess antiulcer and anti-inflammatory activities (10).

Gastric hyperacidity and gastroduodenal ulcers are common global problems. Hyperacidity (hyperchlorhydria) is a pathological condition due to excessive hypersecretion of hydrochloric acid from the parietal cell of the gastric mucosa through the proton pumping H^+/K^+ ATPase (11). Peptic ulcer occurs due to imbalance between offensive acid-pepsin secretion versus impaired mucosal resistance which includes mucin-bicarbonate secretion, life span of cells, cell proliferation, mucosal blood flow, mucosal glycoproteins, sulfhydryl groups, anti oxidant status etc. Most of the commonly used drugs such as H_{2-} blockers (ranitidine, famotidine etc), M_{1-} blockers (pirenzepine, telenzepine etc), proton pump inhibitors (omeprazole, pentoprazole, Rabeprazole etc), decrease secretion of acid while, drugs like sucralfate and carbenoxolone promote mucosal defenses. It is now assumed that these drugs ultimately balance the aggressive factors (acid, pepsin, *H. pylori*, bile salts) and defensive factors (mucin secretion, cellular mucus, bicarbonate secretion, mucosal blood flow, cell turnover, etc.) (12). Reports on clinical evaluation of these drugs show that there are incidences of relapses, adverse effects and danger of drug interactions during ulcer therapy. Hence, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules, which could afford better protection and decrease the incidence of relapse.

We earlier reported the ulcer protective and healing effects of an aqueous extract of *A. indica* leaves against various acute and chronic gastric ulcer (GU) models in normal and diabetic rats (13). The present work pertains to the detailed study of AIE and omeprazole, a standard antisecretary agent on offensive acid-pepsin and defensive mucin secretion and cell shedding in gastric juice of 4 h pylorus ligated rats. Further, the effect of AIE was also studied on pentagastrin-stimulated acid secretion and gastric mucosal proton pump (H^+/K^+-ATPase) activity in rats.

MATERIAL AND METHODS

Animals: After approval of the Institutional Ethics Committee, Albino rats (CF strain) of either sex weighing between 150–200 g and albino mice (CF strain) weighing between 20–30 g were obtained from the central animal house of Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house at 26°±2°C and relative humidity 44–56%, light and dark cycles of 10 and 14 hr respectively. Animals were provided with standard rodent pellet diet. Food was withdrawn 18–24 hrs before the experiment though water was allowed

ad libitum. 'Principles of laboratory animal care' (NIH publication no. 82–23, revised 1985) guidelines were followed.

Drug collection and extraction : One kg of fresh tender green leaves of *Azadirachla indica* (AI) was collected from the Ayurvedic Garden of the Institute and was dried under shades and powdered. The powdered leaves were grounded in 4 liters of distilled water and allowed to soak overnight. Then it was filtered through Whatman filter paper and the aqueous extract of *Azadirachla indica* (AIE) was then dried. The yield was 2.4%.

Drug treatment : A fixed oral dose of 500 mg/kg of AIE was used on the basis of earlier reported work from our laboratory (13). AIE and standard proton pump inhibitor omeprazole (OMZ, 2 mg/kg) were suspended in 1% carboxymethyl cellulose (CMC) in distilled water. The test and standard drugs were administered orally, once daily for six days and the last dose of the test drugs was given 1 h prior to experiment to 18–24 h fasted rats. Control group of animals received 1% CMC only. AIE 200, 500, 1000 and 2500 mg/kg doses were used in acute toxicity (mice) study and 1000 mg/kg dose was used for sub acute toxicity study (rat).

Gastric secretion study : The gastric juice was collected after 4 h PL and the juice was centrifuged for 5 min at 2000 rpm. The volume of the supernatant was expressed as ml/100g body weight. Total acid concentration and output was determined by titrating the gastric juice with 0.01 *N* NaOH, using phenolphthalein as indicator and expressed as µEq/ml as concentration or µEq/4 h as output. Peptic activity was determined using haemoglobin as substrate and was been expressed as µmol of tyrosine/ml as concentration or µmol of tyrosine/4 h as output (14). Dissolved mucosubstances were estimated in the 90% alcoholic precipitate of the gastric juice. The precipitate, thus obtained was either dissolved in 1 ml of 0.1 *N* NaOH or 1 ml of 0.1 *N* H_2SO_4. The former was used for the estimation of protein (15) total hexoses, hexosamine and fucose, while the latter was used for the estimation of sialic acid (16). The results are expressed in µg/ml. The ratio of total carbohydrate (TC) (sum of total hexoses, hexosamine, fucose and sialic acid) to protein (P) has been taken as the index of mucin activity (16). DNA content was estimated and expressed as µg/ml gastric juice of rats (17).

