Antiulcer activity of Daucus carota (Carrot) and Raphanus sativus (Radish) on Alcohol Induced Ulcer in Albino Rat Models

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ABSTRACT: ***The effect of ethanol extract of carrot and radish powder was investigated in albino rats in order to an alternative for treatment for alcoholic patients. Gastric ulcer was artificially induced in the albino rats by administrating ethanol and antiulcer activity of these vegetables was evaluated by assessing the free acidity, total acidity, ulcer index and histoarchitecture of stomach. 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. Thus, it is concluded that ethanol extract of both the carrot and radish significantly decreases the volume of gastric acid secretion, free acidity, total acidity and ulcer index with respect to control rats.***

Key words: Daucus carota, Raphanus sativus, Antiulcer activity, Ulcer index, Curation index

INTRODUCTION

Peptic ulcer is among the most serious diseases in the world. Ulceration occurs when there is an imbalance between protective (mucus secretion, blood flow, prostaglandins, enzymatic and non-enzymatic antioxidants) and aggressive mechanisms in the stomach (acid-pepsin, leukotrienes and reactive oxygen species) (Repetto and Llesuy, 2002), which is affected by factors such as excessive ingestion of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol, infection by *Helicobacter pylori* and emotional stress (Rao *et al.*, 2004). The therapy used for treating gastric ulcers includes the control of the *H. pylori* bacterium, the control of the H+/K+-ATPase pump and the acid secretion, as well as the damage and inflammation reversal to the mucosa. The results indicate that the ethanolic extract of *Zanthoxylum rhoifolium* exhibits a significant gastroprotection, because it inhibits the formation of gastric lesions using different models (Freitas *et al*., 2011).

Radish, *Raphanus sativus* L. (Brassicaceae Family) commonly known as *Fijl* or *muli* 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 (Mayer, 1981; Poterton, 1983). There were no available scientific reports in literature on the traditional claims of this plant material in regard to its antiulcer potential (Alqasoumi *et al*., 2008). *Daucus carota* L. belongs to family Apiaceae. The roots of *Daucus carota* has been traditionally used as a local stimulant for indolent ulcer (Kirtikar and Basu, 1993; Nadkarni, 1976). The study was undertaken to determine the gastroprotective potential of the aqueous extract from the roots of *Daucus carota* (DCE) using three experimental gastric ulcer models viz., pylorus ligation-, aspirin- and ethanol- induced gastric lesion (Nayeem Khatib *et al*., 2010). Revathi *et al* (2017) studied the curative effect of the ethanol extract of *Daucus carota* (Carrot) and *Raphanus sativus* (Radish) using aspirin induced rat model. The present study was aimed to find a natural remedy for gastric ulcer. Thus, this study was under taken to determine the curative effect of the ethanol extract of *Daucus carota* (Carrot) and *Raphanus sativus* (Radish) using alcohol induced rat model.

MATERIALS AND METHODS

Preparation of Extracts

The fresh carrot (*Daucus carota*) and radish (*Raphanus sativus*) were selected and purchased from local vegetable market. The dried carrots and radishes were coarsely powdered using domestic grinder. The carrot powder was extracted by cold extraction method using ethanol. The mixture was filtered and evaporated the solvent using distillation unit to get the extract in powder form. The extract were stored in a desiccator for further use. The similar procedure was made in the preparation of radish extract.

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 *ad libitum*. 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

In the present study, a total of 20 rats were used. The rats were divided in to five groups and each groups consist of 4 rats, among these, ulcer was induced in four groups (group II, III, IV and V). Rats were fasted for 24 hours prior to experiment started.

Group-I: Rats received only normal saline (0.9%).

Group-II: Gastric ulcer rats (induced with absolute alcohol).

Group-III: Gastric ulcer induced rats treated with ranitidine (20mg/kg body weight of rats).

Group-IV: Gastric ulcer induced rats treated with ethanol extract of *Daucus carota* (150mg/kg body weight of rats).

Group-V: Gastric ulcer induced rats treated with ethanol extract of *Raphanus sativus* (150mg/kg body weight of rats).

After 1 hr of alcohol administration, experimental rat groups were treated with extracts and ranitidine. The animals were sacrificed after 4 hrs of alcohol administration using chloroform anesthesia followed by removal of stomach and collected gastric juice from the stomach.

Collection of gastric juice

The rats were sacrificed and gastric juice was collected by puncture and sucks the juice from the stomach used by a syringe. The gastric juice was collected for the estimation of free acidity and total acidity.

