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Preclinical screening of Antiulcer activity of *Asparagus racemosus* extract on phenylbutazone induced ulceration in experimental animals

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Abstract
The present study aimed to evaluate the effect of 50% ethanolic extract of *Asparagus racemosus* whole plant extract (ARE) on Phenylbutazone induced chronic gastric damage in experimental rats. Animals were induced for gastric ulcer with Phenylbutazone (100mg/kg bodyweight) and treated orally with ARE (250 and 500 mg/kg bodyweight). The effective dose i.e. 500 mg/kg elicited a maximum reduction in lesion index. The gastroprotective effect of ARE was assessed from the levels of different physicochemical parameters. The levels of DNA, protein bound carbohydrate complexes-hexose, hexoseamine, sialic acid, fucose in gastric juice were assessed. A significant reduction (p<0.001) in lesion index was observed in ulcer induced animals treated with plant extract compared to control group. A significant increase was observed in pH, protein bound carbohydrate complexes, nucleic acids with a significant decrease in volume of gastric juice, free and total acidity, pepsin concentration and acid output. From the data presented in this study it could be concluded that 50% ethanolic extract of *Asparagus racemosus* whole plant extract (ARE) acts as a gastroprotective agent.

Keywords: Antiulcer, *Asparagus racemosus*, phenylbutazone, plant extract, ranitidine

1. Introduction
Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Peptic ulcer represents a major health problem, both in terms of morbidity and mortality [1]. It occurs due to imbalance between offensive (acid-pepsin secretion. H. pylori, bile, increased free radical and decreased antioxidants) versus impaired mucosal resistance (mucus, bicarbonates secretion, prostaglandins, blood flow and the process of restitution and regeneration after cellular injury). Various reports have shown that commonly used drugs for peptic ulcers such as H2 blockers (ranitidine, famotidine etc.), M1-blockers (pirenzepine, telenzepine etc.) and proton pump inhibitors (omeprazole, pantoprozole etc.) have danger of drug interaction, adverse effect and increased incidence of relapses during ulcer therapy [2]. Since herbs are the mines of useful drugs and Indian people have a tremendous passion for medicinal plants and use them for a wide range of health related applications from common cold to memory improvements and enhancement of general immunity [3].

Asparagus racemosus Linn (Family: *Asparagaceae*) an under-shrub, climbs up to 1-3 m high, with stout and creeping root stock found in plains to 4,000 ft high, in tropical, sub tropical dry and deciduous forests and in Himalayas. It is common in the plains from the coast to scrub jungle and cultivated in most of the states of India. Ayurvedic literature indicates the use of the tuberous root in gout, puerperal infections, lactic disorders, haematuria, bleeding disorders and also recommends for treatment of hyperacidity. Roots have oleaginous, cooling, antispasmodic, indigestible, appetizer, alliterative, stomach, tonic, aphrodisiac, astringent, antidiarrhoeatic, antidysenteric, laxative properties and is useful in tumors, inflamations, diseases of blood and eye, throat complaints, tuberculosis, leprosy, epilepsy, night blindness and kidney troubles [4]. Scientifically, the plant has been investigated for its antidepressant activity [5] Immuno-stimulant activity [6] cytotoxic activity [7] antiulcer [8] antiamnesic [9] and antidiarrhoedal activity [10]. The methanolic extract of the roots was found to possess antitussive activity [11]. Chemically the plant contains Sarsasapogenin, Shataverin,

Adscendin A & B, Asparnin, A, B, & C, Asparagamine A. Five steroidal saponins, shatavarins VI–X, together with five known saponins, shatavarin I (or asparoside B), shatavarin IV (or asparinin B), shatavarin V, immunoside and schidigerasaponin D5 (or asparanin A), have been isolated from the roots of *Asparagus racemosus* [12]. three steroidal saponins, racemosides A (**1**), B (**2**) and C (**3**), from the methanolic extract of the fruits of *Asparagus racemosus* [13]. A 9, 10-dihydrophenanthrene derivative, named racemosol isolated from the roots of *Asparagus racemosus* [14]. In the limelight of its traditional and scientific use, the present investigation was carried out to evaluate the anti-ulcer activity of the 50% ethanolic extract of *Asparagus racemosus* Linn. against phenylbutazone chronic ulcer model in wistar rats.

2. Materials and Methods

2.1 Collection and authentication of plant material

The whole herb of *Asparagus racemosus* were collected in month of July 2012 from Company garden, Allahabad and identified by Dr. P. Arjun Tiwari, Scientist, Botanical Survey of India Allahabad. A voucher specimen GC 0950222 is preserved in BSI herbarium for future reference. The herb was dried under shade and powdered with a mechanical grinder. The powder was then passed through sieve no. 40 and stored in an airtight container.

2.2 Preparation of the extract

The powder (1 kg) of *Asparagus racemosus* was extracted with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with 50% ethanol by hot percolation method. The extract was concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extracts were transferred to beaker and the remaining solvent was evaporated on a water bath. Then they were cooled and placed in desiccators to remove the excessive moisture and thus 125.0 g of solid residue (yield 12.5 % w/w) was obtained. The dried extracts were packed in airtight containers and used for further studies such as phytochemical screening and pharmacological activities.

2.3 Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the identification of various active constituents by using standard procedures. [15]

2.4 Drugs and chemicals

Phenylbutazone was purchased from Wanbury limited, Mumbai. The standard drug Ranitidine was procured from Viva laboratories Pvt. Ltd. Ahmedabad as a gift sample. All other chemicals and reagents used were of analytical grade.

2.5 Experimental Animals

Wistar albino rats (180-250 g) of either sex and of approximate same age, used in the present study, were procured from animal breeding centre, Indian Institute of Toxicology Research, Lucknow. All the animals were housed in polypropylene cages under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for one week before starting the experiment. The animals were fed with standard pellet diet (Amrut, India) and water *ad libitum*. The experimental protocols were approved by Institutional Animal Ethics Committee of United Institute of Pharmacy, Allahabad (UIP/CPCSEA/Nov-2012/03) after scrutinization. The animals received the drug treatment by oral gavages tube.

2.6 Oral acute toxicity study

The lethal median dose (LD_{50}) determination was done in rats by OECD guidelines 423 [16]. A single dose of the extracts (5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg) in suitable quantity of water was given orally by gavage to different group of rats (three each). The animals were allowed free access to water and food. However, all the animals were deprived of food for 2 hr before and 4 hr after dosing. The animals were continuously monitored during first 4 hrs and every one-hour during the first 12 hrs for any undesirable effects. Later they were monitored (daily twice) for any abnormal changes throughout the study period of 14 days.

2.7 Evaluation of anti-ulcer activity

2.7.1 Phenylbutazone induced ulceration

Gastric ulceration, according to the method described by Liu *et al*, 2001 with slight modification [17] was induced in 36 hour fasted rats by the administration of ulcerogenic drug phenylbutazone 100 mg/kg, p.o. Groups of six animals each were pre-treated with 50% ethanolic extract of *Asparagus racemosus* 30 minutes before the ulcerogenic procedure in the following manner.

Group I - Control rats received vehicle solution
Group II - Rats received standard drug ranitidine 50 mg/kg
Group III - Rats treated with extract 250 mg/kg body weight
Group IV - Rats treated with extract 500 mg/kg body weight

Phenylbutazone is suspended in 1% carboxy-methylcellulose in water (20 mg/ml) and administered orally (gavage) in a dose of 100 mg/kg in 36 hours fasted rats. Two doses are given at an interval of 15 hours and 6 hours. Gastro-protective effects have been studied by administering the plant extract 30 min before each dose of phenylbutazone. After the dose administration, animals are sacrificed and assessed for the gastric mucosal damage. Gastric juice was collected by holding the stomach over a funnel placed on a graduated centrifuge tube and puncturing it with a scissors. Volume of the juice was noted and then centrifuged at 2500 rpm for 15 minutes and the supernatant was used for various biochemical estimations. After juice collection the stomach was opened along the greater curvature, washed under running water and the inner surface was carefully observed with a magnification lens. Number of ulcer in both glandular and fundic region and severity of ulceration was noted. The ulcer index was calculated according to the method [18] the lesion were counted with the aid of hand lens (10x) and scoring of ulcer will be made as follows:

Normal colored stomach	0
Red coloration	0.5
Spot ulcer	1
Hemorrhagic streak	1.5
Deep Ulcers	2
Perforation	3

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

Ulcer index (UI) was measured by using following formula:

$$UI = UN + US + UP \times 10^{-1}$$

Where, UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Test}})}{\text{Ulcer index}_{\text{Control}}} \times 100$$

2.7.2 Estimation of Biochemical parameters

Measurement of various physicochemical and biochemical parameters viz. gastric volume, pH determination, estimation of total acid, pepsin, DNA content, total proteins and estimation of protein bound carbohydrate complexes were determined for ascertain its gastroprotective activity by standard procedure [19-22].

2.8 Statistical analysis

The statistical analysis of the pharmacological analysis was carried out using GraphPad Prism version 5.04 for windows. The values are represented as mean ± S. D. for six rats data were analyzed by Student *t* test and ANOVA with post-hoc difference was analyzed using Newman-keuls method

3. Results & Discussion

3.1 Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of carbohydrate, fixed oils, glycosides, Phytosterols, saponins and flavonoids in 50% ethanolic extract of whole plant. These secondary metabolites are scientifically isolated and investigated for the antiulcer activity in previous literatures.

3.2 Acute toxicity study

The LD_{50} determination was done by OECD guidelines 423. The extract was devoid of any toxicity in animals when given in dose up to 2000mg/kg. Hence for further study 250 & 500 mg/kg doses of extract were selected.

3.3 Antiulcer activity

Table 1 shows the dose dependent changes in lesion index of experimental group of rats. Rats administered with Phenylbutazone showed marked increase in lesion index. A significant reduction (p<0.001) in the incidence of lesions was observed in rats treated with ARE (500 mg/kg) compared to Phenylbutazone induced rats. Levels of acid secretary parameters in gastric juice of control and experimental group of rats were shown in table 1. The volume of gastric juice, free and total acidity, pepsin concentration and acid output of gastric juice were increased significantly (p<0.001) in Phenylbutazone toxicity induced rats with a significant decrease (p<0.001) in pH compared to control rats. Phenylbutazone toxicity induced + ARE (500 mg/kg b.w.) treated rats showed a significant decrease (p<0.001) in volume of gastric juice, free and total acidity, pepsin concentration and acid output with a significant increase (p<0.001) in pH compared to Phenylbutazone toxicity induced rats.

The levels of protein bound carbohydrate complexes and DNA in the gastric juice of control and experimental group of rats. Phenylbutazone caused a significant decrease (p<0.001) in TC:P with a concomitant increase (p<0.001) in protein and DNA. In Phenylbutazone + ARE rats, a significant increase (p<0.001) in the carbohydrate concentration in terms of TC:P ratio and significantly lowered (p<0.001) protein concentration in gastric juice was observed. ARE tended to increase mucin activity in Phenylbutazone + ARE group. The concentration of DNA in gastric juice was significantly decreased (p<0.001) in Phenylbutazone + ARE.

Table 1: Effect of 50% ethanolic extract of entire herb of *A. racemosus* on pH and ulcer index

S/No.	Treatment/ Dose (mg/kg)	Ulcer Index (mm ^{2}/rat)	Percent Protection	pH
1.	Control	22.12±0.66	-	2.11±.481
2.	Ranitidine(50)	4.25±0.71	76.49	5.43±0.067^c
3	ARE (250)	17.5+.66	17.06^a	2.71±.127^b
4	ARE (500)	12.08+.73	42.74^b	3.54±.077^c

The value represents the mean ± S.D. for 6 rats per group. $^a p$<0.05, $^b p$<0.01; $^c p$<0.001 compared to standard group.

Table 2: Effect of 50% ethanolic extract of *A. racemosus* on gastric secretions

Treatment/ Dose (mg/kg)	Volume (ml/100 gm)	Acid		Pepsin		DNA (µg/ml)
		Conc. (µEq/ml)	Output (µEq/4h)	Conc. (µmol/ml)	Output (µmol/4 h)	
Control	1.80±.1.08	98.35±0.899	142.78±2.49	496.245±9.766	779.63±5.693	118.55±3.955
Ranitidine(50)	1.57±0.122	74.411±1.77^c	116.405±1.405	295.646±7.250	427.366±5.1808	74.468±1.455
ARE(250)	1.72±.020^b	94.59±.76	138.76±1.51	474.958±6.842^b	742.1733±6.942	104.05±2.61^b
ARE (500)	1.68±0.038^b	85.43±1.33^c	130.59±1.40	445.781±7.107^c	635.626±4.410	93.556±2.86^c

The value represents the mean ± S.D. for 6 rats per group. $^a p$<0.05, $^b p$<0.01; $^c p$<0.001 compared to standard group.

Table 3: Effect of 50% ethanolic extract of *A. racemosus* on different ratio of carbohydrates and proteins

Treatment/ Dose (mg/kg)	Protein (µg/ml)	Total hexose (µg/ml)	Hexosamine (µg/ml)	Fucose (µg/ml)	Sialic acid (µg/ml)	TC (µg/ml)	TC:P
Control	4382.814±8.955	2475.672±258.53	1164.48±18.329	231.96±2.058	58.028±1.044	3930.14±279.961	0.8967±31.263
Ranitidine (50)	4013.844±7.958	2930.377±84.86	1328.963±6.819	267.211±6.319	99.11±0.975	4625.661±98.973	1.152±12.436
ARE (250)	4314.412±8.347^b	2424.195±35.365	1178.05±6.042^b	237.451±1.684^b	64.555±1.305	3904.251±44.396	0.904±5.318
ARE (500)	4212.1±6.076^c	2701.205±130.075^c	1241.89±7.952^c	246.455±1.990^c	74.736±2.004	4264.286±142.021^c	1.012±23.374^c

The value represents the mean ± S.D. for 6 rats per group. $^a p$<0.05, $^b p$<0.01; $^c p$<0.001 compared to standard group.

In the present study, the gastro protective effect of asparagus extract was assessed in rat model. Efficacy was measured using a index of ulceration and various gastric secretary parameters like pH, pepsin, total acid contents etc. Minimum dose producing statistically significant benefits was found to be 250 mg/kg for ulcers induced by 100 mg/kg Phenylbutazone. (p<0.01 compared to control). There is increase in pH and decline in acidity, acid output and pepsin concentration were confirmed in ulcerated animals treated with plant extract in dose dependent manner. In the present study, increase in pH and decrease in acidity, acid output and pepsin concentration were evidenced in ulcerated animals treated with ARE, which is highly desirable for gastroprotection and antiulcer effect. Ulcerated rats showed an alteration in the peptic activity which is in accordance with previous report [23-24]. The modification in pepsin

concentration on ARE treatment depicts the efficacy of ARE on gastric secretions. The gastroprotective effect of plant extract may be due to the direct action on the acid producing cells. 50% ethanolic extract of plant exhibited ulcer healing activity by increasing hexosamine and carbohydrate protein ratio and adherent mucus content against phenylbutazone induced ulcer. This results in the increase in mucus secretion. The importance of mucus secretion as a response to gastric mucosal trauma has long been recognized [25]. Mucosal barriers are the most significant factors for gastric protection [26]. More production of mucous is responsible for the less degree of ulceration. Mucus also defends the mucosa and sub-mucosa from inflammatory reaction. The higher the mucin contents the lower is the free acidity. Mucosal defense agents are a new dimension in the treatment of gastro-duodenal diseases [27]. In the present study we can conclude that the biochemical parameters reduced after the treatment of extract might be a preventive measure of ulceration.

4. Conclusion

From the above study, it was confirmed that the 50% ethanolic extract of whole plant of *Asparagus racemosus* plant is the most effective in prevention of ulceration in experimental animal model. The beneficial multiple properties present in medicinal plant offer exciting opportunity to develop them into novel therapeutics for ulcer.

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