World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 6, Issue 8, 2017 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

PHARMACOLOGICAL AND PHARMACOGNOSTICAL EXTRACTION AND EVALUATION FOR ANTI ULCER, ANTI OXIDANT ACTIVITY OF BEETAVULGARIS EXTRACTS IN EXPERIMENTAL ANIMALS

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Received on: 19-07-2017; Revised and Accepted on: 03-08-2017

ABSTRACT

Peptic ulcer disease is a serious gastrointestinal disorder that requires well targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H2 receptor antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapse, side effects and drug interactions. This has been rational for the development of new anti ulcer drugs and search for novel molecules has been extended to herbal that offer better protection and relapse. The present study is to evaluate the anti ulcer activity by using herbal remedy beta vulgaris. The ethanolic extract of beta vulgaris treated groups shows a significant effect when compared to control group animals which indicating that the plant having the anti ulcer activity. And also the results showed that the ethanolic extract of the beta vulgaris having the antioxidant activity. The acute toxicity study conducted for ethanolic extract of beta vulgaris indicates that safe up to 2000mg/kg body weight. Ulcer can minimize by some life style changes like, avoid eating at least two hours before bed time and whatever foods might cause discomfort, such as alcohol, caffeine beverages (coffee and pop), fatty foods, and highly seasoned foods. It is important to try to stop smoking, since smoking has been linked to ulcer formation, reduced healing, and ulcer recurrences. Also try to minimize stress in life. Stress may worsen ulcer symptoms.

Keywords: *Beta Vulgaris, Antioxidant, Anti Ulcer, Ethanolic extracts.*

INTRODUCTION

Peptic ulcer is one of the major gastro-intestinal disorders. Peptic ulcer is a lesion of gastric or duodenal mucosa, it occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors [1]. Most injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products and certain drugs and pathological condition such as Zollinger –Ellison Syndrome, they cause the ulcers in gastric or duodenal mucosa [2]. The erosion on the stomach, it is referred to as a gastric ulcer. If it is in the duodenum (the part of the small intestine just after the stomach), it is called a duodenal ulcer.

Peptic ulcer disease is a worldwide problem, affecting about 1 in 10 people. In the early 20th century peptic ulcers were thought to be caused by emotional stress and spicy foods. Peptic ulcer is more Occurs frequently in men than in women. After 45 years of age peoples have less sex differences probably because the incidence of ulcer increases in post menopausal women. The ulcer differences between sexes are related in some way to sex hormones and that the female sex hormones protect against ulceration [3]. Duodenal ulcers are more common than gastric ulcers and usually occur in people aged fewer than 50.Gastric ulcers are more common in people aged over 50. Duodenal ulcers are the most common ulcers found in the Western world. In 1982, Australian doctors Robin Warren and Barry Marshall first discovered a link between ulcers and H. Pylori [4].

Usually Ulcer occurs by many causative agents. But now a day's ulcer is mainly caused by five reasons.

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1. Alcohol consumption
2. NSAIDs consumption
3. Smoking consumption
4. Skipped meals and poor sleep

Multiple mechanisms of protective action and anti-oxidant properties of drugs are minimizing tissue injury in human disease. Absolute ethanol induced gastric lesions in stomach. Gastric lesion is accompanied with the formation of the free radicals (FRs) and reactive oxygen species (ROs). These radicals in particular seem to play an important role in ulcerative and erosive lesions of the gastrointestinal tract. Therefore, treatment with anti-oxidants and FR scavengers can decrease ethanol induced gastric mucosal damage

Anti Oxidant Activity:

Free radicals in Health and Disease: A free radical is defined as any molecular species that contains an unpaired electron in the atomic orbital [5, 6]. Radicals are highly reactive that either donate an electron to or extract an electron from other molecules, and therefore, behave as oxidants or reductants. As a result of their high reactivity, most radicals have a very short half life (10-6 seconds or less) in biological systems [7, 8]. The most important free radicals produced in the body are oxygen derivatives, particularly superoxide and the hydroxyl radical. Examples of free radicals and reactive oxygen species include: superoxide anion radical, hydroxyl radical, nitric oxide, thiyl radical, trichloromethyl radical, hypochlorite radical, hypochlorous acid, and also some potentially dangerous non-radicals such as hydrogen peroxide, singlet oxygen, hypochlorous acid and ozone. Radical production in the body occurs by both endogenous and environmental factors.

Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids. Lipoprotein particles or membranes characteristically undergo the process of lipid peroxidation, giving rise

to a variety of products including short chain aldehydes such as malondialdehyde or 4-hydroxynonenal, alkanes and alkenes, conjugated dienes and a variety of hydroxides and hydroperoxides [9-13]. Oxidative damage to proteins and nucleic acids similarly gives rise to a variety of specific damage products as a result of modifications of amino acids or nucleotides [14-16]. Such oxidative damage might also lead to cellular dysfunction and contribute to the pathophysiology of a wide variety of diseases.

Oxidative stress has been implicated in the etiology of a host of degenerative diseases including cardiovascular disease, diabetes, cancer, alzheimer's disease, neurodegenerative disorders and in aging [17, 18]. In addition, they also play a role not only in acute conditions such as trauma, stroke and infection but also in physical exercise and stress [20, 21].

Since free radicals are causally involved in the disease state, it is believed that antioxidants should be effective in preventing or delaying their occurrence. Indeed, investigations at the cellular, tissue and whole animal level as well as epidemiological studies, strongly support the concept that nutritional antioxidant status is inversely related to the occurrence of free radical–mediated diseases.

MATERIALS AND METHODS

Materials:

Aspirin, Standard drug ranitidine, 0.01N NaOH, phenolphthalein indicator, Topfer's reagent, 80% ethanol, Formalin, gum acacia, Anaesthetic ether obtained from Zeal chemicals, wargal. Benedict's reagent, barfoed's reagent, million's regent, wager's regent, Hager's regent. Mayer's regent.

Beet roots (*Beta vulgaris L.*) were purchased from local markets in wargal. It was identified and authentificated by Professor Dr. Md. Mustafa, Department of botany, Kakatiya university, Wargal, AP.

Animals:

Healthy wistar albino rats weighing between 200-250g were used for the study. The animals were procured from Sainath agencies, laboratory animals, Hyderabad and the animals were kept in polypropylene cages (6 in each cage) and animals were acclimatized to our lab environment for about a week prior to the study, so that they could adapt to the new environment. Animal house were maintained under standard hygienic conditions, at 25 ± 2^{0}C, humidity (60 ± 10 %) with 12 hrs day and night cycle, with food and water *ad libitum*. The experiments were carried out prior approval from Institutional Animal Ethical Committee (IAEC).

Methodology:

Acute Toxicity Studies:

The acute toxicity was determined on female albino rats by fixed dose method of OECD Guide line no 420 given by CPCSEA. Groups of 6 rats were administered test drug by oral route at a dose of 2000, 300mg/kg (6 animals in each dose) and mortality was observed after 24 hr. The safe dose was found to be mg/kg body weight. For this study two doses were selected.

Alcohol-induced gastric ulcer:

First group treated with 1ml of 80% ethanol orally on the day of experiment at about 10 AM with the help of an oral feeding tube. 2nd, 3rd, 4th groups of animals were treated with ranitidine, low and high doses of beet root extracts respectively one hour before ethanol administration. One hour after drug treatment of 2nd, 3rd, 4th groups of animals were treated with 1 ml of 80% ethanol by p.o, to induce ulcers. The animals were sacrificed after 1hr of ethanol administration. The stomach was opened and calculates the ulcer index and percentage inhibition of ulcer.

Aspirin induced ulcer mode:

First group treated with Aspirin in a dose of 250 mg/kg was administered orally on the day of experiment at about 10 AM with the help of an oral feeding tube in the form of an aqueous water suspension. 2nd, 3rd, 4th groups of animals were treated with ranitidine, low and high doses of beet root extracts respectively one hour before aspirin administration. One hour after drug treatment of 2nd, 3rd, 4th groups of animals were treated with 250mg/kg aspirin by p.o, to induce ulcers. The animals were sacrificed after 4hr of aspirin administration. The stomach was opened and calculates the ulcer index and percentage inhibition of ulcer.

