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Effect of *Alectra Parasitica* Var. *Chirakutensis* on Aspirin Induced Ulceration in Rats

Ch V Rao*[1], G Rajesh Kumar[1], Manju Sharma[1], Shyam Sundar Gupta[1] and RLS Sikarwar[2]

[1]Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, (Council of Scientific and Industrial Research) Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, Uttar Pradesh, India.
[2]Arogyadham, Deendayal Research Institute (DRI), Chitrakooot 485 331, Madhya Pradesh, India.

Article

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***For Correspondence**

Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, (Council of Scientific and Industrial Research) Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, Uttar Pradesh, India.
E-mail: chvrao72@yahoo.com,
Phone No: +91 522 2297976
Fax No: +91 522 2205836

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ABSTRACT

The effect of 50 % ethanolic extract of *Alectra parasitica* var. *Chirakutensis* was assessed in different acute gastric ulcer models in rats. *Alectra parasitica* administered orally at dose levels of 50 – 200 mg/kg, twice daily for 3 days showed dose dependent ulcer protective effect 48.89 – 82.22% protection on aspirin –induced acute ulcers. Besides, *Alectra parasitica* reduced the ulcer index with significant ($P < 0.01$ and <0.001) protection of lipid peroxidation and superoxide dismutase and increased in catalase activity, respectively. Preliminary phytochemical screening of the *Alectra parasitica* gave the positive test for steroids, alkaloids, terpenoids, saponins and tannins. The results indicate that *Alectra parasitica* possesses antiulcer activity.

INTRODUCTION

Alectra parasitica A. Rich Var. *Chitrakutensis* M.A. Rau is a parasitic plant, belonging to the family of Scrophulariaceae and it grows on the roots of *Vitex negundo* Linn. in the vicinity of Chitrakut along the borders of Satna District of Madhya Pradesh and Banda district of Uttar Pradesh. It is 4-8 ft in height and bears small yellow flowers [1,2,3]. The plant has been used in the Ayurveda in treatment of various ailments[4]. 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. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood [5]. Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage is caused by physical, chemical and psychological factors that leads to gastric ulceration in human and experimental animals [6]. To the best of our knowledge there were no scientific reports available in support of its traditional claims. Therefore, present study was designed to demonstrate the effect of *Alectra parasitica* extract on aspirin –induced gastric ulceration in rats.

MATERIALS AND METHODS

Materials

Alectra parasitica, collected by from Arogyadham, Deendayal Research Institute (DRI), Chitrakooot Madhya Pradesh. The plant sample was authenticated using voucher specimens (R L S Sikarwar DRI 2341) deposited in herbarium of Arogyadham (DRI). Fig 1 shows the live species of *Alectra parasitica* var. *Chirakutensis* growing in garden.

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Fig 1: *Alectra parasitica* var. *Chirakutensis* growing in garden

Preparation of Extract

Air-dried powdered aerial parts of *Alectra parasitica* (1000g) were powdered and exhaustively extracted by overnight maceration with 10 volumes of 50% ethanol and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure to obtain 78 g of solid residuce (yield 7.8% w/w). Preliminary qualitative phytochemical screening of *Alectra parasitica* extract gave the positive test for steroids, alkaloids, terpenoids, saponins, and tannins.

Test Animals

Sprague-Dawley rats (140-180g) were kept in the departmental animal house at 26 ± 2 °C and relative humidity 44 - 56%, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was with drawn 18-24 h before the experiment though water was allowed *ad libitum*.

Experimental Procedure

Alectra parasitica in doses of 50, 100 and 200 mg/kg and H_2 receptor blocker ranitidine in the dose of 50 mg/kg were administered orally twice daily at 1000 and 1600 hrs respectively for three days for acute ulcer protective studies. Control group of animals received suspension of 1 % carboxymethyl cellulose in distilled water (10 ml/kg).

