Ann. For. Res. 65(1): 7563-7575, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

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

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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 etc.,.

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.

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 (Halliwell and Gutteridge, 1999). 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 (Halliwell and Gutteridge, 1999). 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 (Esterbauer, 1996). 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 (Griffiths et al., 2002). 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 (Scalbert et al., 2005b). 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 (Sahnoun et al., 1998).

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:

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 ± 20C, 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 model

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 µg/ml. Incubation was carried out at room temperature (250C) 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. EC50 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].

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

Where,

ADPPH is the absorbance of the 0.1 mM of DPPH solution and

Atest is the absorbance in the presence of the extract or ascorbic acid.

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:

Table 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 2:Effect of ethanolic 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

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.

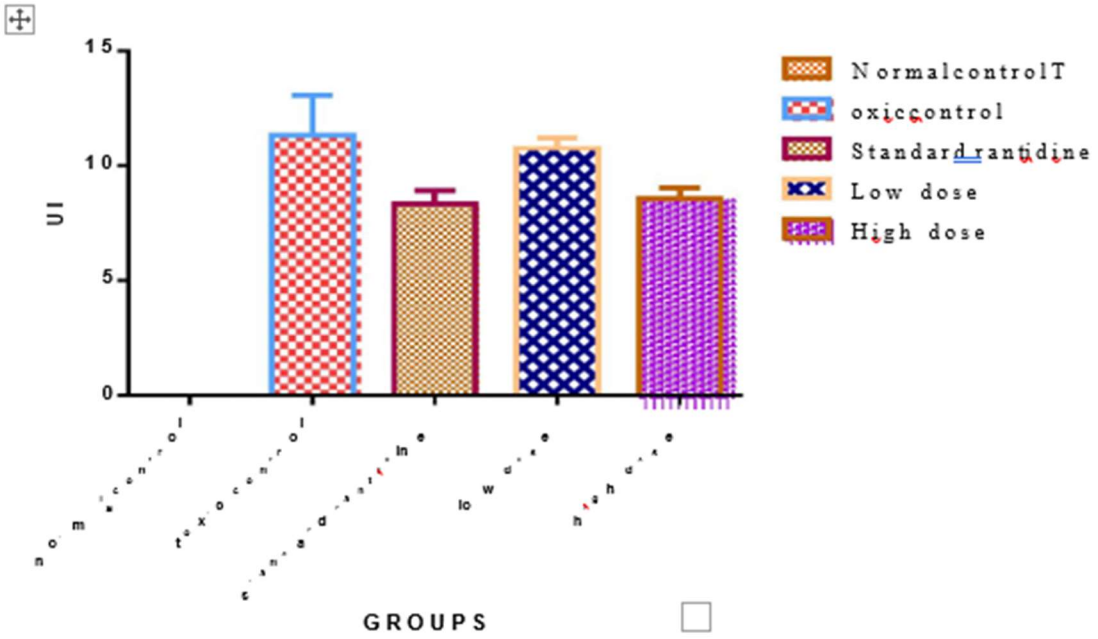


Fig 1:Effect of ethanolic extract of Beta vulgaris on ulcer index in ethanol induced gastric ulcer.

Table 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±0.5627ns	6.613
IV	***Beta vulgaris*** 400mg/kg	8.516±0.42816**	23.16

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

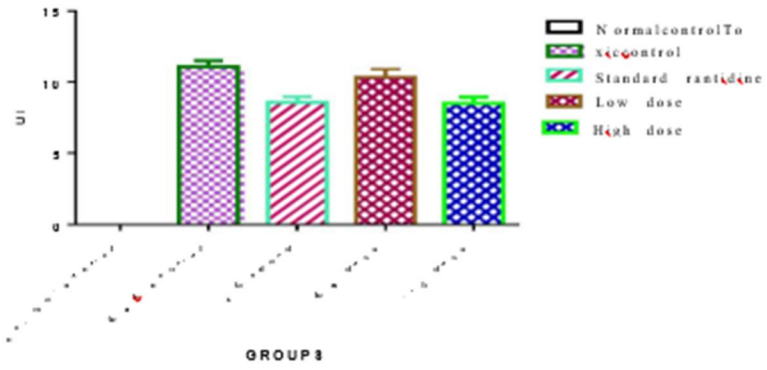


Fig 2:Effect of ethanolic extract of Beta vulgaris on ulcer index in aspirin induced gastric ulcer.

Table 4: Effect of ethanolic extract of Beta vulgaris on ulcer index and %ulcer protection in pylorus ligation induced gastric ulcer.

Groups (n=5)	Treatment	UI	% ulcer protection
I	Toxic control	11.99±0.6009	0.00
II	Standard	8.532±0.4944**	28.840
III	***Beta vulgaris*** 250 mg/kg	10.732±1.138ns	10.49
IV	***Beta vulgaris*** 400mg/kg	8.66±0.6667**	27.77

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.

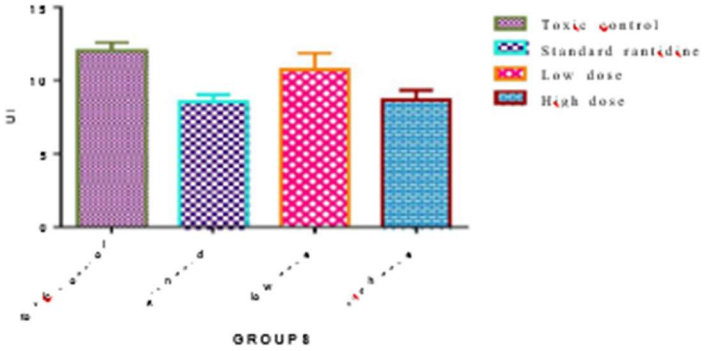


Fig 3:Effect of ethanolic extract of Beta vulgaris on ulcer index in pylorus ligation induced gastric ulcer.

Table 5: Effect of ethanolic extract of Beta vulgaris on gastric content volume

Groups	Treatment	Volume of gastric content
I	Control	3.2±0.1291
II	Standard	1.983±0.113**
III	***Beta vulgaris*** 250 mg/kg	2.433±0.233**
IV	***Beta vulgaris*** 400mg/kg	2.15±0.1945**

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

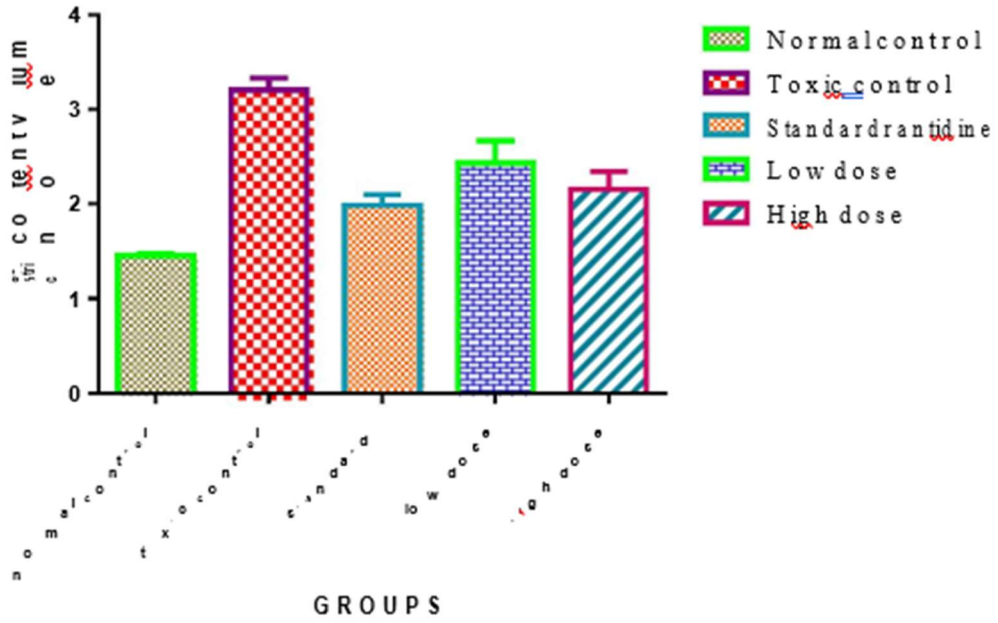


Fig 4:Effect of ethanolic extract of Beta vulgaris on gastric content

Table 6: Effect of ethanolic extract of Beta vulgaris on gastric juice volume

Groups	Treatment	Volume of Gastric juice
I	Control	2.133±0.1022
II	Standard	0.95±0.08466**
III	***Beta vulgaris*** 250 mg/kg	1.4±0.2817*
IV	***Beta vulgaris*** 400mg/kg	1.183±0.1327**

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

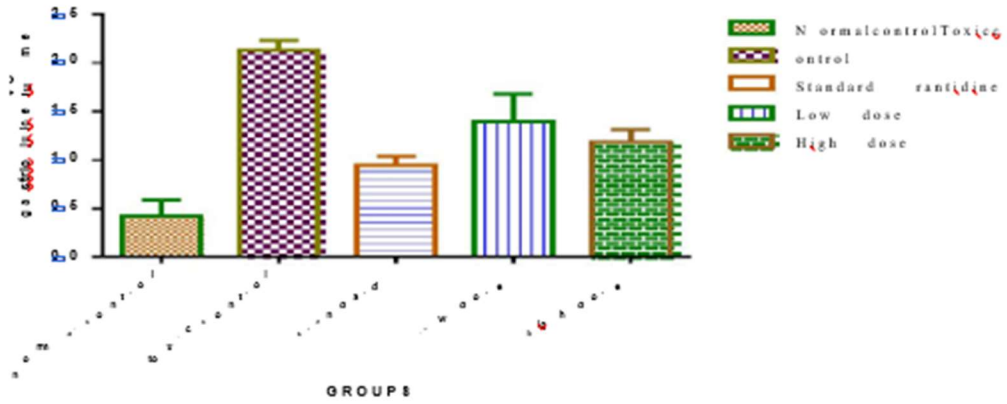


Fig 5:Effect of ethanolic extract of Beta vulgaris on gastric juice

Table 7: Effect of ethanolic extract of Beta vulgaris on gastric juice PH

Groups	Treatment	PH
I	Control	1.865±0.1018
II	Standard	4±0.1238**
III	*Beta vulgaris* 250 mg/kg	2.8±0.1932**
IV	*Beta vulgaris* 400mg/kg	3.46±0.1936**

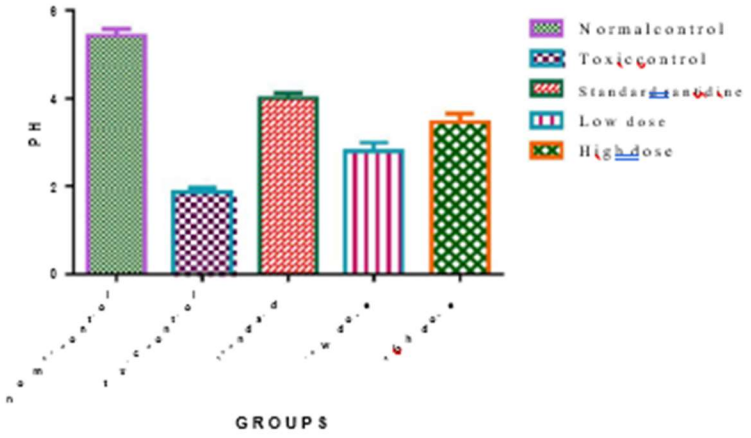


Fig 6:Effect of ethanolic extract of Beta vulgaris on gastric juice PH

Table 8: Effect of ethanolic extract of Beta vulgaris on total acidity

Groups	Treatment	Total acidity(mEq/lit)
II	Control	705±1.478
III	Standard	44.6±0.2186**
IV	***Beta vulgaris*** 250 mg/kg	162.5±1.315**
V	***Beta vulgaris*** 400mg/kg	52.8±0.2358**

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

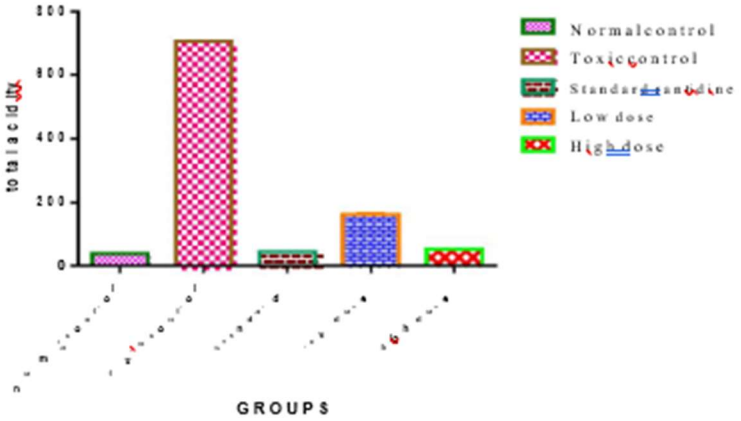


Fig 9: Effect of ethanolic extract of Beta vulgaris on total acidity

Table 9: Effect of ethanolic extract of Beta vulgaris on free acidity

Groups	Treatment	Free acidity(mEq/lit)
II	Control	283±2.171
III	Standard	24±0.1880**
IV	***Beta vulgaris*** 250 mg/kg	66.3±0.4447**
V	***Beta vulgaris*** 400mg/kg	21.76±0.2088**