In vitro Antioxidant activity:

DPPH free radical-scavenging activity:

The methanolic solution of DPPH (0.1 mM, 1 ml) was incubated with 3 ml of different concentrations of the root extract ranging from 10-100 µ g/ml. Incubation was carried out at room temperature (25^{0}C) for 30 min. For each concentration, the assay was run in triplicate. At the end of the incubation period, the optical density of each sample was determined at 517 *nm*. Ascorbic acid solution was used as a standard. EC_{50} values (concentration required to scavenge 50% of the free radicals) for both ascorbic acid and the root extract were determined. The radical scavenging activity of the tested sample was expressed as an inhibition percentage (IP)[102].

$$\text{DPPH Scavenged (\%)} = (A_{DPPH} - A_{test} / A_{DPPH}) \times 100$$

Where,

A_{DPPH} is the absorbance of the 0.1 mM of DPPH solution and

A_{test} is the absorbance in the presence of the extract or ascorbic acid.

IC_{50} value was determined from the graph obtained using standard ascorbic acid by using the "y = mx + c" formula from the slope of the graph.

RESULTS

Phytochemical Analysis:

Table No. 1: Phytochemical Analysis

Phytoconstituents	Present or Absent
Carbohydrates	Present
Glycosides	Present
Fats	Present
Gums & mucilages	Absent
Proteins & amino acids	Present
Saponins	Present
Tannins & Phenolic compounds	Present
Phytosterols	Absent
Flavonoids	Present
Alkaloids	Absent

Table No. 2: Effect of ethanolic and aqueous extract of *Beta vulgaris* on ulcer index and % ulcer protection in ethanol induced gastric ulcer

Groups (n=5)	Treatment	UI	% ulcer protection
I	Control	11.33±1.732	0.00
II	Ranitidine 20mg/kg	8.33±0.577**	26.47
III	***Beta vulgaris*** 250 mg/kg	10.76±0.4282ns	5.03
IV	***Beta vulgaris*** 400mg/kg	8.55±0.477**	24.53
V	***AEBV 300mg/kg***	9.76±0.410	4.95

*Values express as mean ± SEM; n=6 in each group, statistical comparisons as follows: significant at P<0.01** compared to control group, P>0.05 ns-non significant.*

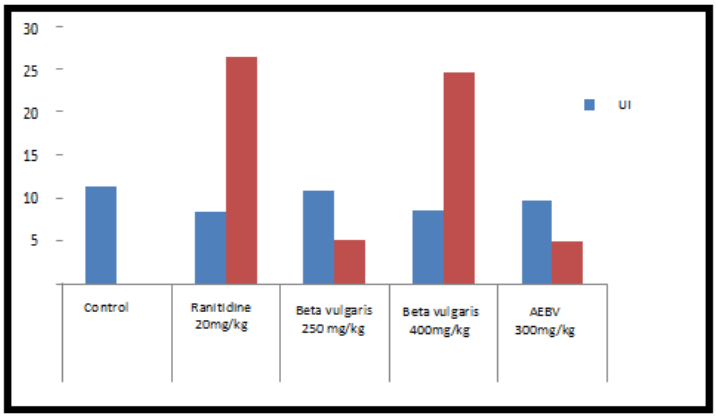

Fig. 1: Effect of ethanolic and aqueous extract of *Beta vulgaris* on ulcer index in ethanol induced gastric ulcer.

Effect of ethanolic extract of *Beta vulgaris* in Aspirin induced gastric ulcer:

In aspirin induced gastric ulcer model, the ulcer index of control group is 11.083±0.4282. The animals treated with ethanolic extract of *beta vulgaris* at 400 mg/kg dose showed significant (*P<0.01*) reduction in the number of ulcer and ulcer index is 8.516±0.42816. Ranitidine at 20mg/kg showed significant (*P<0.01*) reduction in the number of ulcer and ulcer index is 8.516±0.42816. Extract at 250mg/kg shows the protection against the aspirin induced gastric ulcer, ulcer index 10.35±0.5627. Administration of beet root 1 h before the induction of gastric lesions by aspirin showed significant activity, and inhibited the ulcer index in dose dependent manner. The ethanolic extract of *beta vulgaris* was found to possess remarkable ulcer-protective properties at 250 mg/kg and 400 mg/kg when compare to toxic control group.