Aspirin (ASP)- Induced Ulcers

ASP in dose of 200 mg/kg (20 mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after 4 h. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5.0 ml of 0.9 % NaCl and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach. Ulcer index has been calculated by adding the total number of ulcers per stomach and the total severity of ulcers per stomach. The total severity of the ulcers was determined by recording the severity of each ulcer after histological confirmation as follows: 0, no ulcer; +, pin point ulcer and histological changes limited to superficial layers of mucosa and no congestion; ++, ulcer size less than 1 mm and half of the mucosal thickness showed necrotic changes; +++, ulcer size 1-2 mm with more than two-thirds of the mucosal thickness destroyed wih marked necrosis and congestion, muscularis remaining unaffected; ++++, ulcer either more than 2 mm in size or perforated with complete destruction of the mucosa with necrosis and haemorrhage, muscularis still remaining unaffected and pooled group ulcer score was then recoreded [7,8].

Determination of Gastric Wall Mucus

The glandular segments from stomachs were removed, weighed and incubated in tubes containing 1 % Alcian blue solution (0.16M sucrose in 0.05 M sodium acetate, pH 5.8) for 2 h. The alcian blue binding extract was centrifuged at 3000 rpm for 10 min and the absorbency of supernatant was measured at 498 nm. The quantity of alcian blue extracted (g/g of glandular tissue) was calculated [9].

Estimation of Lipid Peroxidation (LPO)

The fundic part of the stomach was homogenized (5%) in ice-cold 0.9% NaCl with a Potter-Elvehjem glass homogenizer for 30 seconds. The homogenate was centrifuged at 800 x g for 10 min and the supernatant was again centrifuged at 12,000 x g for 15

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min and the obtained mitochondrial fraction was used for the following estimations [10]. A volume of the homogenate (0.20 ml) was transferred to a vial and was mixed with 0.2 ml of a 8.1 % (w/v) sodium dodecyl sulfate solution, 1.50 ml of a 20 % acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.50 ml of a 0.8 % (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10 % trichloro acetic acid were transferred into a centrifuge tube and centrifuged at 1000 x g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water [11]. 1,1,3,3-Tetraethoxypropan was used as standard for calibration of the curve and is expressed as nmoles/mg protein.

Assay of Antioxidant Enzymes

The fundic stomach was homogenized (5%) and mitochondrial fraction was prepared as described above. Decomposition of H_2O_2 in presence of catalase (CAT) was followed at 240 nm [12]. One unit of (U) catalase was defined as the amount of enzyme required to decompose 1µmol of H_2O_2 per min, at 25 °C and pH 7.0. Results are expressed as units (U) of CAT activity/mg protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide (reduced)-phenazine methosulphate-nitrobluetetrazolium reaction system [13,14]. One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25 °C ± 2 °C) and the results have been expressed as units (U) of SOD activity/mg protein.

Statistical Analysis

All the data were presented as mean ± SEM and analyzed by Wilcoxon Sum Rank Test [15] and unpaired Student's t-test for the possible significant interrelation between the various groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of *Alectra parasitica* at dose of 50-200 mg/kg, twice a day for 3 days prevented the acute gastric ulcers in a dose related manner. The range of percent protection of aspirin 48.89 – 82.22% ($P < 0.001$) and the percent protection of ranitidine is 57.78 ($P < 0.001$), respectively in various gastric ulcer models (Table 1). Secretion of mucus and bicarbonate by surface epithelial constitute a mucus-bicarbonate barrier, which is regarded as first line of defense against potential ulcerogens. The gastric wall mucus was significantly ($P<0.001$) enhanced by *Alectra parasitica* and is regarded as a first line of defence against aspirin-induced gastric ulcers showing cytoprotective property. However, the H_2 receptor blocker ranitidine (50 $mgkg^{-1}$) also produces a significant protective effect (Table 1).

Table 1: Effect of *Alectra parasitica* on aspirin -induced changes in gastric ulcers and gastric wall mucus in rats

Treatment and dose (mg/kg)		Ulcer Index	% protection	Gastric wall mucus (g/g wet glandular tissue)
Control	-	0.0 ± 0.0	-	267.5 ± 15.0
Aspirin	-	22.5 ± 4.3	-	153.1 ± .9.6^{z}
Alectra parasitica	100	11.5 ± 3.2^{a}	48.89	207.8 ± 10.0^{a}
Alectra parasitica	100	8.0 ± 4.3^{b}	64.44	235.5 ± 12.7^{b}
Alectra parasitica	200	4.0 ± 1.2^{b}	82.22	269.9 ± 14.9^{b}
Ranitidine	50	9.6 ± 2.5^{a}	57.78	218.7 ± 10.5^{a}

Values are mean ± SEM for six rats. P: $^{z}<0.001$ compared to respective control group, P: $^{a}<0.01$ and $^{b}<0.001$ compared to respective aspirin group

A summary (Table 2) is included to indicate the severity of ulcer index as well as enzyme activities. While studying the role played by reactive oxygen species on aspirin induced gastric damage, lipid peroxidation and SOD were increased significantly of the ulcerated stomachs ($P < 0.001$). Pretreatment with *Alectra parasitica* and general antioxidant reduced glutathione significantly reduced the ulcer index, LPO, SOD levels and increased in CAT activity in comparison to the ulcer control ($P < 0.01$ – $P < 0.0011$).

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Table 2: Effect of *Alectra parasitica* on LPO, CAT, and SOD activities in aspirin-induced ulcers

Treatment and dose (mg/kg)		Ulcer Index	LPO	CAT	SOD
Control		0.0 ± 0.0	0.36 ± 0.01	29.5 ± 2.0	97.6 ± 7.6
Aspirin		23.2 ±3.2^{y}	0.50 ± 0.01^{y}	16.2 ± 1.4^{y}	197.8 ± 9.8^{y}
Alectra parasitica	100	7.8 ± 1.1^{b}	0.27 ± 0.01^{b}	24.1 ± 1.3^{a}	156.0 ± 4.5^{b}
Alectra parasitica	200	4.2 ± 0.9^{c}	0.18 ± 0.01^{c}	32.6 ± 1.2^{c}	101.2 ± 3.6^{c}
Ranitidine	50	8.5 ±2.2^{c}	0.26 ± 0.02^{c}	30.5 ± 1.4^{c}	131.5 ± 4.8^{c}

Values are mean ± SEM for six rats, P: ^{x}<0.05 and ^{y}<0.001 compared to respective control group, P: ^{a}<0.05, ^{b}<0.01 and ^{c}<0.001 compared to respective aspirin group

DISCUSSION AND CONCLUSION

The present study showed for the first time that the ethanolic extract of *Alectra parasitica* possess gastroprotective activity as evidenced by its significant inhibition in the formation of ulcers induced by drugs. Synthetic NSAIDs like aspirin cause mucasal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H^{+} ions [16,17]. Aspirin –induced depletion of gastric wall mucus has been prevented by *Alectra parasitica*. A copious amount of gastric mucus is secreted during superficial mucosal damage and provides a favorable microenvironment in repair by restitution. Therefore it is conceivable that the observed gastric ulcer protection of *Alectra parasitica*, provides a general evidence for the close relationship between these factors. As aetiopathogenesis of these ulcer models are different, mechanism of *Alectra parasitica* should then include number of predisposing factors. On the other hand, the mucosal protection induced by nonprostanoid compounds was perhaps mediated through the mobilization of endogenous prostaglandins [18]. Free radicals affect lipids by initiating peroxidation. Superoxide (O^{-}_{2}), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) are important ROS causing tissue damage and lipid peroxide level is an indicator for the generation of ROS in the tissue. The experimental data stated that the aspirin aggravated the ulcer severity and lipid peroxidation as compared to control rats. The higher lipid peroxidation and SOD levels indicated increased production of O_2^{-} within the tissue as elevated O_2^{-} level was thought to increase the concentration of cellular radical level. These radicals functioned in concert to induce cell degeneration via peroxidation of membrane lipids, breaking of DNA strands and denaturing cellular proteins [19]. This effect was significantly reversed by prior administration of *Alectra parasitica* providing a close relationship between free radical scavenging activity and the antiulcer activity. The more work is required for the clear understanding on alcohol and acetic acid induced chronic ulcers.

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