Original Article

Pharmacology

Comparative Study on Neem Leaf Extract and Nimolicin (NC) on Gastric Mucosa of Albino Rats

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ABSTRACT

Objective: Compare the anti ulcer effect of Methanolic Neem (Azadirachta indica, A Juss, Meliaceae) Leaf Extract (NLE) and Neem compound Nimolicin on gastric mucosa of albino rats.

Azadiradione also called Nimolicine coded as NC has been studied for its anti insect effect but anti ulcer effect has never been studied.

Study Design: Experimental study

Place and Duration of Study: This study was carried out at the Pharmacy and Physiology Department of Baqai Medical College for duration of two years.

Materials and Methods: Gastric ulcers in albino rats were induced in group-1 (check group) by a single oral dose of 1 ml 100% ethanol. After 24 hours the treatment was started. Group-2 was treated with oral administration of pea nut oil 1ml/day for 5 days (control of the treated group). Group-3 was treated with NLE (1ml/day for 5 days) and Group-4 with NC 1% (1ml/day for 5 days). The healing effects of neem were compared to oral administration of anti ulcer drugs ranitidine (50mg/kg daily for 5 days) and omeperazole (2.5 mg/kg daily for 10 days). Histopathology of the stomach was performed to confirm the presence or healing of ulcers. Ulcers were scored and indexed on the basis of histopathology.

Results: Ranitidine had the highest ulcer inhibition of 84%. NLE proved to be better than omeperazole by showing an ulcer inhibition of 82 % compared to 73% ulcer inhibition of omeperazole. NC showed least anti ulcer activity with an ulcer inhibition of only 69%. HPLC was performed to show the tissue concentration of NC, omeparazole and ranitidine showing their retention time, area and concentration compared to their controls.

Conclusion: It is concluded that NLE proved to be better anti ulcer agent as compared to NC and can be used as an anti ulcer drug after clinical trials.

Key Words: Gastric ulcer**,** neem (Azadirachta indica, A Juss, Meliaceae) leaf extract, azadiradione, nimolicin, ranitidine, omeperazole, ulcer inhibition.

INTRODUCTION

Neem (Azadirachta indica A. Juss) has been known for its medical values for the last 2000 years. Even today neem has been the centre of attraction especially for workers of medical field. All parts of neem are important particularly the leaves which is "storehouse of organic compounds"[1]. The leaves are easily available through out the year and preparation of extracts is easy. Thus they have been used extensively for medicinal applications[2].

More than 300 different compounds of neem have been isolated from different parts of tree about one third are tetranortriterpinoids (Liminoids).[3] Nimolicine coded as NC is a known neem compound. The neem fruit is divided into two parts. Fleshy outer part pericarp and mesocarp and inner part seed coat and kernel. The active ingredient of seed coat and kernel is Azadirachtin (AZ).[4] NC (Azadiradione) was isolated from fresh fruit coatings (after separation of seeds from fruits). Thus in the present study the anti ulcer effects of neem leaf extract were studied and compared with NC. The potent anti ulcer and anti secretory effect of neem leaf extract has been attributed to a glycoside.[5] Plant glycosides are known to inhibit chloride transport in gastric juice reducing gastric acidity.[6] The mechanism of action of NLE in healing of gastric ulcers is possibly because of its antioxidant effect which is independent of acid suppression.[7] This effect may be similar to the effects of omeperazole providing gastroprotection due to its antioxidant and anti apoptotic role.[5]

This study shows a comparison of anti ulcer effects of methanolic extract of neem leaves with neem compound NC showing the healing effects by histopathology.

MATERIALS AND METHODS

Wistar strain of rats were purchased from HEJ Institute of Chemistry, Karachi University and kept under optimum conditions in the animal house of Baqai Medical University. The animals were acclimatized and had free access to food and tap water ad libitum. Principals of laboratory animal care (NIH publication no.82-83, revised 1985) guidelines were followed. The animal experimentation approval was obtained from University Animal Ethical committee. NLE and NC were obtained from HEJ Institute of Chemistry, Karachi.

