Protective Effect of Daucus Carota (Carrot) And Raphanus Sativus (Radish) on Aspirin Induced Gastric Ulcer In Albino Rat Models

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Abstract- *The present study was designed to validate the antiulcer potential of ethanolic extract of carrot and radish on alcohol induced gastric ulcer in albino rats in order to an alternative treatment for alcoholic patients. Gastric ulcer was artificially induced in the albino rats by administering aspirin and antiulcer activity of the selected vegetables was evaluated by assessing the free acidity, total acidity, ulcer index and histoarchitecture of the stomach. Ethanol extract and fresh juice of carrot significantly decreased the free acid level and total acid output in aspirin induced ulcer rats. Similarly, fresh juice and ethanolic extract of radish remarkably decreased the free acid level and total acid output in aspirin induced ulcer rat model. Ulcer score was comparatively found to be low in the ethanol extract of carrot and radish treated rats. The percentage of curation index was observed to be high in the radish treated rats and in the carrot extract treated rats as well. Further the histological studies also supported by these results and they recovered the cellular damage caused by aspirin. From the results, it is concluded that both the radish and carrot have antiulcer activity and thus it may be recommended for the treatment of gastritis and gastric ulcer in alcohol abuse patients.*

Keywords- Daucus carota, Raphanus sativus, gastric ulcer and antiulcer activity.

I. INTRODUCTION

Gastric ulcer is a most prevalent gastrointestinal disorder in the world. It is influenced by various aggressive factors (acid, pepsin, stress, *Helicobacter pylori*) and defensive factors (mucus bicarbonate, blood flow, prostaglandins, etc.) (Kulkarni Goyal, 1996; Repetto and Lesuy, 2002). The modern drugs used in the treatment of gastric ulcers are based on the inhibition of gastric acid secretion by histamine receptor blockers, proton pump inhibitors (Omeprazole) or enhancing the mucosal production like prostaglandin analogues (Hoogerwerf and Pasricha, 2001). In recent years, there has also been growing interest in alternative therapies and the use of natural products, especially those derived from plants (Rates, 2001). Herbal medicine is a fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness. Radish, *Raphanus sativus* L. (Brassicaceae) is a common pungent ingredient used in various abdominal disorders. Almost all parts of the plant including leaves, seeds and roots are utilized in medicine and the fresh juices obtained from roots are used for urinary complaints, hemorrhoids, gastrodynic pains and various gastric ailments (Porterton, 1983). *Dacus carota* L. belongs to the family Apiaceae, is an annual or biannual herb commonly known as carrot. The roots of *Daucus carota* has been traditionally used as a local stimulant for indolent ulcer (Shastri, 1952).

The present study was aimed to find a natural remedy for gastric and intestinal ulcer. Thus, this study was undertaken to determine the curative effect of the ethanol extract of *Daucus carota* (Carrot) and *Raphanus sativus* (Radish) using different experimental ulcer rat models (aspirin induced rat model).

II. MATERIALS AND METHODS

Collection of Study Material

The fresh carrot (*Daucus carota*) and radish (*Raphanus sativus*) were selected and purchased from local vegetable market.

Preparation of Cold Extracts

The dried carrots and radishes were coarsely powdered using domestic grinder. The carrot powder was soaked in absolute ethanol for 48 hours under the refrigerator. The mixture was filtered and evaporated the solvent using distillation unit to get the extract in powder form. The extract fractions were stored in a refrigerator until use. The similar procedure was made in the preparation of radish extract fractions.

Preparation of Fresh Juice

The fresh carrots (*D. carota)* and *(R. sativus)* were cleanly washed and they were cut into small pieces. They were homogenized using mortar and pestle without adding water. The homogenate carrots were squeezed and filtered. The filtrate (fresh juice) was immediately used for the study. The similar procedure was adopted for the preparation of radish juice.