Table No. 3: Effect of ethanolic extract of *Beta vulgaris* on ulcer index and % ulcer protection in aspirin induced gastric ulcer

Groups (n=5)	Treatment	UI	% ulcer protection
I	Control	11.083±0.4282	0.00
II	Standard	8.562±0.4216**	22.74
III	***Beta vulgaris*** 250 mg/kg	10.35±0.5627	6.613
IV	***Beta vulgaris*** 400mg/kg	8.516±0.42816***	23.16
V	***AEBV 300 mg/kg.***	9.65±0.7265**	9.156

*Values express as mean ± SEM; n=6 in each group, statistical comparisons as follows: significant at P<0.01** compared to control group, P>0.05 ns-non significant.*

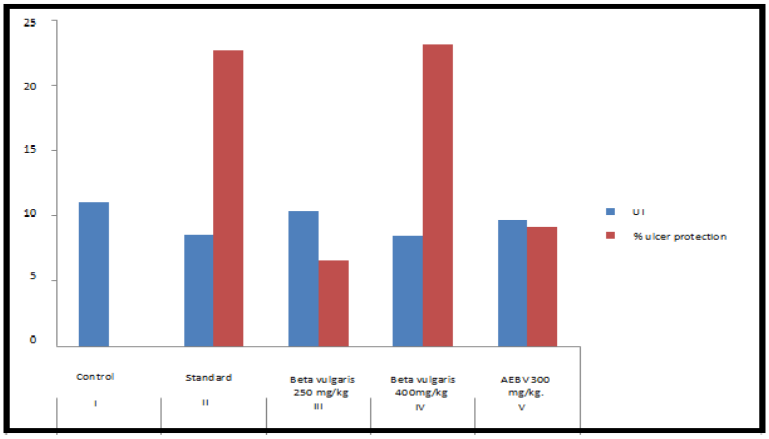

Fig. 2: Effect of ethanolic extract of *Beta vulgaris* on ulcer index in aspirin induced gastric ulcer

In Vitro Anti oxidant activity:

Table No. 4: *In vitro* Antioxidant Activity of various Extractives of *Beta vulgaris* in different Methods

Extractives / Standard	IC_{50} values ± SEM (µg/ml)*			
	Nitric Oxide	H_2O_2	DPPH	ABTS
Pet. Ether	130.6 ± 1.76	188.0 ± 1.15	>500	18.0 ± 1.15
Chloroform	83.0 ± 3.60	91.33 ± 3.5	>500	14.0 ± 1.15
Ethyl Acetate	74.66 ± 2.40	76.0 ± 1.15	11.0 ± 1.73	6.66 ± 0.66
Aqueous Alcohol	77.0 ± 1.0	70.0 ± 1.76	5.33 ± 0.66	15 ± 1.73
Water	90.0 ± 4.6	141.33 ± 2.40	10.66 ± 0.66	18.0 ± 1.15
Rutin	65.0 ± 0.57	35.33 ± 1.33	2.66 ± 0.66	0.52 ± 0.05
Ascorbic Acid	-	180.66 ± 1.76	3.90 ± 0.57	11.25 ± 0.49

