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Antioxidant activity of leaf extracts of *acalypha indica* in animal models of gastric ulcer

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ABSTRACT

In the present study, aqueous and ethanolic extracts of *acalypha indica* leaves were investigated for its antioxidant activity in animal models of gastric ulcer with the aim of exploring possible correlation between its antioxidant and antiulcer activities. Gastric ulcers were produced in rats by pyloric ligation method as described by *Shay* et al. The animals were divided into four groups of six animals each. Group I served as disease control in which the animals received only distilled water. Group II received standard drug ranitidine 10mg/kg orally. Group III and IV received aqueous and ethanolic extracts of *Acalypha indica* 400mg/kg orally that served as test groups. The antioxidant activity was evaluated qualitatively by TLC spray reagent method using β-carotene linoleate mixture oxidation when exposed to sunlight and its protection by alcoholic and aqueous extracts of *Acalypha indica*. *Acalypha indica* administered animals produced significant antiulcer effects in pylorus ligated model and it also showed good antioxidant effect by TLC method. The antioxidant and antiulcer activity was correlated for the reduction in ulcer level. Various parameters like mean volume of gastric secretion, mean pH and mean total acid were calculated and was concluded that both the extracts of *Acalypha indica* significantly reduced the antiulcer parameters when compared to standard drug ranitidine. The mean volume of gastric secretions, mean pH and mean total acid for aqueous extract was calculated as 2.93±0.48 ml, 4.89±0.72, 107.50±1.70mEq/L, and for the alcoholic extract was found to be 3.23±0.78ml, 5.22±0.51 and 112.05±1.60mEq/L.

KEYWORDS

Acalypha indica leaf extract;
Antioxidant activity;
Gastric ulcer.

INTRODUCTION

Ayurveda the ancient medicinal system of our country explored the maximum extent of the medicinal use of herbs for alleviation of human diseases. Today we find in every country the folklore living in every part of the world are habituated to use their plant resources. They have developed ethnic systems of medicine. Some of them have developed on prolonged experience, while some on rational and scientific basis. In modern era, the medicinal plants have been gradually replaced by synthetic drugs. But of late, it is being realized that several disease were found to develop drug resistance to synthetic drugs and also responsible for many of adverse effects. Nearly 70% of the people in India still rely on herbal drugs, necessitating the need to conduct research on isolation and identification of bio active principle of plant drugs. This has lead to the investigations in this field have gained great importance.

Acalypha indica[1] has been investigated for its

antiulcer and antioxidant property in a view to correlate its effects with each other which is claimed in folklore medicine. *Acalypha indica*[2] is a common annual shrub in Indian gardens, backyards of houses and waste place throughout the plains of India. Leaves, roots, stalks (young shoots) and flowers are used in medicine. Fruits are used in asthma, cough, bronchitis, ear ache. Whole plant is used as an expectorant, laxative, diuretic, pneumonia, rheumatism; leaves are used in skin diseases like scabies.

Acalypha indica[3] contains cyanogenetic alkaloids and glycosides, acalyphine and triacetonamine possibly a degradation product of glucoside, 2 methyl anthraquinone, tri-O-metlryl ellagic acid and its D-glucoside (leaves) acalyphamide (as acetate), stigmasterol (root), tannins (whole plant), the other constituents are n- octacosanol, beta sitosterol, kaempferol, tannins, resins and essential oils.

In animal experimental studies and in clinical use[4], beneficial effects of flavonoids have been claimed in the treatment of capillary fragility, retinal hemorrhage in hypertension, diabetic retinopathy, purpura, rheumatic fever, arthritis, radiation disease, habitual abortion, frost bite, anaphylactic shock, experimental cancer and in the prevention of chromadocryorrhea produced by dietary and environmental stress. Flavonoids have been reported to exert multiple biological effects like anti-inflamm atory, anti-allergic, antiviral and anti-cancer activities. It has been suggested that flavonoids activity depends heavily on their antioxidant and chelating properties.

Preliminary chemical tests were performed to detect the presence of flavonoids. In this research an attempt was made to correlate the antioxidant and antiulcer properties of *acalypha indica* leaf extracts and to explore its possible mechanism of action.

MATERIALS AND METHODS

1. Plant collection

The plant *Acalypha indica* was collected from RFRC, Rajahmundry which was authenticated by botanist at RFRC, the fresh leaves were taken and they were shade dried and then dried leaves were powdered coarsely and were used for the extraction.

2. Extraction

The extraction was carried out by two methods using the coarsely powdered leaf powder. They were cold maceration and Soxhlet extraction. In cold maceration process, the dried leaves were macerated in aqueous medium with 0.3% chloroform as preservative for 7 days.

In soxhlet extraction process, the plant material was packed in the soxhlet apparatus. Then the organic solvent ethanol was poured and extraction was carried out until the complete alcoholic extract is collected. Then it was vacuum dried to remove the organic solvents.

