Evaluation of the anti-ulcer activity of NR-ANX-C (a polyherbal formulation) in aspirin & pyloric ligature induced gastric ulcers in albino rats

Vinod Nair*,+, Albina Arjuman**, H.N. Gopalakrishna*, P. Dorababu*, Mirshad P.V.*, Divya Bhargavan* & Dipsanker Chatterji*

Departments of *Pharmacology & **Biochemistry, Kasturba Medical College, Mangalore, India

Background & objectives: The aetiology of gastric ulcers is not completely understood and continuous use of anti-ulcer agents leads to many side effects. In this study we evaluated the anti-ulcer efficacy of a polyherbal formulation with potent antioxidant activity in aspirin and pyloric ligature induced gastric ulcers in rats.

Methods: The efficacy of the polyherbal formulation NR-ANX-C (composed of the extracts from *Withania somnifera, Camellia sinensis, Ocimum sanctum*, shilajith and triphala) was evaluated in terms of antioxidant potential as assessed in terms of protection from lipid peroxidation and the antiulcer activity as seen by the area of gastric lesions, gastric juice volume, gastric *p*H, total acidity and total adherent gastric mucus content.

Results: In our study, NR-ANX-C (25 and 50 mg/kg) was more efficacious than ranitidine in reducing ulcer index in both the models. At the highest dose tested (50 mg/kg), NR-ANX-C was comparable to omeprazole in preventing ulcer formation in the pyloric ligature model. NR-ANX-C showed a dose-dependent decrease in gastric juice volume and total acidity in both the models. A dose-dependent increase in gastric *p*H and total adherent gastric mucus was also seen in NR-ANX-C treated groups. The extent of lipid peroxidation was also reduced in the test drug treated groups.

Interpretation & conclusion: Based on our findings, we presume that the cytoprotective, anti-secretary and antioxidant properties of NR-ANX-C were responsible for its anti-ulcer activity. These findings suggest the potential for use of NR-ANX-C as an adjuvant in the treatment of gastric ulcer.

Gastric ulcers are mucosal lesions that result from an imbalance between aggressive factors such as acid and pepsin, and defensive mechanisms like gastric mucous, high mucosal blood flow and high mucosal

---

turnover rate that work towards maintenance of mucosal integrity[1]. Another factor that has been implicated in the pathogenesis of gastric ulcers is oxidative stress in the gastric mucosa. Studies have shown a positive correlation between increased free radicals and the extent of gastric ulceration in experimental animals[2-4]. Agents that are currently available for the treatment of gastric ulcers act by either reducing gastric acid secretion (H2 blockers, proton pump inhibitors, anti muscarinic agents), acting as physical barriers (sucralfate, colloidal bismuth subcitrate), or increasing the mucous and bicarbonate secretion (prostaglandin analogues, carbenoxolone). Even though these agents are effective in healing of gastric ulcers, continued use is required to prevent recurrence. Continued use of these agents can in turn lead to a plethora of side effects ranging from dryness of mouth to achlorhydria, atrophic gastritis, osteodystrophy and encephalopathy[5]. A new approach would be the use of cytoprotective agents that can also modulate the antioxidant defenses in the body and thus prevent mucosal damage and gastric ulceration. As plants are a rich source of active principles and antioxidants, there has been a growing interest to identify and scientifically validate agents that have traditionally been used in folk medicine in the treatment of gastric ulcers and related diseases.

NR-ANX-C is a polyherbal formulation (a test drug supplied by Natural Remedies Pvt. Ltd., Bangalore, India) containing aqueous extracts of *Withania somnifera* root, triphala and shilajith and the ethanolic extracts of *Ocimum sanctum* and *Camellia sinensis* leaves. All the individual components of the above mentioned polyherbal formulation have been shown to possess significant antioxidant potential[6-11]. In this study we evaluated the efficacy of NR-ANX-C in preventing aspirin and pyloric ligature induced gastric ulcers in rats. As various factors including gastric acidity, free radical damage and loss of mucosal defense have been implicated in the development of gastric ulcers, we evaluated the test drug so as to assess its cytoprotective, anti-secretory and antioxidant properties.