Gastric perfusion study : The acid inhibitory effect of AIE and OMZ was seen against acid stimulatory effect of continuous infusion of pentagastrin (PENTA, 5 µg/kg/hr) in the rat gastric perfusate following the method of Ghosh & Schild (1958) and as described earlier (18). Briefly, food was withheld for 24 h before experimentation but water was allowed *ad libitum*. On the day of the experiment rats were anesthetized with urethane (1.6 g/kg) administered intramuscularly. Abdomen was opened by a midline incision and a glass cannula was introduced in the stomach through the pyloric end. The glass cannula was connected via a polythene tube draining in a test tube. Abdomen was closed in two layers. A polythene orogastric tube was next passed into the stomach through the mouth. Through this orogastric tube, *N*/2000 NaOH was perfused at a constant rate of 10.5 ml per 15 min, with the help a slow continuous injector. The NaOH solution was passed

through a coiled tube kept in a water bath maintained at 37°C. Another small polythene tube was passed into the jugular vein for slow intravenous infusion at a constant rate of 3.5 ml per hour of either normal saline (NS) or PENTA in normal saline. PENTA was administered at a rate of 5 µg/kg/h. The gastric perfusate was collected in the test tube at every 15 min and was used to estimate the acid concentration. After the experiment was set up, NS was infused intravenously till the acid output comes to a steady state level. Further, it has been seen that this steady state level usually comes within 90 min of NS infusion with *N*/2000 NaOH perfusion. The last 30 min samples were taken as the basal rate of acid secretion. After this basal rate of gastric output was obtained, saline infusion was either continued or replaced by PG in normal saline to note the rate of basal or stimulated acid secretion respectively. This infusion was carried out for the next three hours and effect on acid output was noted. Acid concentration was estimated by titration with AVI00 NaOH using phenolphthalein as indicator. Acid output was calculated as µEq/30 min and also expressed as percent normal control (18).

Proton pump (H^+/K^+-ATPase) inhibitory activity: On 6th day, one hour after the last dose administration of the test drugs, the rats were sacrificed by cervical dislocation and stomach was taken out. The gastric mucosa was rinsed with cold saturated sodium chloride solution for 3–5 minutes. The superficial cells, cell debris plus the mucus were wiped off with the edge of a plastic ruler. The mucosa in the fundic region was scrapped off. Scrapped tissue was weighed and suspended in 20 ml of sucrose-EGTA buffer containing 250 mM sucrose, 2 mM magnesium chloride, 1 mM EGTA and 2 mM Tris (pH 7.4). The tissues were homogenized for 3 minutes. The homogenate was fractionated by differential centrifugation. The nuclei, mitochondria and the microsomes were obtained by successive centrifugation at 3000 rpm for 10 min and at 20,000 rpm for 30 min twice. The supernatant was discarded and the pellet was weighed and homogenized in 10 ml of mannitol buffer containing 250 mM mannitol, 2 mM magnesium chloride and 2 mM Tris (pH 7.4) (19). 0.1 ml of the homogenized solution was used for estimation of ATPase enzyme activity by using Tuassky-Shorr colour reagent (20). Results were expressed as µmol phosphate liberated/g tissue/min and calculated from standard curve prepared by using phosphorus standard solution.

Toxicity studies: Acute toxicity study was carried out in albino Charles-Foster strain mice (10 mice of both sexes in equal number) using the following doses of 200, 500, 1000 and 2500 mg/kg. The animals were observed for 24 h for any behavioral changes and mortality. Sub acute toxicity study was carried out in rats and AIE was given orally in the dose of 1000 mg/kg, once daily for 4 weeks. Food and water intake, body weight changes and mortality were also observed in this group at an interval of one week up to 4 weeks of treatment with AIE in the above dose. At the end of 4 weeks, the rats were sacrificed by decapitation, blood was collected for hematological studies and serum was used for estimation of enzymes and other assays by standard procedures using commercial kits. Organs were collected, weighed and preserved for

histopathological studies.