Experimental animal

The albino rats, *Rattus norvegicus* were used for the present study. Five months old male albino rats weighing about 175 to 200g were selected for the experiments. The animals were fed with *ad libitum* of standard laboratory feed (Sri Sai Durga Feeds and Foods, Bangalore) and supplied with water. The bedding was changed every alternative day to maintain hygienic conditions. The study was approved by the Animal Ethical Committee of the Institute (790/03/ac/CPCSEA).

Evaluation of Antiulcer Activity of Carrot and Radish

Totally 28 male rats were used and they were divided into seven groups. Each group consisted of 4 rats. Rats were fasted for 24 hours prior to experiment started.

Group-I: Rats received only normal saline (0.9%).
Group-II: This group rats received only normal food and water for eight days. Further they were received aspirin on eighth day.
Group-III: This group rats received ranitidine (20mg/kg body weight of rats) by oral route. Further, they were received aspirin on the eighth day.
Group-IV: This group rats received an alcoholic extract of carrot (150mg/kg body weight of rats) by oral route. Further, they were received aspirin on the eighth day.
Group-V: This group rats received an alcoholic extract of radish (150mg/kg body weight of rats) by oral route. Further, they were received aspirin on the eighth day.
Group-VI: This group rats received radish fresh juice (250mg/kg body weight of rats) by oral route. Further, they were received aspirin on the eighth day.
Group-VII: This group rats received carrot fresh juice (250mg/kg body weight of rats) by oral route. Further, they were received aspirin on the eighth day.

Aspirin induced ulcer model and experimental design

All the rats were fasted for 36 h before administration of aspirin. The animals were divided into seven groups, each consisting of four rats. Rats in group I, served as a negative control group, received normal saline (1 ml) orally. Group II rats received aspirin (1ml/kg body weight) and it is maintained as treated control rats. Rats in group III were administered with ranitidine (20 mg/kg body weight), a standard reference drug. Rats in group IV and group V received an ethanolic extract of carrot and radish respectively at a single dose of 150 mg/kg body weight. Rats in group VI to VII received fresh juice of carrot and radish, respectively at a single dose of 250 mg/kg body weight. Further, they were received aspirin on the eighth day. Gastric ulcerations were induced using the method of Robert *et al.,* (1979). One hour after extract / fresh juice / drug administration, 250mg/kg body weight of aspirin was given by oral to each rat on the eighth day. The animals were sacrificed four hours later using anesthesia followed by removal of stomach and various parameters of gastric juice (gastric volume, free acidity and total acidity) and ulcer index was evaluated

Collection of gastric juice

The rats were sacrificed and gastric juice was collected by puncture and sucks the juice from the stomach used with a new syringe. The gastric juice was collected for the estimation of free acidity and total acidity. The stomach was washed thoroughly with saline and collected in small bottles containing 10% formalin solution and was subjected to histopathological examination.

Estimation of free acidity and total acidity

An aliquot of 1 ml gastric juice was taken into a 50 ml conical flask. To this supernatant, 9 ml of water was added and mixed well. And then two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until an orange colour appears which indicates the free acidity. Again titrate with NaOH until the permanent pink color was established, it indicates that the total acidity. The volume of 0.01N NaOH consumed was noted and the free acidity and total acidity was calculated by the following formula and expressed as milli equivalent.

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

Where N is volume of NaOH consumed in total acidity titration + the consumption of NaOH in the free acidity titration. 0.01 is the normality of NaOH and 100 is the factor to be represented in liter (Okabe *et al.,* 1978)

Ulcer index

Each rat stomach was removed and inflated with 10ml of 10% buffered formalin solution to fix the outer layer of the stomach. Each stomach was cut open along the greater curvature, rinsed with tap water to remove stomach contents and the mucosa were examined under the dissecting microscope with a square grid eyepiece to access the formation of ulcer hemorrhage lesions. The maximum length of all lesions in mm for each stomach was expressed as the ulcer index as recommended by (West, 1982; Hara, 1985)

Ulcer Index (UI):

0 mm — Normal colored stomach
0.5mm — Red coloration
1 mm — Spot ulceration
1.5mm — Haemorrhagic streak
2 mm — Ulcers > 3mm
3 mm — ulcers > 5mm (perforation)

Percentage of curation was determined by following formula.