Material & Methods

Animals: The study protocol was approved by the Institutional Animal Ethical Committee, Kasturba Medical College, Mangalore. Male Wistar rats weighing 180-200 g were procured from the institutional animal house and were housed in groups of 3. The animals were acclimatized for a duration of 7 days at $25 \pm 1^{\circ}$C and 12:12 h light-dark cycle with free access to food and water. The animals were deprived of food 24 h before the study and transferred to metabolic cages so as to avoid coprophagy.

Test drug: The composition of test drug NR-ANX-C (supplied by Natural Remedies Pvt. Ltd, Bangalore), a polyherbal formulation, was as follows: *Withania somnifera* 17 per cent (aqueous extract of root: withanolides 2% w/w), *Ocimum sanctum* 17 per cent (70% alcohol extract of leaves: ursolic acid 3% w/w), *Camellia sinensis* 33 per cent (70% alcohol extract of leaves: total polyphenols 60% w/w)*, triphala 25 per cent (aqueous extract: total tannin 34% w/w) and shilajit 8 per cent (aqueous extract: fulvic acid 53% w/w; humic acid 17% w/w). The standardization of the plant extracts was done by gravimetry and HPLC.

Experimental protocol: A pilot study was carried out using the test drug and a minimum effective anti-ulcer dose was calculated. This was taken as the lowest dose and the efficacy of the test drug was further evaluated in doses of 10, 25 and 50 mg/kg body weight by using aspirin and pyloric ligature induced gastric ulcer models. The anti-ulcer efficacy of NR-ANX-C was compared against standard drugs *viz.*, ranitidine (27 mg/kg body weight) and omeprazole (1.8 mg/kg body weight). The dose for ranitidine and omeprazole was calculated from their corresponding ulcer healing doses (300 mg and 20 mg per day respectively) used in man. The test drug and the standard drugs were suspended in 1 per cent gum acacia and were administered in a dose of 2ml/kg body weight *per orally*.

Aspirin-induced gastric ulcer: One day before the induction of ulcers, animals were divided into groups (n=6) and drugs/vehicle was administered as follows. Group I received 2 ml/kg vehicle (1% gum acacia), group II received ranitidine (27 mg/kg body weight), group III received omeprazole (1.8 mg/kg body weight), groups IV, V and VI received NR-ANX-C in doses of 10, 25 and 50 mg/kg body weight respectively, *per orally*. The animals were then fasted (with free access to water) for a period of 24 h so as to ensure complete gastric emptying and a steady state gastric acid secretion. The 24 h fasted animals were again administered with the drugs/vehicle on the morning of the experiment. Sixty minutes after administration of the drugs/vehicle, aspirin was administered in a dose of 500 mg/kg body weight orally to all the animals[12]. Food was withheld for a duration of 5 more hours. Animals were then sacrificed by an overdose of anaesthetic ether. The stomach was dissected out and a small opening was

made along the greater curvature. All the gastric content was drained into a graduated centrifuge tube and used for biochemical estimations. The stomach was then cut open along the greater curvature and evenly spread out on a dissection board. A transparent film was placed over it and the boundary of the stomach and ulcerated area was traced on the film. The mucosal surface was then gently scraped with a blunt surface to collect the adherent mucus.

Pyloric ligature induced gastric ulcers: One day before the induction of ulcers, the animals were divided into groups (n=6) and drugs/vehicle was administered as mentioned under aspirin induced gastric ulcers. The animals were then fasted (with free access to water) for a period of 24 h so as to ensure complete gastric emptying and a steady state gastric acid secretion. The 24 h fasted animals were again administered with the drugs/vehicle on the morning of the experiment. Sixty minutes after administration of the drugs/vehicle, the animals were anaesthetized using anaesthetic ether and a midline incision was made just below the xiphoid process. The stomach was lifted out and ligated at the level of the pylorus following which it was replaced and the abdomen wall was closed by interrupted sutures[13]. The animals were then housed separately and food and water was withheld for a duration of 4 h following which they were sacrificed by an overdose of anaesthetic ether. The stomach was then dissected out, gastric contents were collected and the boundary and ulcerated area was traced as mentioned above.