A total of 60 albino rats weighing 180Gm to 200Gm were divided into six groups. In each group 5 male and 5 female rats were included. Group-1 was the check group which was given 1 ml of 100% ethanol orally and sacrificed after 24 hrs to check for ulcers. Macroscopic and microscopic examination for the confirmation of ulcers was done. Once it was established that oral administration of ethanol caused gastric ulceration in rats, the next part of the study was undertaken. All the other groups were first given oral ethanol for ulcer production and then treated orally after 24 hours accordingly and then sacrificed. Group no 2 (control of the treated group) was treated with 1 ml of peanut oil daily for 5-7 days. Group-3 (test group) was treated with 1ml NLE daily for 5 days. The rats of fourth group (test group) were treated with 1% NC daily for 5 days. The fifth group (comparison group) of rats was treated with ranitidine (purity 50%) 50mg/Kg daily for 5 days. The rats of the 6th group were treated with Omeperazole (purity 83.33%) 2.5 mg/kg daily for 10 days. After completion of the respective treatment rats of Group-2 to Group-6 were sacrificed and stomach was incised. The gastric ulcer was examined in all the rats by a hand lens (power 10). The gastric tissues were then processed for histopathological examination by staining procedure of[8]. Gastric ulcers were scored on the basis of histopathology and indexed as Ulcers = 3, Heavy infiltration of polymorpho nuclear (PMN) cells = 2, Mild infiltration of (PMN) cells = 1, no ulcers = 0. The ulcer index and ulcer inhibition was determined.[9,10] The experiments were carried out in the Pharmacology Department of the Baqai Institute of Pharmaceutical Sciences and were repeated three times. The total duration of the study was three years.

High Performance Liquid Chromatography (HPLC): HPLC was performed to demonstrate the concentration of drugs in tissues. This was then compared with the standard solution prepared. One Gm of gastric tissue was homogenized in a homogenizer (OSK 9258) at 500RPM and centrifuged at 3500RPM (Labofuge-200 Heraeus). Soxhalation of the supernatant fluid was done. Sorption was done by passing the solution through Alluminium Oxide and Silica[11]. Pure methanol was used as a solvent for the mobile phase with a flow rate of 1 ml/min. A UV detector was used at a wavelength of 214 nm, pressure 200 kg/cm^2 and absorbance 0.08 with chart speed 2.5mm/min. Standard samples were prepared and run for comparison. The samples were filtered by Swiney syringe a micro filter pore diameter of 0.42 nm (Millipore Corporation Bedford MA01730). 20µL of the sample were injected by a special 25µL chromatographic syringe via the injector pore. HPLC apparatus Shimadzu (Japan) SPD-10A UV spectrophotometer with Merck reverse phase column (RP-C_{18}, 25cm X 0.46mm) was used. The chromatographic data were processed with Class LC-10 software (Shimadzu, Japan) and CBM (communication bus model) to the monitor. Peaks were recorded by software programme Real Time Analysis. The peaks were compared on the basis of retention time (RT) with the standard peaks. The area of each peak was noted to quantify the different compound residues in the samples.

RESULTS

In normal healthy rats ulcers were induced by giving 100% ethanol in the check group. This group had the highest ulcer score as shown in table-1 seen on naked eye and later confirmed on microscopic examination (Fig.2). There was no ulcer inhibition because no treatment was given to this group. The remaining 5 groups were treated with pea nut oil, neem, omeperazole or ranitidine after the induction of ulcers. The comparison of anti ulcer effects of neem with anti ulcer drugs on gastric tissue of rats have been shown in table-1 and figures 1-7.

Table No.1: Comparison of ulcer inhibition of NLE, NC, Omeperazole and Ranitidine with the control.

	Ethanol (Check Group) n=17	PNO (Control Group) n=17	NC (Test Group) n=7	NLE (Test Group) n=9	OMP (Known Antiulcer Group) n=6	Ranitidine (Known Antiulcer Group) n=7
Ulcer Score	31	31	4	3	3	2
Ulcer Index	1.82	1.82	0.57	0.33	0.5	0.29
Ulcer Inhibition	Nil	Nil	69%	82%	73%	84%

Table No.2: Shows concentration of NC, omeperazole and ranitidine in gastric tissue compared to the control by HPLC (Schimadzu)

	NC	Ranitidine	Omeprazole
Gastric tissue Conc (µg/20 µl)	3.37	3.62	7.11
Control Conc. (µg/20 µl)	7.08	14.64	9.22

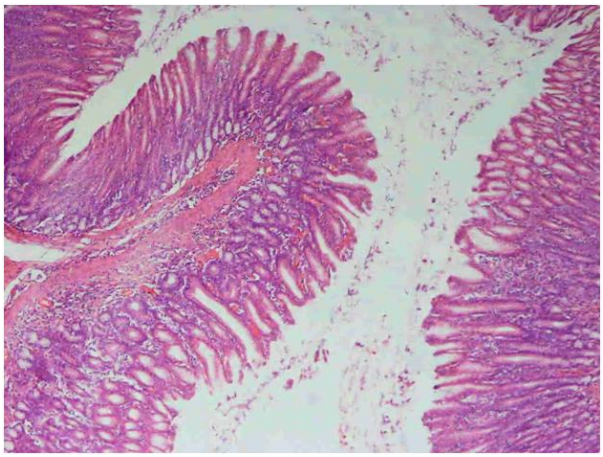

Figure No. 1: H & E stained, 5 micron thick paraffin section of stomach in an adult female rat untreated showing normal gastric mucosa. (Photomicrograph H&Ex10)

