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Original Research Article

Anti-ulcer activity of *Nardostachys jatamansi* against pylorous ligation induced gastric ulcer

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Abstract: The present study was designed to evaluate the effects of hydroalcoholic extract of *Nardostachys jatamansi* on experimentally induced ulcer by pylorous Ligation method. The extract (500 mg/kg) was administered orally in rats 60 minutes before the induction of ulcer using pyloric ligation method. Volume of gastric secretion, pH of gastric juice, Free acidity, Total acidity, Ulcer index were calculated to examine ulcer preventive ratio of the extract. The extract of *Nardostachys jatamansi* (500 mg/kg) significantly inhibited ulcer formation in Pyloric ligation models. These findings justify the use of this *Nardostachys jatamansi* traditionally in the treatment of ulcer.
Keywords: Gastric ulcer, Diarrhea, *Nardostachys jatamansi*, Ulcer index, Castor oil, Pyloric ligation.

INTRODUCTION

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors[1]. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs[2]. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility[3].

Nardostachys jatamansi (family Valerianaceae), an indigenous medicinal plant induces in organism a state of resistance against stress. It helps to promote physical and mental health augment resistance of the body against disease and has shown potent antioxidant activity. It has also shown marked tranquillizing activity, as well as hypotensive, hypolipidemic, antiischemic, antiarrhythmic, hepatoprotective, anticonvulsant, neuroprotective, antioxidant activities[4-7].

Various sesquiterpenes (such as Jatamansic acid and Jatamansone), lignans, alkaloids, coumarins and neolignans have been reported to be present in the roots of the plant[8,9]. In addition, volatile oils like jatamansic acid and other chemical substances have been isolated from various fractions of roots and rhizomes of the herb[10]. These components provide protection against reactive oxygen species (ROS) induced damage in cells. With this background our aim of the study is to find the protective effect of *Nardostachys jatamansi* root extract against electron beam radiation (EBR) induced cellular damage.

MATERIALS AND METHODS

Plant Collection and Authentification

The Plant *Nardostachys jatamansi* rhizomes have been collected and the plant material was identified and authenticated in Department of Botany, Osmania University, and Hyderabad.

Extract Preparation

The rhizomes were shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size and then used for extraction. A weighed quantity (500 g) of the powder was then subjected to continuous hot extraction in

Soxhlet apparatus with hydroalcoholic solvent (ethanol 50% + water 50%) and the residual marc was collected. The extract was filtered through a cotton plug, followed by whatman filter paper (no.1). The extract was evaporated under reused pressure using a rotovac evaporator at a low temperature (40-60^{0}C) until all the solvent had been removed to give an extract sample with a yield of 18% w/w, 16 %w/w and 13% w/w in relation to the dried starting material. Preliminary Phytochemical

analysis was carried out to identify presence of Phytoconstituents in the crude extract[11].

Phytochemical Screening of Hydroalcoholic Extract of *Nardostachys jatamansi* rhizomes

It is planned to carry out the preliminary phytochemical investigation for rhizomes extracts of *Nardostachys jatamansi* Linn. for detection of various phytoconstituents and the tests is done to find out the presence of common phytochemicals by following standard method described in practical pharmacognosy by C.K.Kokate and R.K.Khandelwal.

Experimental Protocol

Albino Wistar rats (150-200g) of both sexes were obtained from the animal house. Before and during the experiment rats were fed with standered diet(gold mohar, lipton Ind Ltd.) after randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standered environmental conditions of temperature, RH, dark/light cycle. Animals described as fasting were deprived of food and water for 16hrs ad libitum. All animals experiment were carried out in accordance with the guidelines of CPCSCA and study was approved by the IAEC (Institutional Animal Ethical Commity)

Animal

Albino wistar rats of either sex weighing between 150 to 200 gm are used for studying antiulcer activity. The animals were housed under standard conditions of temperature (25±2°C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet (VRK Nutrition, Pune) and water ad libitum.

Anti-Ulcer Activity

1) Pylorus ligation method induced gastric ulcer [12]:

Procedure:

Animals were divided into 4 groups, each comprised 6 rats.