Determination of ulcer index: The tracing of the stomach boundary and the ulcerated area on the transparent film was placed on top of a graph paper. The total surface area of the stomach and the lesions was determined in mm² from the graph paper. The ratio of total surface area and the total ulcerated area was determined and scoring of the ulcer index was done according to the method described by Ganguly[14]. Percentage protection was calculated in the drug treated groups against control using the formula:

% protection = (1-ulcer index in test/ulcer index in control) x 100

Estimation of gastric volume, pH, and total acidity: The gastric content that was transferred into centrifuge tubes was used for estimation of gastric volume, *p*H and total acidity. The tubes were centrifuged at 1000 rpm for 10 min and the gastric volume was directly read from the graduation on the tubes. The supernatant was then collected and *p*H was determined by using a digital *p*H meter (ECOSCAN: EC-PH510, Thermo Fisher

Scientific, Mumbai). Total acidity was determined by titrating 1.0 ml of gastric juice against N/10 NaOH to *p*H 7 using phenolphthalein as the indicator and was expressed in terms of clinical units, *i.e.*, the amount (ml) of N/10 NaOH required to titrate 100 ml of gastric secretion[15].

Determination of adherent gastric mucus: The mucosal scrapings were weighed and incubated with 1 per cent alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, *p*H 5.8) for 2 h. The tubes were then centrifuged at 1450 *g* for 10 min and the absorbance of the supernatant was measured at 489 nm to determine the total adherent mucopolysaccharide content[16].

Estimation of tissue malondialdehyde: The stomach of the animals was weighed, homogenized (10% in cold 2 mM phosphate buffer, *p*H-7.2) and centrifuged to pellet out organic debris. The supernatant was then collected and malondialdehyde (MDA) was estimated in it as thiobarbituric acid reactive substances (TBARS)[17].

Statistical analysis: Comparison of means was done by One-way ANOVA followed by Dunnett's multiple comparison. $P<0.05$ was considered to be significant.

Results

Aspirin induced gastric ulcers in rats: NR-ANX-C showed a dose dependent protection against aspirin (500 mg/kg body weight) induced ulcers in rats (Table I). Maximum protection was seen in the omeprazole treated group. Even though NR-ANX-C produced a significant reduction of ulcer index only in the higher dose treated groups (25 and 50 mg/kg body weight), all the tested doses produced a decrease in ulcer index as compared to the control. NR-ANX-C in a dose of 25 and 50 mg/kg body weight was more efficacious than ranitidine in reducing aspirin induced gastric ulcers. NR-ANX-C also showed a dose dependent and significant reduction in lipid peroxidation products in the stomach tissue. The volume of gastric secretion and total acidity was significantly ($P<0.01$) reduced in all drug treated groups as compared to control. NR-ANX-C produced a dose dependent reduction in gastric juice volume and total acidity, but maximum reduction in these parameters was produced by ranitidine and omeprazole respectively. Gastric *p*H was also found to be significantly ($P<0.01$) increased in all drug treated groups as compared to control, with maximum increase being produced by omeprazole. However, NR-ANX-C was superior to ranitidine in reducing the

gastric *p*H and total acidity of gastric juice. Adherent gastric mucus content was also significantly increased in all the drug treated groups as compared to control. NR-ANX-C produced a dose dependent increase in the adherent mucus and was superior to both omeprazole and ranitidine in the higher two doses (25 and 50 mg/kg) tested (Table I).