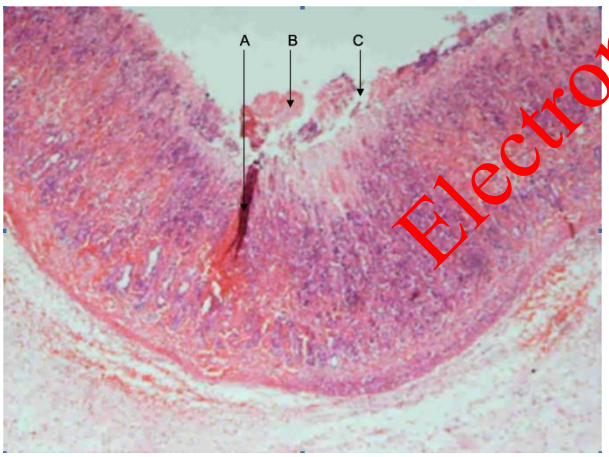

Figure No.**2:** H & E stained, 5 micron thick paraffin section of stomach in an adult female rat given oral Ethanol 100% showing massive erosions (A). The section reveals ulceration of mucous membrane with infiltration of the base with neutrophils (B & C) and dilatation and congestion of blood vessels. (Photomicrograph H&E x 10)

The normal gastric tissue Fig.1 has been compared with ulcerated gastric epithelium in Fig.2. Treated gastric epithelium has been shown in Fig.3-7. Pea nut oil used as control of treatment group has not caused a healing effect and the persistence of ulcers has been demonstrated in Fig.3. The healing effect of neem has been shown in Fig.4 & Fig.7 and this gastric tissue can be compared to the normal gastric mucosa fig.1 and healing effect of ranitidine and omeperazole Fig 6 & Fig 7. Table-1 shows that ranitidine had the highest ulcer inhibition of 84%. The ulcer inhibition of NLE was better than omeperazole by showing an ulcer inhibition of 82% compared to 73% of omeperazole. NC showed least anti ulcer activity with an ulcer inhibition of only 69% (Table-1). The ulcer score of the check group and control is the same. There was no ulcer inhibition with pea nut oil which served as the control of treated group. Tissue concentration of NC, ranitidine and omeperazole on HPLC has been shown in table -2 compared to their controls.

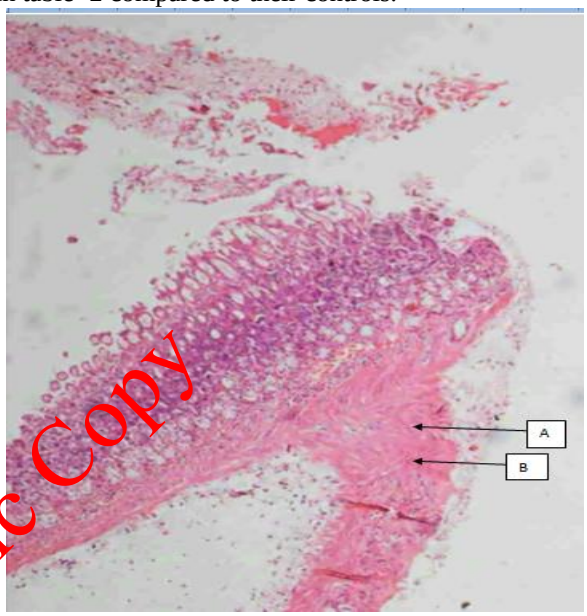

Figure No.3: H & E stained 5 micron thick paraffin section of stomach in an adult female rat treated with Pea nut oil for 5 days. The section shows the presence of chronic inflammatory cells showing a non healing effect of PNO. Ulcers are seen. A & B. (Photomicrograph H&E x 10)

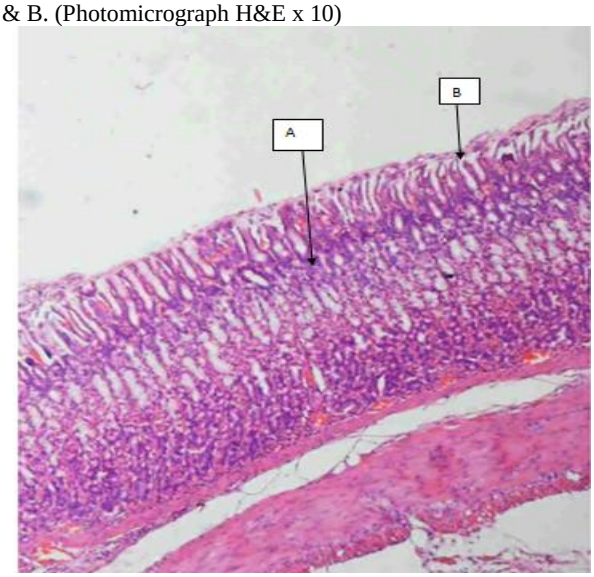

Figure No.5: Photomicrograph of H & E stained, 5 micron thick paraffin section of stomach in an adult female rat treated with neem compound nimolicin (NC). The section reveals

mild infiltration of mucosa by PMN cells, lymphocytes and occasional plasma cells. No ulcers visible. A & B. (H & E X 10).