Group I - Control

Group II - Pyloric ligation

Group III - Pyloric ligation + Hydroalcoholic Extract of *Nardostachys jatamansi* rhizomes 500 mg/kg body weight

Group IV -Pyloric ligation + Standard drug (Famotidine 10 mg/kg)

Hydroalcoholic leaf Extract of *Nardostachys jatamansi* rhizomes (500 mg/kg) was administered for a period of 7 days. Group I were fed with saline solutions for 7 days, Group III were given extract 500mg for 7 days, Group 4 were fed with standard famotidine for 7 days. GroupII, III & IV were induced ulcers by Pyloric Ligation. On the 7th day normal saline, Famotidine and Hydroalcoholic extract of rhizomes were administered 1hr prior to pyloric ligation. Animals were anaesthetized using diethyl ether and the abdomen was opened and pylorus was ligated without causing any damage to its blood vessels. The stomach was replaced carefully and the abdomen wall was closed with interrupted sutures. After 4hrs of ligation, the animals were sacrificed by cervical dislocation. The abdomen was opened and a ligature was placed around the cardiac sphincter. The stomach was removed. Gastric juice is collected and drains into test tubes and then centrifuged at 1000 rmp for 10 minutes and the volume noted. The pH of the gastric juice is recored by pH meter. Then the contents are subject for the analysis of free and total acidity. The stomachs are then washed with running water to see for ulcers in the glandular portion of the stomach. The number of ulcers per stomach is noted and severity of the ulcer scores microscopically with the help of hand lens (10x).

In this model the following parameters are plan to study.

- pH of gastric juice.
- Volume of gastric secretion
- Free acidity
- Total acidity
- Ulcer index

Estimation of Gastric Volume and Free and Total Acidity in Pylorus Ligation Model :

Animals were sacrificed 4 h later and the stomach was opened to collect the gastric contents. The total volume of gastric content was measured. Thegastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatent liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued using 1% solution of phenolothalein till the solution gained pink colour. The volume of NaOH required was noted and was taken as corresponding to the total acidity. Acidity was expressed as:

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality x 100 mEq/1}}{0.1}$$

Estimation of Gastric Ulcerative Index Changes in Pylorus Ligation Model :

Ulcrative index is measured by method of Takagi et al, 1969, briefly, the stomach was opened along the greature curvature. The stomach was washed with running tap water. Then it was placed on a flat wooden plate to count the ulcerative area.

The ulcer index was determined using the formula:

Ulcer index: 10/ X

Where X = total mucosal area

total ulcerated area

Percentage ulcer protection was calculated using the formula:

Ulcer protection (%) = 100 - Ut/ 100 – Uc X 100

Where:

Ut = Ulcer index of treated group

Uc = Ulcer index of control group

Statistical Analysis

All the biochemical results were expressed as mean + standard error of means(SEM). Data were analysed by turkey's range tests using sigma stat version- 3.5 software. A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Table 1. Phytochemical Screening of *Nardostachys jatamansi* rhizomes Linn.

S.NO	Constituents	Presence/absence
1	Alkaloids	+
2	Saponins	-
3	Flavanoids	+
4	Glycosides	+
5	Phenols	+
6	Tanins	+
7	Steroids	+
8	Proteins	+
9	Starch	+