Pyloric ligature induced gastric ulcers in rats: The extent of gastric ulceration in the control group was more severe in the pyloric ligature model (Table II) as compared to the aspirin induced gastric ulcer model. NR-ANX-C produced a dose dependent and significant (*P*<0.01) reduction in the ulcer index. Here also, maximum protection was seen in the omeprazole treated group. Higher doses of NR-ANX-C (25 and 50 mg/kg body weight) were more efficacious than ranitidine in reducing ulcer index in the treated animals. NR-ANX-C showed a dose dependent and significant (*P*<0.01) reduction in lipid peroxidation products in the stomach tissue compared to control. Even though omeprazole produced maximum protection from ulcers, NR-ANX-C (50 mg/kg body weight) produced maximum reduction in lipid peroxidation products. The volume of gastric secretion and total acidity was significantly (*P*<0.01) reduced in all drug treated groups as compared to control. NR-ANX-C produced a dose dependent reduction of gastric juice volume and total acidity. NR-ANX-C in the highest dose tested (50 mg/kg) was superior to both ranitidine and omeprazole in reducing

Table I. Efficacy of NR-ANX-C in aspirin induced gastric ulcers in rats

Group	Treatment	Ulcer index (% protection)	MDA (nM/g tissue)	Gastric volume (ml/100 g body weight)	Gastric *p*H	Total acidity (clinical units)	Adherent gastric mucus (mg/g tissue)
I	Control	0.61 ± 0.03	116.39 ± 0.49	4.76 ± 0.12	2.23 ± 0.14	93.23 ± 2.89	139.56 ± 3.47
II	Ranitidine (27 mg/kg)	0.44 ± 0.05 (27.86)	82.13 ± 0.29**	2.79 ± 0.04**	3.62 ± 0.09**	58.96 ± 1.62**	157.34 ± 3.82**
III	Omeprazole (1.8 mg/kg)	0.19 ± 0.03** (68.85)	53.65 ± 0.47**	3.19 ± 0.07**	5.12 ± 0.03**	32.73 ± 1.07**	166.87 ± 2.40**
IV	NR-ANX-C (10 mg/kg)	0.43 ± 0.06 (29.50)	82.19 ± 0.52**	3.37 ± 0.11**	3.65 ± 0.07**	68.20 ± 1.56**	156.28 ± 1.98*
V	NR-ANX-C (25 mg/kg)	0.36 ± 0.02** (40.98)	67.08 ± 0.23**	3.12 ± 0.06**	3.98 ± 0.09**	54.13 ± 1.23**	172.46 ± 3.72**
VI	NR-ANX-C (50 mg/kg)	0.34 ± 0.03** (44.26)	56.14 ± 0.51**	2.89 ± 0.05**	4.13 ± 0.15**	47.83 ± 2.12**	178.29 ± 3.69**

Data expressed as mean ± SE (n=6). Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison
P*<0.05, **<0.01 compared to control

Table II. Efficacy of NR-ANX-C in pyloric ligature induced gastric ulcers in rats

Group	Treatment	Ulcer index (% protection)	MDA (nM/g tissue)	Gastric volume (ml/100 g body weight)	Gastric *p*H	Total acidity (clinical units)	Adherent gastric mucus (mg/g tissue)
I	Control	0.83 ± 0.04	147.05 ± 0.39	5.36 ± 0.19	2.02 ± 0.09	119.54 ± 3.76	197.23 ± 4.17
II	Ranitidine (27 mg/kg)	0.45 ± 0.07** (45.78)	101.52 ± 0.22**	3.52 ± 0.08**	4.16 ± 0.13**	71.25 ± 2.34**	223.30 ± 3.96**
III	Omeprazole (1.8 mg/kg)	0.24 ± 0.03** (71.08)	69.45 ± 0.34**	4.09 ± 0.11**	5.21 ± 0.18**	37.82 ± 2.09**	217.67 ± 2.92**
IV	NR-ANX-C (10 mg/kg)	0.52 ± 0.02** (37.34)	99.87 ± 0.24**	3.96 ± 0.14**	3.12 ± 0.15**	84.13 ± 3.05**	203.56 ± 4.30
V	NR-ANX-C (25 mg/kg)	0.35 ± 0.03** (57.83)	72.54 ± 0.36**	3.58 ± 0.09**	3.97 ± 0.13**	65.67 ± 4.06**	215.67 ± 2.58*
VI	NR-ANX-C (50 mg/kg)	0.29 ± 0.06** (65.06)	60.95 ± 0.24**	3.42 ± 0.16**	4.23 ± 0.11**	52.34 ± 2.89**	229.34 ± 3.09**