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.

$$\text{Total Acidity (mEq/L)} = \frac{\text{N x Normality of NaOH x 100}}{0.1}$$

Where N is volume of NaOH consumed in total acidity titration + the consumption of NaOH in the free acidity titration. 0.1 is the normality of NaOH and 100 is the factor to be represented in liter (Okabe *et al*., 1978).

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 = $[UI_{Control} - UI_{treated} / UI_{Control} \times 100]$

Histological evaluation of gastric lesions

The stomach was washed thoroughly with saline and specimen of the gastric walls from each rat were fixed in 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.

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 alcohol induced ulcer rat models. This method of ulcer induction is being widely used and is a convenient way of assessing antiulcer activity of drug (Tan *et al*., 1996; Umamaheswari *et al*., 2007). Absolute ethanol method of inducing gastric lesions is rapid and convenient way of screening plant extracts for antiulcer potency and cytoprotection in macroscopically and microscopically visible lesions. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals (Soll, 1990). Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water. 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 (Kannappan, 2008).

Ethanol extract of *D. carota on* ethanol induced ulcer rats

Free Acidity: The free acidity was measured in control and all experimental rats at the end of the experiment. The effect of *D. carota* and ranitidine on free acidity level in alcohol induced ulcer rats were given in table-1. The free acidity of the ranitidine treated rats (group-III) and ethanol extract of *D. carota* treated rats (group-IV) was 0.1 and 0.3 mEq/L, respectively. The free acidity of the extract treated rat group was observed to be close to the value of standard drug treated rat group and it was lower than that of ulcer control group-II (0.6 mEq/L).

Total Acidity: The effect of *Daucus carota* and ranitidine on total acidity level in alcohol induced ulcer rats were given in table-1. The total acidity of the ethanol extract of *D. carota* treated rats was higher (0.5 mEq/L) than that of control rats (0.2 mEq/L). On the other hand, the total acidity of extract treated group was lowered when compared to ulcer control rats (0.9 mEq/L). There is no notable difference between ranitidine and extract treated groups.

Ulcer Index: The effect of ethanol extract of *D. carota* and ranitidine on ulcer index in alcohol induced ulcer rats were given in table 2. There is a decreased ulcer index in the ranitidine and *D. carota* extract treated group (0.1 mm and 0.2mm respectively, the ulcer score inferred that the 0.1mm is normal) when compared to ulcer control groups (1.1 mm *i.e* Spot ulceration). There is no notable difference among the ranitidine and extract treated rats in the ulcer score.

Curation Index: The effect of ethanol extract of *D. carota* and ranitidine on curation index level in alcohol induced ulcer rats were given in table 2. The curation index of the carrot extract treated rats was about 79.5 and it was about 88.6 for ranitidine treated rats.

Ethanol extract of *R. sativus* on ethanol induced ulcer rats

Free Acidity: The free acidity of the ranitidine treated rats was lower (0.1 mEq/L) and the *R. sativus* treated rats was higher (0.2 mEq/L) when compared to that of ulcer control group (0.6 mEq/L). On the other hand, the free acidity values are decreased in extract treated groups when compared to ulcer induced group. There is no significant difference among the control, ranitidine treated and *R. sativus* extract treated rats (Table 1).

***Total Acidity*:** The total acidity of the ethanol extract of *R. sativus* (group-V) and ranitidine (group-III) treated rats was higher (0.4 mEq/L respectively) when compared to control rats (0.2 mEq/L) (Table 1). On the other hand, the total acidity was lowered in ranitidine and

ethanol extract treated rats (group III, IV and VI) when compared to ulcer control rats (0.9 mEq/L). The results indicated that the ethanol extract of *R. sativus* caused notable and favorable changes in the total acid output, when compared to control animal.

***Ulcer Index*:** The effect of ethanol extract of *R. sativus* and ranitidine on ulcer index in alcohol induced ulcer rats were given in table 2. There is a decreased ulcer index in the ranitidine and *R. sativus* extract treated group (0.1 and 0.2mm respectively) when compared to ulcer control groups (1.1 mm). There is no notable difference between the ranitidine and extract treated rats.

***Curation Index*:** The curation index of the radish extract treated rats were lowered (74.9 %) when compared to carrot extract treated and ranitidine treated rats (79.5% and 88.6% respectively). There is no notable difference between ranitidine and extract treated rats (Table 2).

Table 1: Student Newman Keuls Post hoc test results showed the variations and similarities in the free acidity and total acidity among different group of rats. Mean values are arranged in ascending order.