Statistical analysis : It was done either by using unpaired student 't' test or by one way analysis of variance (ANOVA) followed by Dunnett' s test.

RESULTS

AIE significantly decreased volume, acid and pepsin concentration and output in 4 h PL rats and was comparable with OMZ (Table I). AIE showed little or no effect either on individual carbohydrates, total carbohydrates, protein or TC:P ratio indicating no effect on mucin secretion. However, it enhanced life span of mucosal cell as evidenced by a decrease in the gastric juice DNA content (Table I).

Continuous infusion of normal saline showed nearly a steady rate of acid secretion up to 3 h of study in rats (Table II & Fig. 1). When pentagastrin (PENTA. 5 µg/kg/h),

TABLE I : Effect of AIE (500 mg/kg, po, od for 6 days) and OMZ (2 mg/kg, po, od for 6 days) on gastric juice volume, acid and pepsin secretion in 4 h PL rats. Values are mean±SEM from 8 animals in each group.

Gastric juice parameters	*Control (1% CMC)*	*AIE*	*OMZ*
Volume (ml/100 g)	2.49±0.28	1.54±0.12*	1.32±0.14
Acid			
Concentration (µEq/ml)	88.6±6.2	51.8±3.1*	38.9±3.6*
Output (µEq/4 h)	221.2±29.4	79.2±7.8*	51.4±6.2*
Peptic			
Concentration (µmol/ml)	298.2±19.4	192.0±15.8*	165.2±17.2*
Output (µmol/4 h)	742.8±87.5	296.8±26.4*	219.1±19.6*
Mucoproteins (µg/ml)			
Total Hexoses	234.2±12.4	252.7±16.2	255.9±11.3
Hexosamine	166.1±4.7	171.0±5.1	166.7±4.3
Fucose	62.5±2.7	66.4±2.4	59.7±1.9
Sialic acid	25.6±1.4	27.8±1.2	28.4±1.5
Total carbohydrate (TC)	488.4±14.8	517.9±15.7	485.0±14.7
Protein (P)	480.2±11.4	485.0±14.7	492.2±10.4
TC:P ratio	1.02±0.03	1.07±0.02	1.04±0.04
Cell shedding (µDNA/ml)	231.4±11.9	179.2±6.9*	240.3±12.1

P<0.05 indicates significantly different from respective control group.
Statistical analysis was done by oneway ANOVA followed by Dunnett's test.

TABLE II : Effect of AIE and OMZ on PENTA (5 µg/kg/h)-stimulated acid secretion in rats.

Treatment	*Acid-output*							
	Basal secretion	*Basal/stimulated secretion*						*Total secretion*
	(–30 to 0 min)	*30 min*	*60 min*	*90 min*	*120 min*	*150 min*	*180 min*	*3 h*
NS	39.6±1.4	42.4±1.9	41.7±2.3	44.6±2.2	39.9±2.7	41.7±1.7	35.0±1.5	245.3±6.2
PENTA	41.2±2.1	58.3±2.4*	84.5±2.6*	90.6±3.0*	88.4±2.9*	83.9±2.6*	66.9±2.5*	472.6±14.9*
AIE+PENTA	34.4±1.2	52.2±2.5	63.1±2.6^{+}	66.8±2.1^{+}	50.6±1.6^{+}	48.0±1.9^{+}	44.7±2.6^{+}	325.4±9.2^{+}
OMZ+PENTA	33.9±1.3	43.8±1.8^{+}	65.3±2.4^{+}	61.5±2.5^{+}	53.9±2.2^{+}	46.3±1.8^{+}	37.4±2.1^{+}	308.2±10.6^{+}

Values are mean±SEM from 5 animals in each group; Acid output is expressed either as µ Eq/30 min or 3 h; *indicates significance from normal saline (NS) (P<0.05) and $^{+}$indicates significance from PENTA (P<0.05). Statistical analysis was done by one way ANOVA followed by Dunnett's test.

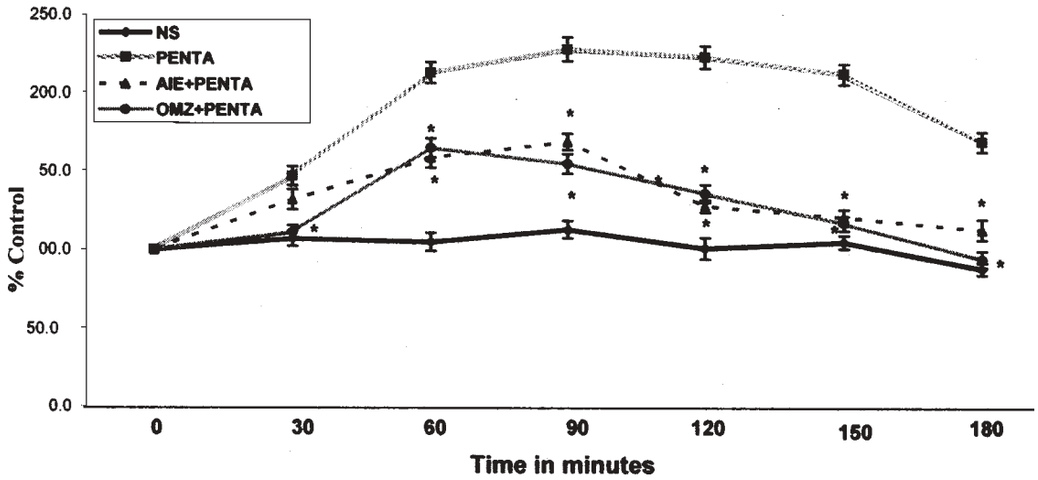

Fig. 1 : Effect of AIE and OMZ on pentagastrin (PENTA)-induced acid secretion in rat gastric perfusate. Statistical analysis was done by ANOVA followed by Dunnett's test. *P<0.05 indicates significantly different from respective PENTA group.

a secretogogue was infused through the jugular vein, it enhanced the 3 hr acid secretion by 92.7% (P<0.05). However, rats pretreated with AIE and OMZ, a proton pump inhibitor, showed significant inhibition of PENTA-stimulated acid secretion (total acid secretion inhibited by 31.1 and 34.8 % respectively) (Table II & Fig. 1).

AIE also showed significant proton pump inhibitory activity in the rat gastric mucosal homogenate. The % inhibition of proton pump activity was 43.2% and the effect was comparable to standard proton pump inhibitor drug, OMZ (71.4% inhibition) (Tablet III).

No mortality was observed with 2.5 g/kg dose of AIE (single administration) or after 28 days of 1 g/kg of AIE indicating that toxic dose was more than 2.5 g/kg compared with the effective ulcer protective dose which was 500 mg/kg. There was an increase in weights of testis in males and of both liver and kidneys in both the sexes of rats but the weight of adrenals was not increased. However, histology of these tissues did not show any gross abnormality between the control and AIE treated groups. Rats neither showed any change in food and water intake nor in their body weight at the end of 4 weeks study. Little or no change was observed in various hematological liver and renal function tests. However, SGOT and blood urea tended to increase and decrease respectively with AIE treatment (Table IV).

TABLE III : Effect of AIE and OMZ on proton pump (H^+/K^+-ATPase) activity in rats.

Oral treatment		*H^+/K^+-ATPase activity*	
(mg/kg, od × 6 days)		*(µmol Pi liberated/g/min)*	*% inhibition*
Control	(1% CMC)	9.12±0.64	–
AIE	500	5.18±0.56*	43.2
OMZ	2.0	2.61±0.30*	71.4

Values are mean±SEM from 8 animals in each group. *indicates significantly different from control group (P<0.05). Statistical analysis was done by one way ANOVA followed by Dunnett's test.

TABLE IV: Sub acute toxicity studies with *Azadiracta Indica* (1 g/kg) in rats (28 days treatment).

Parameters	*Male rats*		*Female rats*	
	Control	*AIE*	*Control*	*AIE*
Organ weight (n=6)				
Liver (g)	2.61±0.24	3.86±0.26	2.87±0.15	3.37±0.23
Kidney (g)	0.69±0.077	0.883±0.18	0.674±0.025	0.845±0.023
Adrenals (mg)	25.0±0.58	24.2±1.11	21.8±1.01	22.7±0.42
Testes (g)	1.39±0.068	1.76±0.075	––	
Haematological parameters (n=8)				
Hemoglobin (g%)	16.8±0.48	15.9±0.53	14.2±0.68	13.9±0.68
WBC ($\times 10^3/mm^3$)	14.0±0.30	13.4±0.57	13.0±0.36	13.0±0.644
Liver function tests (n=6)				
SGOT (IU/L)	0.609±0.07	0.97±0.24	0.690±0.08	1.1±0.13
SGPT (IU/L)	72.5±0.75	74.3±1.39	71.4±2.6	71.9±1.69
Total protein (mg/dl)	5.22±0.36	5.79±0.066	6.76±0.38	6.59±0.48
Albumin (g/dl)	2.33±0.96	2.46±0.15	3.17±0.189	3.12±0.18
Renal function tests (n=6)				
Blood urea (mg/dL)	47.9±6.3	35.9±4.4	48.6±2.3	41.7±2.5
Creatitnine (mg%)	1.16±0.11	1.27±0.99	1.08±0.17	1.03±0.099
General parameters (n=8)				
Body weight (gm/rat)	186±1.50	178±1.47	178±1.21	181±1.07
Food intake (g/rat/day)	27.4±0.711	25.8±0.62	25.6±0.78	26.8±0.94
Water intake (ml/rat/day)	32.6±0.99	31.6±0.67	30.4±0.67	29.8±0.71

Values are mean±SEM from 6–8 animals in each group.

DISCUSSION

Recent works with aqueous extract of leaves (13) and bark (21) of neem *(A. indica)* have indicated its ulcer protective and healing effects against various acute (for ulcer protective effect) and chronic (for ulcer healing effect) gastric ulcer models in rats. Further nimbidin, an active constituent of the oil of *neem* seeds reported to possess antiulcer activity by virtue of its antisecretory action (6, 22). It has also been reported to show H_2-blocking action (6) and shown to decrease both free and total acid output and peptic activity of the gastric fluid (5). Our present work incorporates study on both offensive (acid-pepsin secretion and proton pump inhibitory activity and defensive gastric mucosal parameters like mucin secretion and cell shedding, a good indicator for life span of mucosal cells.

Ulcers are thought to be due to an imbalance between offensive factors like acid and pepsin and defensive factors like mucin secretion, cell proliferation, prostaglandins etc (12). The role of hydrochloric acid in the pathogenesis of gastric ulcer is well established. Acid back diffuses through the compromised defense and destroys the cells, capillaries and vein causing hemorrhagic ulcers (23–26). It also causes release of histamine furthering acid output and thus further damaging the mucosa (27). Presence of acid causes a decrease of gastric pH and leads to activation of pepsinogen to pepsin, which increases the size of the lesion by its proteolytic action (28, 29). The reduction of acid–secretion is necessary for healing and prevention of esophageal reflux disease, gastric and duodenal ulcers (30). AIE decreased the acid-pepsin secretion and output in pylorus ligated rats and

significantly decreased the pentagastrin-stimulated acid secretion in rat gastric perfusate.

The secretion of acid by the parietal cells is achieved by an enzyme hydrogen-potassium adenosine triphosphatase (H^+/ K^+-ATPase), which pumps protons in exchange for potassium ions across the apical membrane (31). H^+/K^+-ATPase is located in the upper microvillus membrane and tubulo-vesicular apparatus of the parietal cell. However treatment with AIE significantly inhibited the proton pump activity in the rat gastric mucosal homogenate and the effect was comparable with the standard proton pump inhibitor, omeprazole.

Flavanoids have been reported to possess both antiulcer and anti-inflammatory activities (10, 32). The ulcer protective effects of them may be due to their various effects of both offensive and defensive mucosal factors which includes prostaglandins, mucin secretion, acid inhibition and inhibition of lipid peroxidation etc (13). We earlier reported the presence of flavanoids (rutin and quercetin) and phytosterols (β-sitosterol, sigmasterol and campasterol) in AIE (13). We did not find any effect of on mucin secretion though it was earlier reported to work through prevention of mucous depletion and mast cell degranulation (21). However, it was found to enhance life span of mucosal cells possibly through decreased secretion of offensive acid-pepsin secretion and subsequent less mucosal damage.

AIE was found to be well tolerated and dose up to 2.5 g/kg (single administration) or 1 g/kg when given for 28 days did not cause any mortality or histological changes in the kidney, liver, testis or adrenals though it either increased or tended to increase their weights. Hematological parameters and liver and kidney function tests as observed through estimation of various serum enzymes, urea or creatinine levels showed little or no change from the control values indicating it to be a safe drug.

Thus, the present study does indicate the ulcer protective effect of aqueous extract of leaves of *A. indica*. The protection may be due to its antisecretory effect and action on gastric mucosal H^+/K^+-ATPase activity rather than on defensive mucin secretion as confirmed by our PL and gastric perfusion studies. Our results are also in conformity of the earlier reported antisecretory and proton pump inhibitory effects of aqueous extract of bark of this plant (21, 23). The extract seems to be well tolerated in animals. A further clinical work may be done to study the safety and efficacy of this extract in peptic ulcer diseases.

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REFERENCES

1. Murty KS, Rao DN, Rao DK, Murty LBG. A preliminary study on hypoglycemic and anti hyperglycemic effect of *Azadirachta indica*. *Indian J Pharmacol* 1978; 10: 247–250.
2. Pillai NR, Santha Kumari G. Hypoglycemic activity of Melia *Azadirachta indica*. *Indian J Med Res* 1981; 74: 931–933.
3. Sen P, Mediratta PK, Ray A. Effects of *Azadirachta indica* A Juss on some biochemical, immunological and visceral parameters in normal and stressed rats. *Indian J Exp Biol* 1992; 30: 1170–1175.

4. Ray A, Banerjee BD, Sen P. Modulation of humoral and cell mediated immune responses by *Azadirachta indica* (Neem) in mice. *Indian J Exp Biol* 1996; 34: 698–701.

5. Pillai NR, Suganthan D. Seshadri C, Santha kumari G. Anti–gastric activity of *nimbidin*. *Indian J Med Res* 1978; 68: 169–175.

6. Balakrishnan V, Narendranathan M, Subair AS, Raji EK, Pillai NR, Santhakumari G. Nimbidin in duodenal ulcer. *Tropical Gastroenterology* 1985; 6: 23–25.

7. Schmutterer H. In *The Neem Tree* edited by Schmutterer H. (Weinheim, Federal Republic of Germany: VCH) 1995; pp. 1.

8. Kraus W. Biologically active ingredients, in *The Neem Tree* edited by Schunutterer H. (Weinheim, Federal Republic of Germany: VCH) 1995; pp. 35.

9. Devakumar C, Sukh Dev, Chemistry, in *Sukh Dev Chemistry Neem* edited by Randhawa NS and Parmar BS. (2nd edition) 1996; pp–77.

10. Kontureck S J, Redecki T, Brzozowski T, Drozdowicz D, Piastuki I, Muramatsu M, Tangka M & Aihara H, Antiulcer and gastroprotective effects of solon, a synthetic flavanoid derivative of sophoradine: Role of endogenous prostaglandins. *Eur J Pharmacol* 1986; 125: 185–192.

11. Sachs G, Maton PN, Wallmark B. Pharmacology of parietal cell, in *Pharmacology of peptic ulcer disease,* edited by Collin MJ and Benjamin SB. (Springer VErlag, New York) 1991; pp–1.

12. Goel RK, Bhattacharya SK. Gastroduodenal mucosal defense and mucosal protective agents. *Indian J Exp Biol* 1991; 29: 701–714.

13. Dorababu M, Prabha T, Priyambada S, Agrawal VK, Aryaa NC, Goel RK. Effect of *Bacopa monniera* and *Azadirachta indica* on gastric ulceration and healing in experimental NIDDM rats. *Indian J Exp Biol* 2004; 42: 389–397.

14. Debnath PK, Gode KD, Govinda Das D, Sanyal AK. Effect of propranolol on gastric secretion in albino rats. *Br J Pharmacol* 1974; 51: 213–216.

15. Lowry OH, Rosenborough NJ, Farr AL, Randal RJ. Protein measurement with folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.

16. Sanyal AK, Mitra PK, Goel RK. A modified method to estimate dissolved mucosubstances in gastric juice. *Indian J Exp Biol* 1983; 21: 78–80.

17. Mukhopadhyaya K, Bhattacharya D, Chakrabarti A, Goel RK, Sanyal AK. Effect of banana powder *(Musa sapientum var.* paradisiaca) on gastric mucosal shedding. *J Ethnopharmacol* 1987; 21: 11–19.

18. Goel RK, Sanyal AK. Effect of $PGF_{2\alpha}$ on basal and pentagastrin stimulated gastric secretion in anaesthetized albino rats. *Indian J Exp Biol* 1982; 20: 901–903.

19. Rabon EC, Im WB, Sachs G. Preparation of gastric H^+/K^+-ATPase. In: *Methods in enzymology,* edited by Fleischer S, Fleischer B, Vol. 157, Academic press. Harcourt Brace Jovanovich, 1988; pp. 649–654.

20. Taussky HH, Shorr EA. Micro colorimetric method for the determination of inorganic phosphorus. *J Biol Chem* 1953; 202: 675–685.

21. Bandyopadhyay U, Biswas K. Chatterjee R, Bandyopadhyay D, Chattopadhyay I, Ganguly CK, Chakraborty T, Bhattacharya K, Banerjee RK. Gastroprotective effect of Neem *(Azadirachta indica)* bark extract: possible involvement of H^+/K^+-ATPase inhibition and scavenging of hydroxyl radical. *Life Sci* 2002; 71: 2845–2865.

22. Pillai NR, Santhakumari G. Effects of nimbidin on acute and chronic gastroduodenal ulcer models in experimental animals. *Planta Medica* 1984; 50: 143–146.

23. Pierson RN, Holt PR, Watson RM, Keatling RR. Aspirin and gastrointestinal bleeding: Chromate 51 blood loss studies. *Am J Med* 1961; 31: 259–265.

24. Rainsford KD. Microvascular injury during gastric mucosal damage by inflammatory drugs in pigs and rats. *Agents Actions* 1983; 13: 5–6.

25. Takeuchi K, Okada M, Ebara S, Osano H. Increased microvascular permeability and lesions formation during gastric hypermotility caused by indomethacin and 2-deoxy-D-glucose in the rat. *J Clin Gastroenterol* 1990; 12: S76–S84.

26. Muira S, Sueatsu M, Tanaka S, Nagata S, Nagata H, Houzawa S, Suzuki M. microcirculatory disturbance in indomethacin-induced intestinal ulcer. *Am J Physiol* 1991; 261: G213–G219.

27. Abdel-Galil AAM, Marshall PB. Phenylbutazone and histamine formation in rat glandular stomach: Its relationship to gastric ulceration. *Br J Pharmacol* 1968; 33: 1–14.

28. Nagashima R, Samloff IM. Aggressive factors II: pepsin. In: *Peptic ulcer disease,* edited by Brocks F. Churchill Livingstone, Edinburgh. 1985; pp. 181–214.

29. Leonard A, Alien A. Gastric mucosal damage by aspirin. *Gut* 1986; 27: A1236–A1237.

30. Baron H. Pathophysiology of gastric acid and pepsin secretion. In: *Magen and magenkrankheiten,* edited by Domschke W, Wormsley KG, Stuttgart, Thieme Verlag, 1981; pp. 131–149.

31. Wallmark B, Larsson H, Humble L. The relationship between gastric acid secretion and gastric H^+/K^+-ATPase activity. *J Biol Chem* 1985; 260: 13681–13684.

32. Goel RK, Pandey VB, Dwivedi SPD, Rao YV. Anti-inflammatory and anti-ulcer effects of Kaempferol, a flavone isolate from *Rhamnus procumbent*. *Indian J Exp Biol* 1988; 26: 121–24.