Percentage of curation = [$UI_{Control}$ - $UI_{treated}$ / $UI_{Control}$ x100]

Histological evaluation of gastric lesions

Specimens of the gastric walls from each rat were fixed at 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 µm and stained with hematoxylin and eosin for histological evaluation.

III. RESULTS AND DISCUSSION

Most of the studies demonstrate the importance of natural products in drug discovery. In this study antiulcer activity of carrot and radish extracts has been studied. The antiulcer study was evaluated using aspirin induced ulcer rat models. This method of ulcer induction is being widely used and is a convenient way of assessing antiulcer activity of the drug (Tan, *et al*., 1996,; Tan, *et al*., 2000). Aspirin method of inducing gastric lesions is a rapid and convenient way of screening plant extracts for antiulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Aspirin induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Aspirin induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhages and necrotic aspects of tissue injury. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium (Soll, 1990).

Effect of *Daucus carota* and *Raphanus sativus* extract and fresh juice on aspirin induced ulcer rats:

Free acidity*:* The free acidity was measured in control and all experimental rats on the 8th day of the experiment. The effect of *Daucus carota, Raphanus sativus* extract and ranitidine on total acidity level in aspirin induced ulcer rats were given in table 1 and are graphically represented in the plate 1.

The free acidity of ranitidine treated, extract treated and fresh juice treated rats were decreased when compared to ulcer induced rats (0.5 ± 0.08 mEq/L). The free acidity of ranitidine treated rats were decreased (0.2 ± 0.08 mEq/L) when compared to extract treated and fresh juice treated rats. The carrot and radish fresh juice treated rats were increased (0.4 ± 0.17 mEq/L and 0.4 ± 0.05 mEq/L) when compared to carrot and radish extract treated rats (0.2 ± 0.08 mEq/L and 0.3 ±0.08 mEq/L). However, there is no significant difference among different groups of rats.

Total acidity*:* The total acidity was measured in control and all experimental rats on the 8th day of the experiment. The effect of *Daucus carota, Raphanus sativus* extract and ranitidine on total acidity level in aspirin induced ulcer rats were given in table 1 and are graphically represented in the plate 1.

The total acidity of the carrot and radish extract and fresh juice and ranitidine treated rats were decreased (0.4 ± 0.05, 0.6 ± 0.12, 0.7 ± 0.12 and 0.9 ± 0.17 mEq/L) when compared to ulcer induced rats (1.2 ± 0.17 mEq/L). Nevertheless, the total acidity was increased when compared to normal control rats (0.3 ± 0.05 mEq/L). The carrot and radish fresh juice treated rats were increased when compared to ranitidine treated rats (0.4 ± 0.12 mEq/L).

Table 1: Effect of *D. carota* extract and *R. sativus* extract and fresh juice for free acidity and total acidity (Mean ± SD) in aspirin induced ulcer rats. (Values in the parentheses are range of respective mean)

Groups	Free Acidity (mEq/L)	Total Acidity (mEq/L)
I	0.1 ± 0.05 (0.10 – 0.20)	0.3 ± 0.05 (0.30 – 0.40)
II	0.5 ± 0.08 (0.40 – 0.60)	1.2 ± 0.17 (1.00 – 1.40)
III	0.2 ± 0.08 (0.10 – 0.30)	0.4 ± 0.12 (0.30 – 0.60)
IV	0.2 ± 0.08 (0.10 – 0.30)	0.4 ± 0.05 (0.40 – 0.50)
V	0.3 ± 0.08 (0.20 – 0.40)	0.6 ± 0.12 (0.50 – 0.80)
VI	0.4 ± 0.17 (0.20 – 0.60)	0.7 ± 0.12 (0.60 – 0.90)
VII	0.4 ± 0.05 (0.40 – 0.50)	0.9 ± 0.17 (0.80 – 1.20)