Student-Newman-Keuls Post hoc test (Subset for alpha = 0.05)					
Parameters	**Groups**				
Free acidity (mEq/L)	0.1 (I)	0.1 (III)	0.2 (V)	0.3 (IV)	0.6 (II)
Total acidity (mEq/L)	0.2 (I)	0.4 (III)	0.4 (V)	0.5 (IV)	0.9 (II)

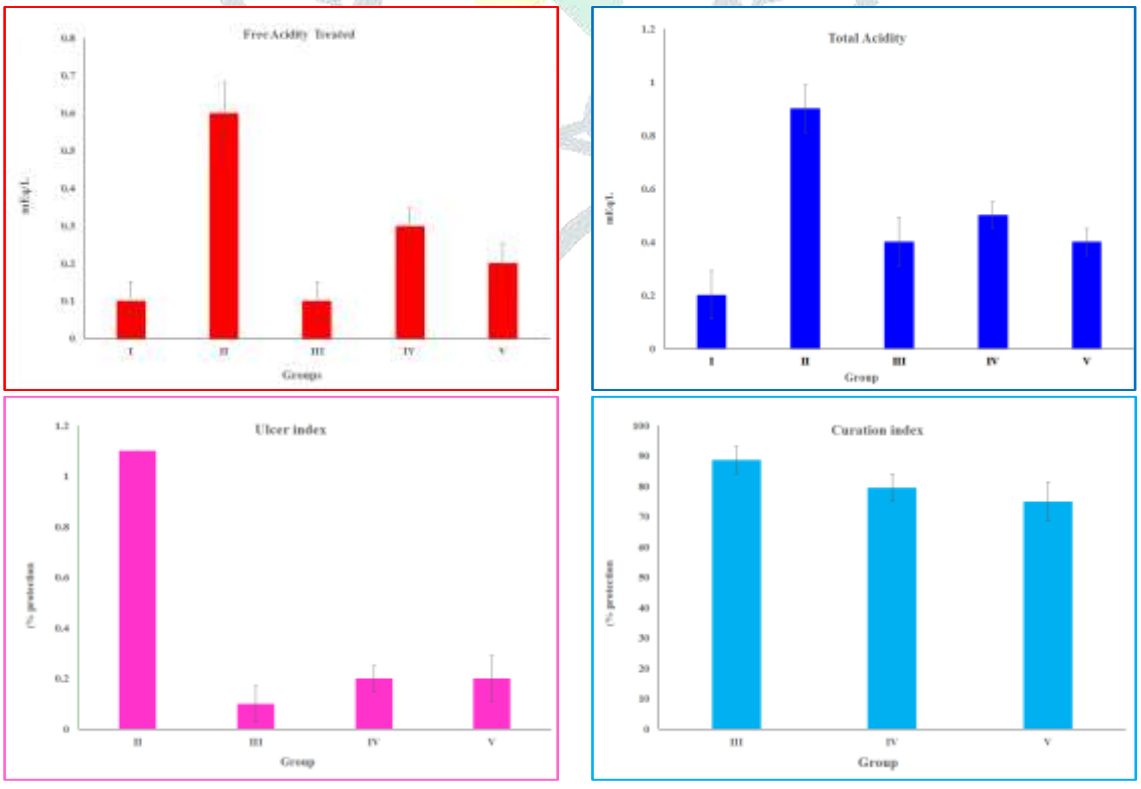
Table 2: Student Newman Keuls Post hoc test results showed the variations and similarities in the curation index among different group of rats. Mean values are arranged in ascending order.

Student-Newman-Keuls Post hoc test (Subset for alpha = 0.05)					
Parameters	**Groups**				
Ulcer Index (%)	0.00 (I)	0.1 (III)	0.2 (IV)	0.2 (V)	1.1 (II)
Curation Index (%)	74.9 (V)	79.5 (IV)	88.6 (III)		

Groups:

I Control, II Ulcer Control, II Ranitidine treated,
IV *D. carota* extract treated, V *R. sativus* treated

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



Groups:
I Control, II Ulcer Control, II Ranitidine treated, IV *D. carota* extract treated, V *R. sativus* treated

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 ethanol extracts of carrot and radish showed congestion of submucosal blood vessels associated with edema. Meanwhile, stomach of rats treated with ethanol 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 the mucosa was found to be almost normal with mild muscularis mucosa in ethanol extracts of carrot and radish treated group. 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 active principle responsible for facilitating healing of wounds. Some of the most important ones include tannins from *Terminalia arjuna* (Fu *et al*., 2005) quercetin, isorhamnetin and kaempferol from H*ippophae rhamnoides* (Jagetia and Rajanikant, 2004) curcumin from *Curcuma longa* (Pillai and Santhakumari, 1984). The presence of flavonoids and other bioactive compounds in *D. carota* may be associated with the gastroprotective effect. The *Daucus carota* possess antioxidants could play a protective role of gastric mucosa from free radical induced damage and acts as antiulcer action (Nayeem Khatib *et al*., 2010).