+ = Presence

= Absence

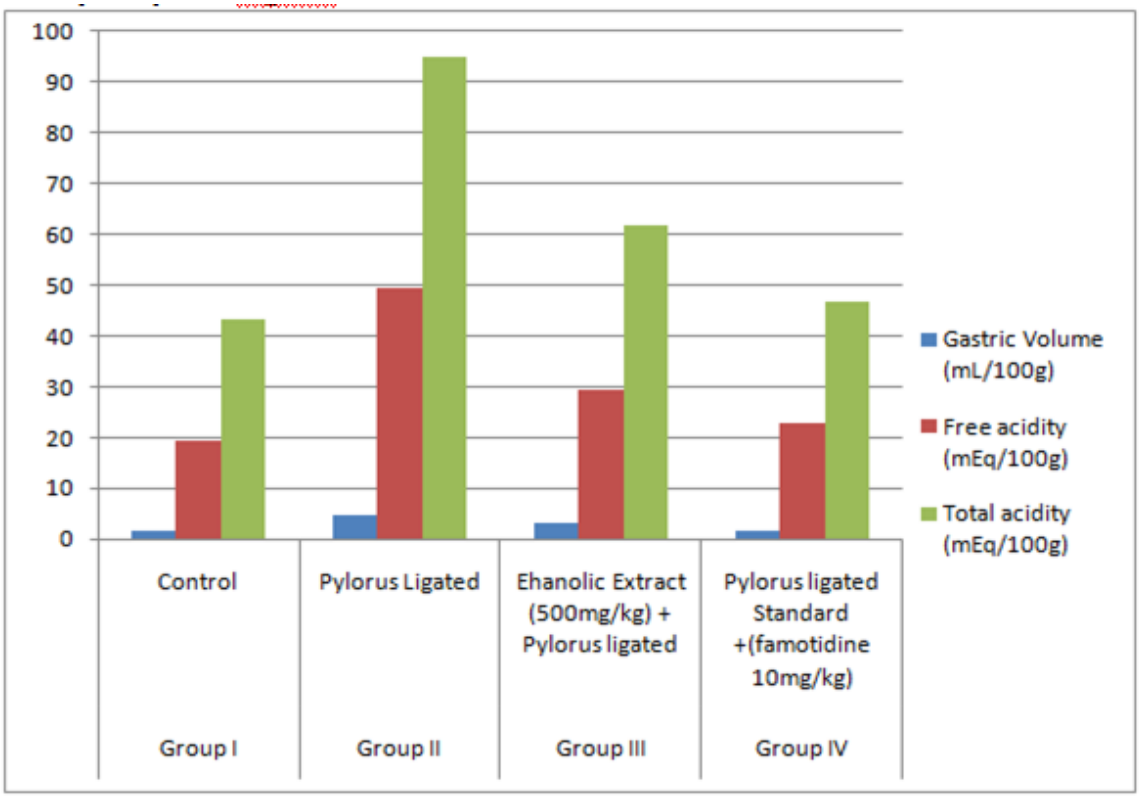
Results of Antiulcers activity

Table 1 : Shows the effect of Hydroalcoholic Extract on Gastric Secretion, Free Acidity, Total Acidity In Pyloric Ligated Rats. The volume of Gastric juice secretion was significantly reduced with a dose 500mg/kg of hydroalcoholic extract of *Nardostachys jatamansi* when compared to Pylorus ligated rats.

The Effect of Control, Pylorus ligated rats, Hydroalcoholic extract (500mg/kg) and Standard drug Famotidine (10mg/kg) on volume of Gastric juice secretions were 1.3 , 4.64, 3.01 and 1.42 ml respectively. Gatric volume ,Free and Total acidity of Hydroalcoholic Extract of *Nardostachys jatamansi* at a dose of 500mg/kg was significantly reduced when compared to the pylorus Ligated rats(Table 2 & Graph 1).

Table 2: Effect of Hydroalcoholic Extract on Gastric Secretion, Free Acidity, Total Acidity in Pyloric Ligated Rats

Groups	Treatment	Gastric Volume (mL/100g)	Free acidity (mEq/100g)	Total acidity (mEq/100g)
Group I	Control	1.4+0.21 ml	19.2 ± 0.28	43.2 ± 2.36
Group II	Pylorus Ligated	4.74+0.54 ml	49.5 ± 2.79	95.1 ± 2.12
Group III	Ehanolic Extract (500mg/kg) + Pylorus ligated	2.91+0.4 ml	29.3 ± 1.75	61.8 ± 1.82
Group IV	Pylorus ligated +Standard (famotidine 10mg/kg)	1.50+0.6 ml	22.6 ± 0.36	46.6 ± 1.28



Graph 1: **Effect of Hydroalcoholic Extract on Gastric Secretion, Free Acidity, Total Acidity in Pyloric Ligated Rats.**

Table 3: Shows the effect of Hydroalcoholic extract on Ulcer Index and Percentage Protection. The Ulcer Index of Hydroalcoholic Extract 500mg/kg were significantly reduced when compared to Pylorus Ligated rats.