Data expressed as mean ± SE (n=6). Statistical analysis by One-way ANOVA followed by Dunnett's Multiple Comparison
P*<0.05, **<0.01 compared to control

the gastric volume, and was superior to ranitidine in reducing total acidity. Gastric pH was also found to be significantly ($P<0.01$) increased in all drug treated groups as compared to control with maximum increase being produced by omeprazole. Adherent gastric mucus content was also found to be significantly increased in all the standard drugs treated groups. Even though NR-ANX-C produced a dose dependent increase in the adherent mucus content, it was statistically significant only in the higher two doses (25 and 50 mg/kg) tested. At the highest dose (50 mg/kg), NR-ANX-C was superior to both omeprazole and ranitidine in increasing the adherent gastric mucus content.

Discussion

Various factors that have been implicated in the pathogenesis of gastric ulcers are an increase in gastric acid secretion, pepsin activity and oxidative stress in the gastric mucosa, and a decrease in mucous and bicarbonate secretion[1-4]. The polyherbal formulation NR-ANX-C is composed of extracts of *Withania somnifera, Ocimum sanctum, Camellia sinensis,* triphala and shilajit. Of these *Withania somnifera*[18] and *Ocimum sanctum*[19] have been shown to exhibit anti-ulcer properties and are regular constituents in ayurvedic rasayana for the treatment of the same. Triphala is also known for its properties contributing to overall gastric care[9]. More so, all the individual constituents are well known for their antioxidant properties[7-11, 18] owing to their phytochemical constituents which either induce antioxidant enzymes or directly contribute to free-radical scavenging.

In the present study, NR-ANX-C was found to be more efficacious than the standard drug ranitidine in aspirin and pyloric ligature induced gastric ulcers in experimental animals. On comparing the two models it was seen that NR-ANX-C and standard drugs afforded more protection against development of ulcers in the pyloric ligature model. This can be explained on the basis of the different mechanisms of ulcer development in both the models. Aspirin induced ulcers develop due to the decrease in mucous production and increased proton back diffusion[20]. On the other hand, pyloric ligation induced ulcers develop as a result of accumulation of the gastric acid and distention of the stomach which in turn weakens the mucosal defenses[13]. In both these models, the adherent gastric mucus plays an important role in preventing development of ulcers due to its cytoprotective action. The estimation of this mucus content is more important in the aspirin induced ulcer model as the secretion of this adherent mucus is

dependent on prostacyclin synthesis which is inhibited by aspirin like drugs[21]. As expected, since both ranitidine and omeprazole produce anti-ulcer effect by reducing gastric acid secretion, these were more effective in the pyloric ligation model where gastric acid is the main causative factor. The test drug NR-ANX-C reduced the gastric juice volume and acid content, and increased the adherent gastric mucus content in both models of gastric ulcer, suggesting a possible cytoprotective action and inhibition of gastric acid secretion. Involvement of oxidative stress in the development of ulcers is evident by the increased levels of lipid peroxidation end products in both, aspirin and pyloric ligation induced ulcer models. NR-ANX-C pre-treatment also reduced the extent of oxidative stress, as there was a decrease in the levels of peroxidation end products when compared to control. Even though the degree of ulcer protection by NR-ANX-C was marginally lower than omeprazole, the test drug produced a greater reduction in lipid peroxidation in the pyloric ligature model. This suggests the involvement of antioxidant potential of bioactives in the test drugs in reducing oxidative stress mediated development of ulcers.