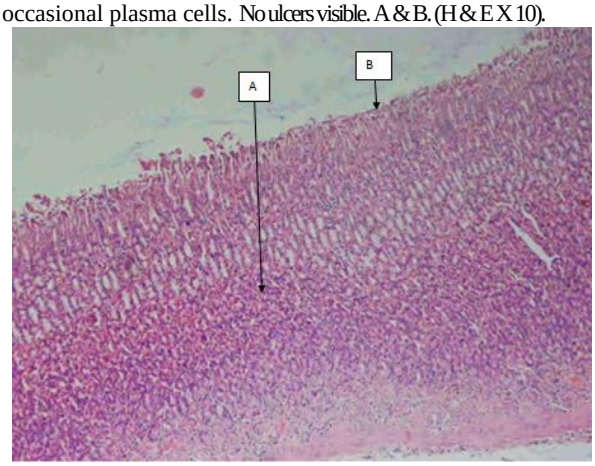

Figure No.**5:** Photomicrograph of H & E stained, 5 micron thick paraffin section of stomach in an adult female rat treated with neem compound nimolicin (NC). The section reveals mild infiltration of mucosa by PMN cells, lymphocytes and occasional plasma cells. No ulcers visible. A & B. (H & E X 10).

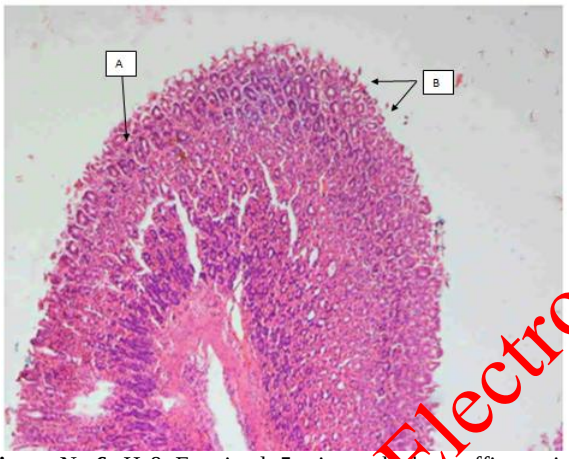

Figure No.**6:** H & E stained, 5 micron thick paraffin section of stomach in an adult female rat treated with ranitidine The section shows mild focal infiltration by chronic inflammatory cells (A). No ulcers seen (B). (Photomicrograph H & E x 10).

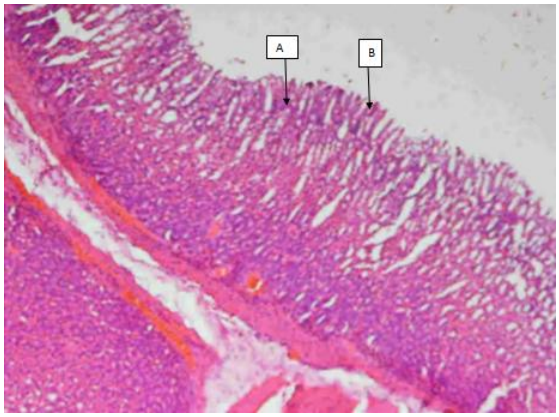

Figure No.**7:** H & E stained, 5 micron thick paraffin section of stomach from an adult female rat treated with omeperazole. The section shows mild focal infiltration by chronic inflammatory cells. No ulcers seen A & B. (Photomicrograph H & E x 10).

DISCUSSION

In the present study ulcer was induced by oral administration of ethanol which is in contrast with the findings of[12] who used mercaptomethylimidazole compound for the production of ulcer which is less potent in ulcer induction than ethanol. The anti ulcer effect of neem has been demonstrated in the present study by the induction of ulcer first, followed by treatment and confirmation by histopathology which is in contrast with the findings of[13] who studied the ulcer protective effect by pretreatment followed by ulcer induction without any histological evidence.

The anti ulcer effects of methanolic extract of NLE in the present study has been reported which has been shown to decrease ulcer index, which is similar with the findings of[14] who used aqueous extract of NLE and bark extract of neem respectively.

Hence the present study demonstrates that NLE is more effective as an anti ulcer agent compared to NC possibly because of its cumulative effect. Resistance is difficult to develop with extracts compared to isolated compounds. Clinical trials may further help in supporting our results.

CONCLUSION

It is concluded that NLE proved to be better anti ulcer agent as compared to NC and can be used as an anti ulcer drug after clinical trials.

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