Plate: 1. Effect of ethanol extract of *D. carota* and *R. sativa* on alcohol induced ulcer in rats

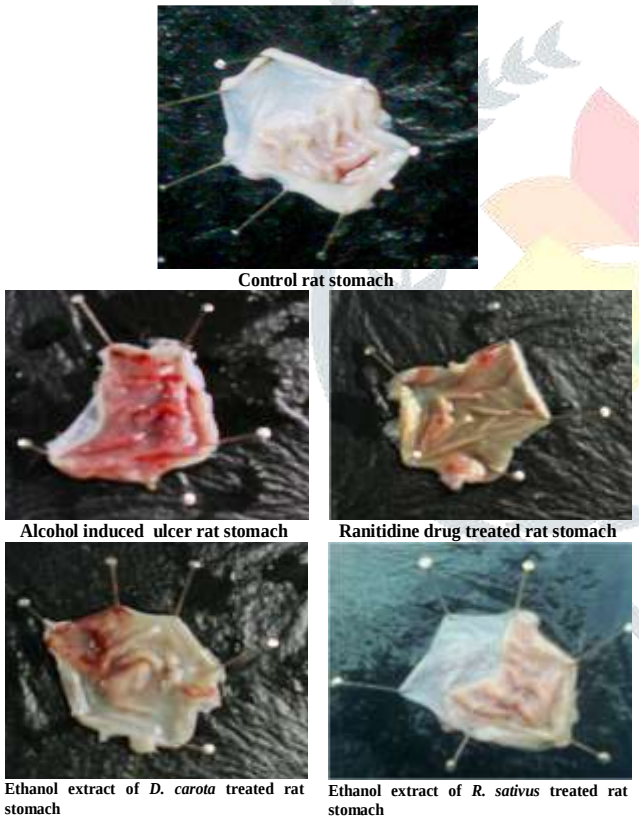
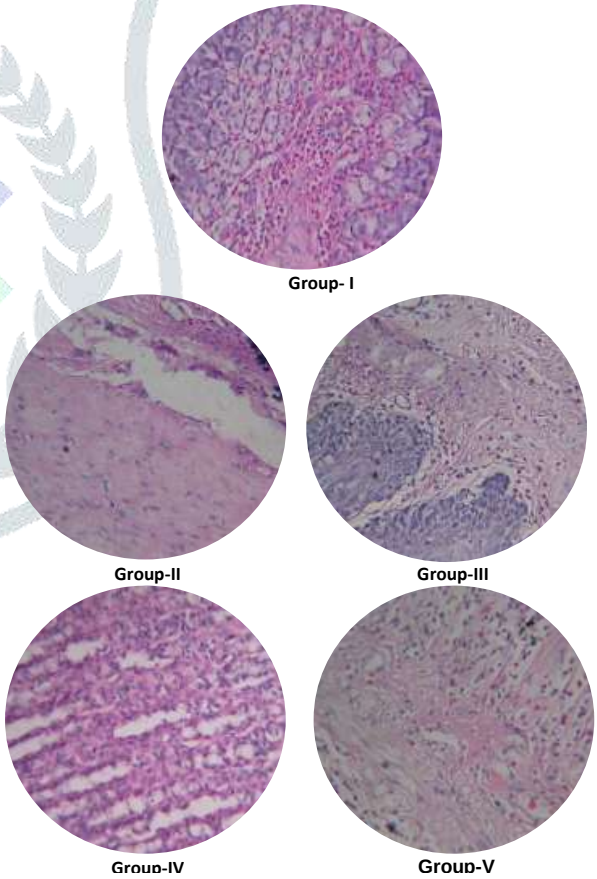


Plate: 2. Histopathology of ulcer stomach in alcohol induced ulcer rats



Groups:
I Control, II Ulcer Control,
III Ranitidine treated,
IV *D. carota* extract treated,
V *R. sativus* treated

Group-I Control -normal epithelial layer and sub mucosal layer and normal mucosa.
Group-II Alcohol induced rats shows mucosal damage, inflammation and necrosis.
Group-III Ranitidine treated shows regeneration of epithelial cell.
Group- IV *D.carota* treated rats shows recovered and regeneration of mucosa, and epithelial layer.
Group-V *R. sativus* treated rats shows almost normal appearance of the cells.

Conclusion

In conclusion the results obtained from the present study demonstrated that *Daucus carota* and *Raphanus sativus* extract has potent antiulcer and gastroprotective activity. This supports the traditional use of *Daucus carota* roots in the treatment of gastric ulcer. *Daucus carota and Raphanus sativus* may be a new alternative remedy for clinical management of gastric ulcer diseases.

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