3. Identification tests for flavonoids

Preliminary chemical tests[5] were performed to detect the presence of flavonoids. The qualitative chemical tests performed were shinoda test, ammonia fuming test, lead acetate test, chalcones test, boric acid test, zirconium oxychloride test, Gibbs test, P- benzoquinone test, and O- nitro benzene test.

4. Anti ulcer property by pyloric ligation method[6,7]

Wistar albino rats of either sex were grouped into 4, each containing 6 animals, They were kept in the animal house at room temperature 25±2^{0}C, with relative humidity of 45-55%, maintained under 12 hrs light and dark cycle and were fed with standard animal feed and were acclimatized for a week before the study. Group I served as disease control in which distilled water was administered orally, group II received ranitidine 10mg/Kg orally and it was considered as standard, group III received 400mg/kg of the drug *Acalypha indica* alcoholic extract orally, group IV received 400mg/kg of the drug *Acalypha indica* aqueous extract orally, The last two groups served as test.

Pyloric ligation method was performed as described by shay et al. Rats were fasted for 36 hours prior to the surgical procedure and kept in raised mesh-bottomed cages to avoid coprophagy. Under ether anesthesia the abdomen was opened by a small mid line incision below the xiphoid process. The pyloric portion of the stomach was identified, slightly lifted, avoiding traction to the pylorus or damage to the blood supply. The stomach was then replaced carefully and the abdominal wall closed by interrupted sutures. Animals were deprived of both food and water during the post operative period and were sacrificed at the end of 19-20 hours after the operation. The stomach was dissected out as a whole by passing a ligature at the esophageal end.

The stomach was separated from the surrounding tissues and organs and thus brought out as a whole along

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TABLE 1: Mean volume of gastric secretion, Mean PH and mean total acidity of aqueous extract, alcoholic extract and standard drug

Group	Mean volume of gastric secretion	Mean pH	Mean total acid
Control	5.09±0.67	2.35±0.51	159±1.89
Standard	2.34±1.04**	4.55±0.66*	94.65±0.917***
Alcoholic extract	3.23±0.78*	5.22±0.51**	112.05±1.60***
Aqueous extract	2.93±0.48*	4.89±0.72**	107.50±1.70**

***P<0.05, **P<0.01, ***P<0.001**

with its contents. The contents were subjected to centrifugation (3000 rpm for 10 min) and then analyzed for mean volume of gastric secretion, mean pH and mean total acid. The pH was estimated using indikrom pH strips (Glaxo India limited, India) with pH ranges of 2.0- 4.5 and 5.0- 8.5 with a difference in range of 0.5. Free acidity and total acidity were estimated by titrating 1 ml of centrifuged sample with 0.01N NaOH, using Topfer's reagent as indicator and phenolphthalein indicator, respectively. Acidity was expressed in clinical units that are the amount of 0.01N NaOH base required to titrate 100 ml of gastric secretion.

5. Antioxidant property by TLC method[8]

Acalypha indica extract was solubilised in ethanol and subjected to TLC on 20 × 20 cm glass plates pre coated with silica gel-G. The developing solvents used were chloroform: methanol (9:1v/v) for flavonoids and chloroform: ethyl acetate: formic acid (5: 4: 1v/v) for free phenolic compounds. The location of the spots was marked under UV light. β-Carotene-linoleate (a mixture of β-carotene in 30 ml of chloroform and 2 ml of purified linoleic acid in 60 ml of 95% ethanol) was sprayed uniformly on the plates and exposed to sun light for about 4 hours. The background was bleached and the spots which contained the flavonoids and phenolic compounds retained the yellow colour which is indicative of anti oxidant activity.

RESULTS AND DISCUSSION

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents were used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucous production, stabilizing surface epithelial cells or interfering with the prostaglandin synthesis.

The causes of gastric ulcer after pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/(or) stasis of acid. According to Shay et al., the volume of secretion is also an important factor in the formation of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid[7].

From the results it is clear that aqueous extract and alcoholic extract have reduced the mean volume of gastric secretion, when compared to control, standard has reduced the mean volume of gastric secretion when compared to control. But the aqueous extract showed significant effect when compared to alcoholic extract. Aqueous extract, alcoholic extract and standard exhibited significant increase in pH when compared to control but when compared to aqueous extract, alcoholic extract showed significant increase in pH. Aqueous extract, alcoholic extract and standard had reduced the total acid significantly when compared to control. But the significance in reduction of total acidity is more for aqueous extract when compared to alcoholic extract.

Qualitative chemical tests and TLC method confirmed the presence of flavonoids in the extract. Antioxidant property of the extracts were confirmed qualitatively by β- carotene linoleate oxidation method by TLC.

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