Ulcer Protective action at a dose of 500mg/kg of Hydroalcoholic Extract of *Nardostachys jatamansi* was found to be closer to the reference drug Famotidine (10mg/kg) respectively.

Table 3: Effect of Hydroalcoholic extract on Ulcer Index and Percentage Protection

Treatment Groups	Ulcer index	% Protection
Group I	0.00+0.00	0.00
Group II	6.38+0.12	0.00
Group III	3.62+0.26	81.2
Group IV	2.16+0.18	94.6

Histopathology of rat stomach:

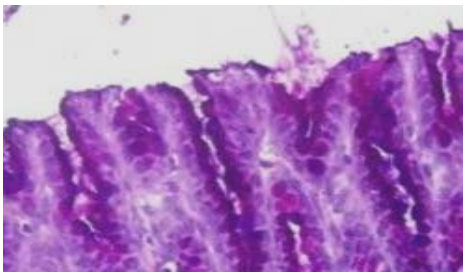


Fig 1 : **Group I – Control**

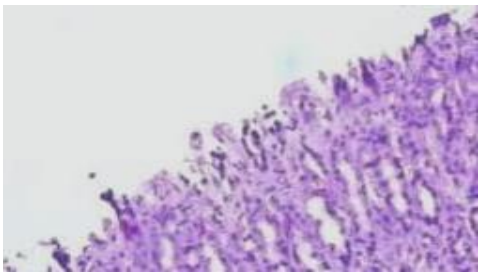


Fig 2 Group II

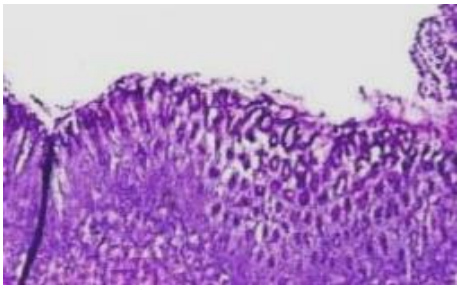


Fig 3 : **Group III**

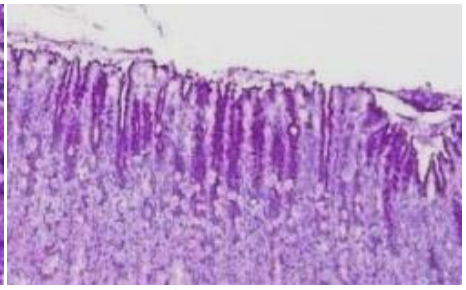


Fig 3 : **Group IV**

Group I: Section of gastric mucosal layers shows normal appearance.
Group II: Pylorus ligation groups shows mucosal ulceration and inflammation.
Group III: Extract treated group show no ulceration.
Group IV: Shows no significance change in histopathology almost normal appearance.

DISCUSSION

The *Nardostachys jatamansi* is used for various gastrointestinal diseases in the folk medicine. Plant extracts are some of the most attractive sources of new drugs, and have shown promising results for the treatment of gastric ulcers in several experimental models[13]. Since, this plant has been reported to contain Flavonoids, Tannins, steroids, carbohydrates, Alkaloids where Flavanoids and tannins have shown potent and anti ulcer activity[14,15].

Peptic ulcer results due to overproduction of gastric acid (or) decrease in gastric mucosal production. Pylorus ligation induced ulcers occur because of an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent mucosal digestion[16]. In folk medicine, *Nardostachys jatamansi* Linn is used for the various gastrointestinal diseases. The present study reveals that hydroalcoholic extract of *Nardostachys jatamansi* rhizomes extract treated groups showed a significant reduces the gastric volume, free acidity and total acidity when compared to pyloric ligated group. *Nardostachys jatamansi* rhizomes extract decreased the ulcer index more effectively in a dose dependent manner.

From the results it has been revealed that pretreatment with 500mg/kg dose of Hydroalcoholic extract of *Nardostachys jatamansi* rhizomes had significantly produced Anti ulcer properties.

CONCLUSION

On the basis of the present results and available reports, it can be concluded that the hydroalcoholic extract of *Nardostachys jatamansi* rhizomes possess Anti ulcer activity could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

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