** All values are average of three determinations, mean ± SEM*

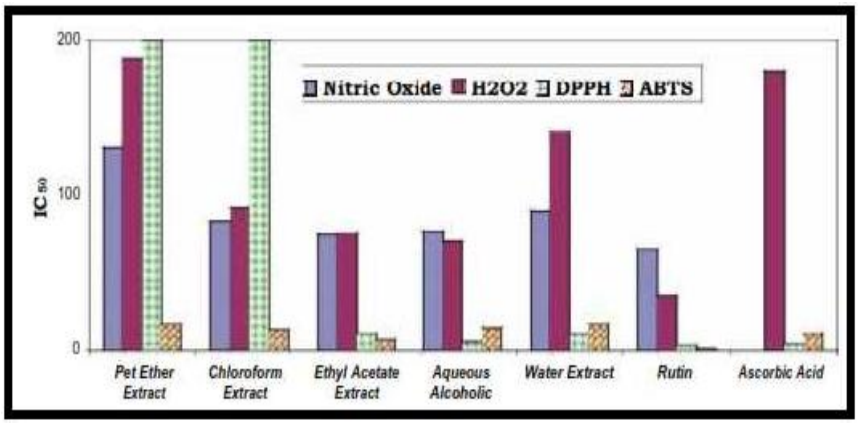

Fig. 3: In Vitro Antioxidant Activity of the various Extractives of Beta vulgaris

In Vitro* Antioxidant Activity of the various Extractives of *Beta vulgaris:

The percentage scavenging of nitric oxide, H_2O_2, DPPH and ABTS radicals by the different extractives of *Beta vulgaris* and standards used at various concentrations are presented in Fig 1, 2, 3 and 4. The percentage inhibition increased with increasing concentrations of the extractives used in all the methods.

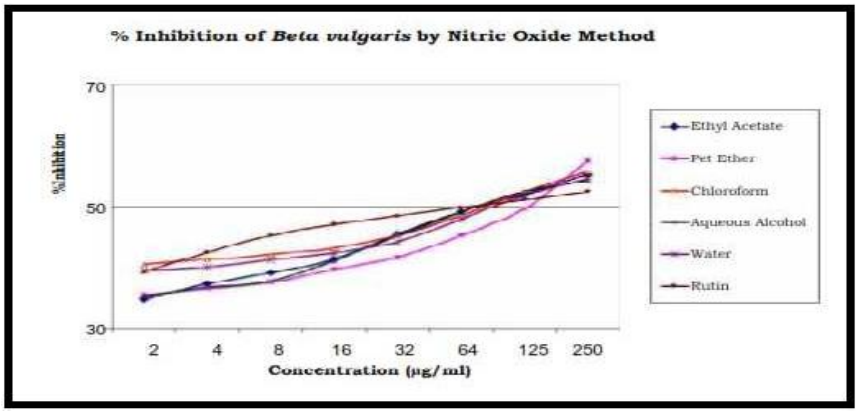

Fig. 4: Percentage Inhibition of various Extractives of *Beta vulgaris* by Nitric Oxide Method

Among the five extractives tested for *in vitro* antioxidant activity, the aqueous alcoholic extractives of *Beta vulgaris* leaves exhibited potent antioxidant activity with low IC50 values in the scavenging of DPPH and hydrogen peroxide.

The IC50 values were found to be 5.33 ± 0.66 and 70.0 ± 1.76 µ g/ml respectively for DPPH and H_2O_2 methods. However, the petroleum ether and chloroform extractives did not exhibit potent antioxidant activity in the DPPH method. In the nitric oxide method, all samples exhibited moderate antioxidant activity with IC50 values of 130.6 ± 1.76, 83.0 ± 3.60, 74.66 ± 2.40, 77.0 ± 1.0 and 90 ± 4.6 µ g/ml respectively for petroleum ether, chloroform, ethyl acetate, aqueous alcohol and water extractives.

In ABTS method all extractives exhibited potent antioxidant activity with low IC50 values which were found to be higher or comparable with those obtained for the standards.

CONCLUSION

In conclusion, the ethanolic extract of *Beta Vulgaris* treated groups shows a significant effect when compared to control group animals which indicating that the plant having the anti ulcer activity. And also the results showed that the ethanolic extract of the *Beta Vulgaris* having the antioxidant activity. The acute toxicity study conducted for ethanolic extract of *beta vulgaris* indicates that safe up to 2000mg/kg body weight.

Ulcer can minimize by some life style changes like, avoid eating at least two hours before bed time and whatever foods might

cause discomfort, such as alcohol, caffeine beverages (coffee and pop), fatty foods, and highly seasoned foods. It is important to try to stop smoking, since smoking has been linked to ulcer formation, reduced healing, and ulcer recurrences. Also try to minimize stress in life. Stress may worsen ulcer symptoms.

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How to cite this article:

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Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil