Original Article

The Effect of Neem (Azadirachta Indica) Leaf Extract and Neem Compound Nimolicine On Gastric Acidity

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ABSTRACT

Objectives: This study has been conducted to look at the anti acid effect of Neem and to compare the effect of leaf extract with the pure compound nimolicine on the gastric acidity. Nimolicine has been studied for its anti acid effect for the first time.

Design of Study: Experimental study.

Place of Study: This study was conducted in the department of Physiology, Baqai Medical College and the Department of Pharmacology in Baqai Institute of Pharmaceutical Sciences, Karachi.

Materials and Methods: Ethanol induced gastric ulcers in albino rats were treated with methanolic neem leaf extract (800mg/day for 5 days) and nimolicine (1%/day for 3 days) and the gastric acid secretion was estimated. The control of the treated group was given peanut oil 1 ml/day for 5 days. The effect on gastric secretion was compared with the effect of anti-ulcer drugs cimetidine® (50 mg/kg for 7-10 days) and omeperazole® (2.5 mg/kg/day for 7-14 days).

Result: Neem leaf extract is a better suppressor of H-ion secretion compared to nimolicine but both neem leaf extract & nimolicine did not show a significant suppression of acid compared to ranitidine and omeperazole. The comparison between control and ranitidine in suppression of acid was significant.

Conclusion: Methanolic NLE and neem compound nimolicine do not decrease gastric acidity and their role as anti-ulcer agents may be because of other mechanisms which need to be studied.

Key words Acid peptic disease, Azadirachta indica, anti ulcer, nimolicine.

INTRODUCTION

Acid peptic disease is a common clinical problem. About 4 million people suffer from this disease in the USA. The current therapy for the disease has limitations and not easily affordable. Thus alternate herbal preparations are gaining popularity. Neem (Azadirachta indica) has been claimed to have an anti ulcer effect but the mechanism is still not clear. Food after chewing is swallowed into the stomach which stores, mixes and empties the chyme into the duodenum. The gastric secretion contains HCl which is responsible for the maintenance of acidic pH. A powerful defensive mechanism is provided by mucus bicarbonate layer which serves as a mucus gel impeding diffusions of harmful substances maintaining the integrity of mucosal membrane [1, 2]. A net imbalance in mucosal offensive and defensive factors play a major role in ulcer production[3]. PG plays a central role in providing this defense[4]. Peptic ulcer disease (PUD) is a mucosal erosion equal to or greater than 0.5 cm of an area in the stomach or duodenum. The life time risk of developing PUD is 10%[5]. The use of anti-ulcer drugs, though they are effective has limitations[6-9]. Thus recent interest has been towards non toxic herbal preparations which is increasing[4]. Azadirachta indica (neem) is an important plant of great medicinal importance and has been shown to posses anti-ulcer and anti secretory effects[10-15]. Thus in this part of the study the effects of methanolic extract of neem leaves and isolated compound of neem Nimolicine (NC) on gastric acidity have been seen while demonstrating the anti-ulcer effects of neem leaf extract NLE and NC. The anti secretory effects of NLE and NC have been compared with cimetidine and omeperazole. The anti secretory effects of NC have been studied for the first time. Nimolicine (Azadiradione) was isolated from the fresh fruit coatings in 0.35% yield (calculated according to the weight of fresh coatings)[16].

MATERIALS AND METHODS

Male and female Sprague Dawley rats weighing 180-200 Gms. were used. They were purchased from the animal house of HEJ Institute of Chemistry, Karachi University. They were kept in the animal house of Baqai Medical University under optimum conditions and temperature ranging from 20 to 22 ^{o}C. The animals were kept in plastic cages and were acclimatized to laboratory conditions and had free access to food and

tap water ad libitum. For weighing of rats an electronic computerized talking weighing machine (Toyo model AT-1020, Japan) (1 to 5000 Grams or 11 lbs.) was used. Animals were fasted for 48 hrs. with water ad libitum before each experiment. During this period rats were transferred in wired cages where the floor was wired to prevent coprophagy. This was done to ensure an empty stomach. The rats were divided into six experimental groups i.e. each group comprised of ten rats i.e., five males and five females. Ulcer induction in Group-1 was done by per oral administration of 1ml of 100% ethanol and the rats were sacrificed after 24 hrs. The other groups each (n=10) were the experimental groups received oral doses of Neem extract/ Neem compounds/ standard ulcer healing drugs as shown in tables. In each test group one control were kept. The control group was given Peanut oil 1 ml/day for 7 days. The test groups received oral NLE 1ml/day (0.88 gm) for 5 days and NC 1%/ day for 3 days (0.01 gm in 1 ml). The H-ion secretion was compared to the effects of oral ranitidine 50 mg/kg per day for 5 days and oral omeperazole 2.5mg/kg per day for 10 days. We used the curative method of treatment instead of protective method as done by most other workers.

Neem leaf extract and neem compound nimolicine NC was obtained from HEJ by the courtesy of Prof. Dr Beena S. Siddiquie. NC in pure powder form. Dissection of animals was carried out according to the protocol for each group. Chloroform was used to anaesthetize the rats. The animals were placed in a glass dessicator containing cotton swabs soaked in chloroform ($CHCl_4$) for a few minutes. The rat was then placed on a dissection board and immobilized by paper pins. A midline incision was given and the stomach was exposed. The upper and the pyloric ends were ligated so that the contents do not escape. The stomach was removed and opened along the greater curvature. Gastric contents were collected and gastric acidity was determined as described (17).

Calculation of gastric acidity

The gastric contents were drained into a centrifuge tube and centrifuged for 1 hour at 45 cycles per second. The supernatant was then collected in a test tube. The volume measured by a 0.5 ml pipette was (V_1). This was titrated with NaOH (N_2=0.1M or 0.40 gms in 100ml. of distilled water) by a 10 ml burette to an end point using Phenolpthaline as an indicator. The NaOH volume (V_2) consumed during titration was used for calculation. The H-ion secretion was expressed as µEq. /100gm.

The study was approved by the Board of advanced study and the ethical committee of Baqai Medical University. There was no funding from external or internal resources.

Statistical analysis of data

The computerised software programme SPSS version 18 was used for the analysis of data. The results were obtained by applying a one way analysis of variance (ANOVA). The significant p value was considered to be ($p<0.05$). Bonferroni correction was used to reduce type-one error.

RESULTS

Gastric Secretion of H-ion (mEq/100 gms)

The Mean values with SE± of H-ion (µEq/100gm) secretion in the control group and the treated group is shown. The pair wise comparison of different groups has also been shown. There is a significant difference ($p<0.05$) on comparison of control group with the ranitidine group (Table-8). The other values are insignificant indicating that the anti secretory effects of NLE and NC as compared to ranitidine® (Ran) and omeperazole® (OMP) are not of importance statistically though the values apparently differ.

Table-1. Mean ± SE of weight and gastric secretion of H-ions (µEq/100 gms) in check, control and test groups.

Group	Wt (gms)	SE ±	H (µEq /100) gms	SE ±	N
Check (Ethanol 100%)	161.09	10.26	1.32	0.04	7
PNO (1ml) (control)	202.31	7.26	6.36	1.42	14
Ran (50 mg/kg)	222.86	13.62	0.38*	0.1	5
OMP (2.5 mg/kg)	213.33	6.15	2.08	0.13	6
NLE (1ml/day)	189	7.14	4.03	0.7	8
NC (1% sol)	231.67	30.6	5.19	0.47	9

* Significantly different from the control ($p<0.05$)

DISCUSSION

Treatment of ulcers is a global problem and needs to be studied in detail specially whether an over secretion of H-ions is the basic cause of ulcers. The treatment of ulcers is aimed at reducing acid secretion. The present study was conducted to look at the anti H-ions secretory effects of methanolic neem leaf extract (NLE) and a newly isolated neem compound nimolicine (NC). In this study the gastric secretion of H-ions (µEq/100gm) in the treated group is lesser compared to the control group but the result is not statistically significant. Significant suppression of H-ion secretion is only of ranitidine showing values of 0.38±0.09. The result of

this study is different from another study where they have shown a dose dependent reduction of gastric acidity augmented by cimetidine indicating a possible H_2-receptor blockade effect[15] of aqueous extract of neem leaves.

The effects of aqueous extract of neem leaves have also been shown to have an anti-ulcer effect by preventing mast cell degranulation and increasing the amount of adherent gastric mucus in stressed animals[12]. The present study differs in results because of the difference that the methanolic extract is different from the aqueous extract of neem leaves. Methanolic extracts are more soluble compared to aqueous extract and are therefore more effective. Mast cells by secreting histamine are responsible for stress induced ulcers but the mechanism of ulcers induced by ethanol is different[33].

It has been shown that the neem (Azadirachta indica) bark extract inhibits H^+-K^+-ATPase activity in vitro, prevents mucus depletion and causes mast cell degranulation but provides gastroprotection by its significant antioxidant effect. Bark extract was shown to be equipotent to ranitdine but more potent to omeperazole in inhibiting pylorus ligation induced acid secretion. It also inhibits MMI (mercapto-methylimidazole) induced acid secretion which acts through histamine release[10]. The potent anti-ulcer and anti secretory effect of Neem Leaf extract has been attributed to a glycoside[18] which inhibits Cl^--transport in the gastric epithelium [19].

The effect of neem bark extract on gastric acidity showing a reduction in gastric volume and pepsin activity in a clinical trial[14] is different from the present study because the bark extract is different in composition compared to leaf extract[20]. The anti-ulcer effect of methanolic extract of neem leaves and NC may be due to their antioxidant effect rather than anti secretory effect.

The present study also differs from the effects of Nimbidin (80 mg/Kg) significantly inhibiting histamine induced DU in guinea pigs[21]. This possibly demonstrates the H_2 receptor blocking effect of Nimbidin which may not be the mechanism involved in anti-ulcer effect of NLE and NC[22]

The lack of significant results of NLE and NC on gastric acidity possibly explains that the mechanism of ulcer inhibition may be due to other reasons. Similar findings have been shown[23] regarding gastroprotection provided by omeperazole was because of its antioxidant and anti apoptotic role. Development of gastric ulcers is a multifactorial process[23] where a balance between acid production and bicarbonate-mucus barrier is disturbed[24]. A disturbance in the antioxidant system[25-26] due to reactive oxygen species (ROS) is the major causative factor[27, 28] which develop due to neutrophil infiltration[29]. Non-ROS-mediated apoptotic cell death occurs in stress ulcer through the involvement of NO[30], caspase-3[31] and an imbalance between antiapoptotic Bcl-2 and apoptotic Bax proteins[32]. TNF-α-induced apoptotic cell death is also noted in ethanol induced gastric damage[33]. Thus a good anti-ulcer agent must have anti secretory, anti inflammatory, anti oxidant and angiogenic effects. Neem is a well known plant having all these properties. But chemical composition of different parts of tree varies and may have different function and mechanism of action. The leaf extract differs in composition to bark extract ie nimbidine is present in bark extract not in leaf extract. The leaf extract containing azadirachtin (AZ) are 3-4 times more potent in effects compared to the pure isolated compound AZ[34]. These differences in chemistry might be a factor in the difference in results shown in present study.

The financial impact of PUD is tremendous with an estimated burden on direct and indirect health care costs of ~$10 billion per year in the United States[4]. Thus further clinical trials to prove the effectiveness of neem extracts must be conducted. This will prove to be a better and cost effective remedy for gastric ulcer therapy specially in the developing world.

CONCLUSION

Methanolic NLE and isolated compound of neem Nimolicine NC do not decrease gastric acidity and their role as anti-ulcer agents may be because of other mechanisms which need to be explored.

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REFERENCES

1. Holzer P. Gastroduodenal mucosal defense. Curr Opin Gastroenterol 2000;16: 469-478.
2. Wallace JL, Granger DN. The cellular and molecular basis of gastric mucosal defense. 1996; FASEB J 10: 731–740.
3. Goel RK, Bhattacharya SK. Gastroduodental mucosal defense and mucosal protective agents. Indian Journal of Experimental Biology 1991; 29: 701-714.
4. Valle JD, Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, et al. "Chapter 287. Peptic Ulcer Disease and Related Disorders". Harrison's Principles of Internal Medicine, 2010;17e: http//www.accessmedicine.com/content.aspx?aID= 2899580.

5. Snowdex FM. Emerging and reemerging disease: "Historical and perspective" Immunol Rev 2008; 225: 9-26.
6. Howden CW, Hunt RH. Peptic ulcer disease. In: Lewis JH editor. A Pharmacologic Approach to Gastrointestinal Disorders. Maryland USA: Williams and Wilkins; 1994: p.1–21.
7. Laheij RJ, Sturkenboom MC, Hassing RJ, Dieleman J, Stricker BH, Jansen JB. Risk of community-acquired pneumonia and use of gastric acid-suppressive drugs. JAMA 2004;292: 1955–1960.
8. Langtry HD, Wilde MI. Omeprazole. A review of its use in Helicobacter pylori infection, gastro-oesophageal reflux disease and peptic ulcers induced by nonsteroidal anti-inflammatory drugs. Drugs 1998; 56: 447–486.
9. Waldum HL, Gustafsson B, Fossmark R, Qvigstad G. Antiulcer drugs and gastric cancer. Dig. Dis. Sci. 2005; 50: 39–44.
10. Bandyopadhyay U, Biswas K, Chatterjee R, Bandyopadhyay D, Chattopadhyay I, Ganguly CK, et al. Gastroprotective effect of Neem (Azadirachta indica) bark extract: Possible involvement of H+-K+-ATPase inhibition and scavenging of hydroxyl radical. Life Sciences 2002; 71:2845-2865.
11. Subapriya R and Nagini S. Medicinal properties of Neem Leaves: A Review. Curr. Med. Chem.-Anti-Cancer Agents 2005;5(2): 1-8.
12. Garg GP, Nigam SK, Ogle CW. The gastric antiulcer effects of the leaves of the neem tree. Planta Med. 1993;59(3):215-217.
13. Spirupa D, Jena J, Patnaik SL and Mukherjee D. The antiulcer effects of Azadirachta Indica in pyloric-ligated rats. XXXIV Annual conference of the Indian Pharmacological Society, Nagpur, January 10-12, 2002. Indian Journal of Pharmacology 2002;34: 141. Abstracts of research papers (part-I) pp.5.
14. Bandyopadhyay U, Biswas K, Sengupta A, Moitra P, Dutta P, Sarkar D, et al. Clinical studies on the effect of Neem (Azadirachta indica) bark extract on gastric secretion and gastroduodenal ulcer Life Sci 2004; 29; 75(24): 2867-2878.
15. Raji Y, Ogunwande IA, Osadebe CA, John G. Effects of Azadirachta indica extract on gastric ulceration and acid secretion in rats. J Ethnopharmacol. 2004; 90(1):167-70.
16. Siddiqui BS, Munawwer Rasheed, Ghiasuddin, Naqvi SNH.and Tariq RM. Biologically active Triterpenoids of Biogenetic Interest from the Fresh Fruit coats of Azadirachta indica. Tetrahedron 2000; 56: 3547-3551.
17. Lai KS. Studies on gastrin. Gut 1964; 5:327-341.
18. Biswas K, Chattopadhyay I, Ranajit K., Banerjee and Bandyopadhyay U. Biological activities and medicinal properties of Neem (Azadirachta indica). Current Science 2002; 82(11):1336-1345.
19. Machen TF, Forte IG. Gastric secretion. In: Guebischil G, Tasteson DC, Using H.H. (Eds.). Handbook of Transport Organs. Berlin Springer 1979; 693-747.
20. Maity P, Biswas K, Chattopadhyay I, Ranajit K., Banerjee and Bandyopadhyay U. Review article: The use of Neem for controlling gasric hyperacidityand ulcer. Phytotherapy Res 2009; 23: 747-755.
21. Pillai NR. Santhakumari G. Effects of Nimbidin on acute and chronic gastro-duodenal ulcer models in experimental animals. Planta Medica 1984; 143-146.
22. Qamer A. To study the anti-ulcer effect of Azadirachta indica leaf extract and isolated neem compound nimolicine (NC) in comparison with ulcer healing drugs on gastric mucosa of albino rats (PhD thesis, Baqai Medical University, Karachi: 2010.
23. Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK. A Novel Antioxidant and Antiapoptotic Role of omeperazole to Block Gastric Ulcer through Scavenging of Hyroxyl Radical J Biol Chem 2003; 278(13): 10993-11001.
24. Tarnawski A, Halter F. Cellular mechanisms, interactions, and dynamics of gastric ulcer healing. J Clin Gastroenterol 1995; 21 (Suppl 1): 93–97.
25. Bhattacharjee M, Bhattacharjee S, Gupta A, Banerjee RK. Critical role of an endogenous gastric peroxidase in controlling oxidative damage in H. pylori-mediated and nonmediated gastric ulcer. Free Radic Biol Med 2002; 32: 731–743.
26. Das D, Banerjee RK. Effect of stress on the antioxidant enzymes and gastric ulceration. Mol Cell Biochem 1993; 125: 115–125.
27. Guslandi M. A radical view of Helicobacter pylori. Am J Gastroenterol 1999 94; 2797–2798.
28. Phull PS, Green CJ, Jacyna MR. A radical view of the stomach: the role of oxygen-derived free radicals and antioxidants in gastroduodenal disease. Eur J Gastroenterol Hepatol 1995; 7: 265–274.
29. Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. Am J Physiol 1990; 259: 462–467.
30. Qiu BS, Pfeiffer CJ, Cho CH. Effects of chronic nitric oxide synthase inhibition in cold-restraint and ethanol-induced gastric mucosal damage in rats. Digestion 1996; 57: 60–66.
31. Slomiany BL, Piotrowski J, Slomiany A. Role of basic fibroblast growth factor in the suppression of

apoptotic caspase-3 during chronic gastric ulcer healing. J Physiol Pharmacol 1998 49; 489–500.
32. Konturek PC, Brzozowski T, Konturek SJ. Apoptosis in gastric mucosa with stress-induced gastric ulcers. J Physiol Pharmacol 1999; 50: 211–225.
33. Piotrowski J, Piotrowski E, Skrodzka D, Slomiany A, Slomiany BL. Gastric mucosal apoptosis induced by ethanol: effect of antiulcer agents. Biochem Mol Biol Int 1997; 42: 247–254.
34. Govindachari TR, Suresh G, Gopalakrishna G. A direct preparative high performance liquid chromatography procedure for the isolation of major triterpinoids and their quantitative determination in neem oil of liquid chromatography 1995; 18:3465-3471.

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