In conclusion, our results showed that the anti-ulcer activity of the test compound was perhaps a result of the interplay between its anti-secretory, cytoprotective and the antioxidant properties. These findings suggest the potential for use of NR-ANX-C as an adjuvant in the treatment of gastric ulcer.

References

1. Wallace JL, Granger DN. The cellular and molecular basis of gastric mucosal defense. *FASEB J 1996; 10 :* 731-40.

2. Granger DN, Hernandez LA, Grisham MB. Reactive oxygen metabolites: mediators of cell injury in digestive system. *Dig Dis* 1986; *18* : 13-6.

3. Tandon R, Khanna HD, Dorababu M, Goel RK. Oxidative stress and antioxidants status in peptic ulcer and gastric carcinoma. *Indian J Physiol Pharmacol* 2004; *48* : 115-8.

4. Hung CR. Modulation of gastric hemorrhage and ulceration by oxidative stress and histamine release in *Salmonella typhimurium*-infected rats. *Inflammopharmacology* 2005; *13* : 235-48.

5. McQuaid KR. Drugs used in the treatment of gastrointestinal diseases. In: Katzung BG, editor. *Basic and clinical pharmacology*. Singapore: McGraw Hill; 2007. p. 1009-40.

6. Mishra LC, Singh BB, Dagenais S. Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): a review. *Altern Med Rev* 2000; *5* : 334-46.

7. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. *Indian J Exp Biol* 2002; *40* : 765-73.

8. Tewari S, Gupta V, Bhattacharya S. Comparative study of antioxidant potential of tea with and without additives. *Indian J Physiol Pharmacol* 2000; *44* : 215-9.

9. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation triphala and its constituents. *Int J Pharmacog* 1997; *35* : 313-7.

10. Tripathi YB, Shukla S, Chaurasia S, Chaturvedi S. Antilipid peroxidative property of shilajit. *Phytother Res* 1996; *10* **:** 269-70.

11. Bhattacharya SK, Sen AP. Effects of Shilajit on biogenic free radicals. *Phytother Res* 1995; *9* : 56-9.

12. Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. *Indian J Pharmacol* 1993; *25* : 120-35.

13. Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration in the rat. *Gastroenterology* 1945; *5* : 43-61.

14. Ganguly AK. A method for quantitative assessment of experimentally produced ulcers in the stomach of albino rats. *Cell Mol Life Sci* 1969; *25* : 1224.

15. Parmar NS, Hennings G. The gastric antisecretory activity of 3-methoxy-5,7,3',4' tetrahydroxyflavan (ME) – a specific histidine decarboxylase inhibitor in rats. *Agents Actions* 1984; *15* : 143-5.

16. Corne SJ, Morrissey SM, Woods RJ. Proceedings: A method for the quantitative estimation of gastric barrier mucus. *J Physiol* 1974; *242* : 116P-7P.

17. Stocks J, Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. *Br J Haematol* 1971; *20* : 95-111.

18. Singh N, Nath R, Lata A, Singh SP, Kohli RP, Bhargava KP. *Withania somnifera* (Ashwagandha), a rejuvenating herbal drug which enhances survival during stress (an adaptogen). *Pharm Biol* 1982; *20* : 29-35.

19. Kath RK, Gupta RK. Antioxidant activity of hydroalcoholic leaf extract of *Ocimum sanctum* in animal models of peptic ulcer. *Indian J Physiol Pharmacol* 2006; *50* : 391-6.

20. Goddard PJ, Hills BA, Lichtenberger LM. Does aspirin damage canine gastric mucosa by reducing its surface hydrophobicity? *Am J Physiol* 1987; *252* : G421-30.

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

Reprint requests: Dr Vinod Nair, Department of Pharmacology, All India Institute of Medical Sciences, Ansari Nagar
New Delhi 110 029, India
e-mail: vinodnair1979@hotmail.com