Groups:
Group I = Normal Control
Group II = Ulcer Control
Group II = Ranitidine + Aspirin
Group IV = *D. carota* extract + Aspirin
Group V = *R. sativus* + Aspirin
Group VI = *D. carota* fresh juice + Aspirin
Group V = *R. sativus* fresh juice + Aspirin

Table 2: Effect of *D. carota* extract and *R. sativus* extract and fresh juice on Ulcer Index and Curation Index (Mean ± SD) in aspirin induced ulcer rats. (Values in the parentheses are range of respective mean)

Groups	Ulcer Index (% protection)	Curation Index (%)
I	0.0 ± 0.00 (0.00 – 0.00)	-
II	4.0 ± 0.00 (4.00 – 4.00)	-
III	0.1 ± 0.50 (0.10 – 0.20)	96.8 ± 1.25 (95.00 – 97.50)
IV	0.2 ± 0.17 (0.10 – 0.40)	93.7 ± 4.33 (90.00 – 97.50)
V	0.1 ± 0.00 (0.10 – 0.10)	97.5 ± 0.00 (97.50 – 97.50)
VI	0.2 ± 0.05 (0.20 – 0.30)	93.7 ± 1.44 (92.50 – 95.00)
VII	0.6 ± 0.18 (0.40 – 0.80)	83.1 ± 4.73 (80.00 – 90.00)

Groups:
Group I = Normal Control
Group II = Ulcer Control
Group III = Ranitidine + Aspirin
Group IV = *D. carota* extract + Aspirin
Group V = *R. sativus* + Aspirin
Group VI = *D. carota* fresh juice + Aspirin
Group VII = *R. sativus* fresh juice + Aspirin

There is no significant difference among control, ranitidine, extract treated and carrot fresh juice treated rats.

Ulcer index*:* The ulcer index was measured in control and all experimental rats on the 8th day of the experiment. The effect of *Daucus carota, Raphanus sativus* extract and ranitidine on total acidity level in aspirin induced ulcer rats were given in table 2 and are graphically represented in the plate 1.

The ulcer index of ranitidine, carrot and radish extract and fresh juice treated rats were decreased (0.1 ± 0.50 mEq/L, 0.2 ± 0.17 mEq/L, 0.1 ± 0.00 mEq/L, 0.06 ± 0.18 mEq/L) when compared to ulcer induced rats (4.0 ± 0.00 mEq/L). The ulcer index of carrot extract and radish extract treated rats were decreased when compared to fresh juice treated rats.

Curation index*:* The curation index was measured in control and all experimental rats on the 8th day of the experiment. The effect of *Daucus carota, Raphanus sativus* extract and ranitidine on total acidity level in aspirin induced ulcer rats were given in table 2 and are graphically represented in the plate 1.

Histology of stomach in experimental rats:

Histoarchitecture of stomach in different group of rats are shown in Plate 2. Microscopically stomach of rats from the ethanol induced ulcer control group showed necrosis of gastric mucosa associated with congestion of submucosal blood vessels, submucosal edema and hemorrhage. Examined stomach of rats from control group revealed necrosis of gastric mucosa associated with hemorrhage. Ranitidine treated section showed the normal mucosa with no ulcer in the submucosa the same observation made by (Chaudhari and Mengi, 2006).

Examined stomach of rats treated with carrot extract and radish extract at a dose of revealed atrophy of gastric mucosa associated with submucosal edema. Moreover, stomachs of rats from orally given ethanolic extracts of carrot and radish showed congestion of submucosal blood vessels associated with edema.

Meanwhile, stomach of rats treated with ethanolic extract of carrot and radish on histopathological results showed favourable changes in the stomach. Rats treated with carrot extract showed submucosal leucocytic cells infiltration. Examined sections from fresh juice groups revealed no histopathological changes.

Histopathological study revealed that ethanolic extracts of carrot and radish treated group, the mucosa was found to be almost normal with mild muscularis mucosa.

The extracts of *D. carota* and *R. sativus* had no observable histopathological effect on the epidermal and dermal layers of the skin apart from partial degeneration of the dermis at the site where it merged with the hypodermis. The ethanol extract of the carrot and radish caused prevent in histoarchitecture of mucosal epithelium from the damage caused by ethanol.

A number of secondary metabolites / active compounds isolated from plants have been demonstrated in animal models (*in vivo*) as the active principle responsible for facilitating the healing of wounds. Some of the most important ones include tannins from *Terminalia arjuna (*Fu, 2005) quercetin, isorhamnetin and kaempferol from H*ippophae rhamnoides* (Jagetia, and Rajanikant, 2004) curcumin from *Curcuma longa* (Pillai, 1984).

D. carota and *R. sativus* extracts and fresh juice have a significant effect on inhibition of gastric acid secretion and also they prevent the damages of mucosal epithelium by ethanol extract of the carrot and radish treatment.

Plate: 1. Effect of *D. carota* extract and *R. sativus* extract and fresh juice on free acidity, total acidity, ulcer index and curation index

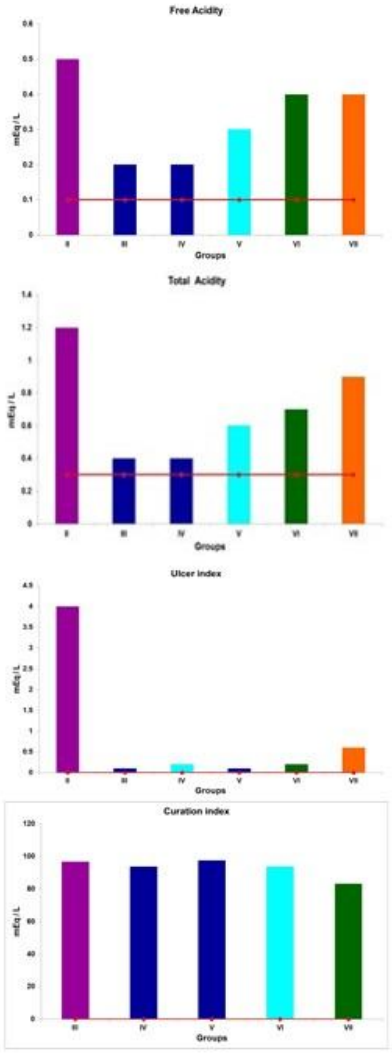
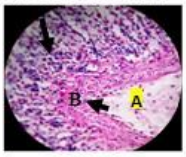
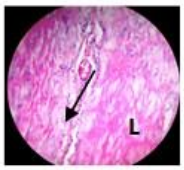


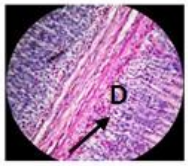
Plate: 2. Histoarchitecture of ulcer stomach of experimental rats.



Group-I Control rat

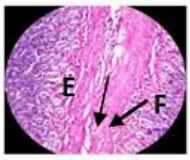


Group-II Aspirin induced rat

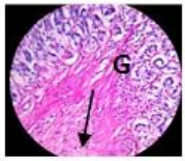


Group-III Ranitidine drug treated rat

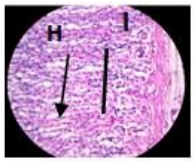
Group-IV Ethanol extract of *D. carota* treated rat



Group-V Ethanol extract of *R. sativus* treated rat



Group-VI *D. carota* fresh juice treated rat



Group-VII *R. sativus* fresh juice treated rat

a.Muscularis mucosa b.mucosa c. Gastric pits d. lumen e. parietal cell f. chief cell g.muscularis externa h. gastric gland i. lamina propria. l. erosion of upper part of epithelium.

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