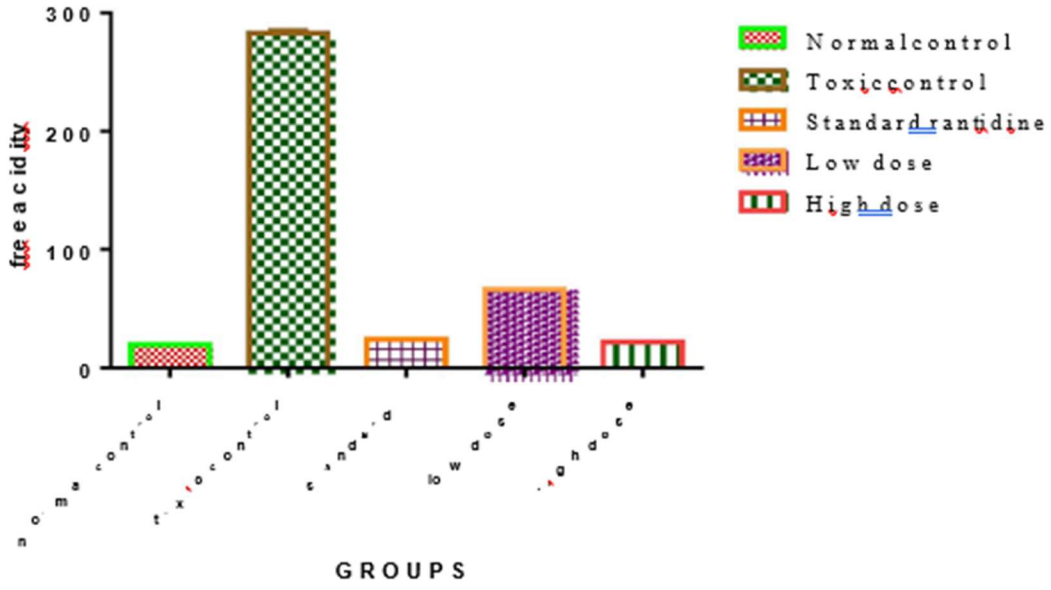


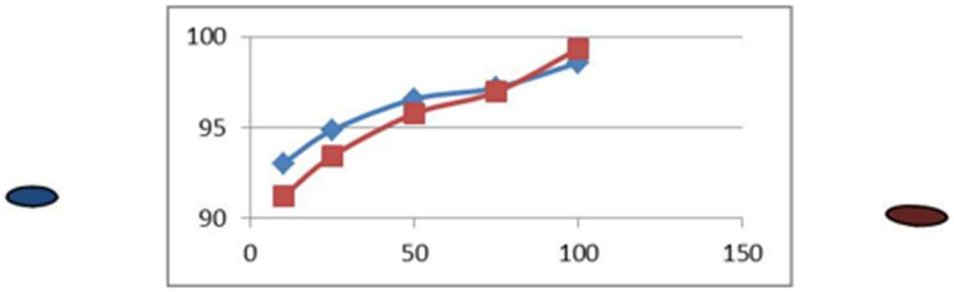
Fig 10: Effect of ethanolic extract of *Beta vulgaris* on free acidity

In Vitro Anti oxidant activity:

DPPH free radical scavenging activity:

Table 10: Showing % scavenging activity of Ascorbic acid and EEBV

Concentration(µg/ml)	% Scavenging activity	
	Ascorbic acid	**EEBV**
10	93.05	91.23
25	94.87	93.45
50	96.58	95.78
75	97.2	96.98
100	98.58	99.34
IC_{50} (µg/ml)	**43.137 µg/ml**	**41.024 µg/ml**



DISCUSSION:

The anti ulcer activity of beta vulgaris was evaluated by employing aspirin, alcohol, and pylorus ligation induced ulcer models. These models cause the gastric ulcer in humans. Many factors and mechanisms are involved in the ulcerogensis and gastric mucosal damage. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. The ethanol-induced ulcers is predominant in the glandular part of stomach and was reported to stimulate the formation of leukotriene C4 (LTC4), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa. Alcohol rapidly penetrates the gastric mucosa causing cell and plasma membrane damage leading to 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 [104]. The beet root extracts shows protection in dose dependent manner against characteristic lesions produced by ethanol and reduced values of ulcer index as compared control group suggesting its potent cytoprotective activity.

Aspirin causes mucosal damage by decreasing cytoprotective prostaglandin levels through inhibition of PG synthesis and also results in back diffusion of H+ ions into the gastric mucosa and inhibits the release of mucus [105]. In this model ethanolic extract of beta vulgaris was produced its ulcer protective effect by counteracting the inhibition of PG synthesis and enhancing the mucus release. Beet root extract was significantly reducing the ulcer index compare to control group.

Pylorus ligation induced ulcer was used to study the effect of beet root extract on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The fasting of rats for 24 h followed by ligation of pyloric end of the stomach, the ulcer index is determined 4 h after pylorus ligation [4]. The lesions produced by this method are located in the lumen region of the stomach. The Ethanolic extract of beta vulgaris and ranitidine significantly decreased the total acidity and free acidity; and significantly enhance the PH; this suggests that it having an antisecretory effect. Its antiulcer activity is further supported by histopathological study shows that protection of mucosal layer from ulceration and inflammation. Pylorus ligation induced ulcer control rats shown perforated ulcer, deep ulceration of granular epithelium and almost reducing the sub-mucosa. The Ethanolic extract of beta vulgaris at 250 mg/kg dose has shown mucosal erosion, the partial healing of ulcer with few inflammatory cells

and the dose 400 mg/kg has shown the healed ulcer, normal mucosa and no inflammatory cells. Beet root extracts have been reported to possess antioxidant activity [106] and to contain various types of compounds such as flavonoids and polyphenolic compounds, saponins and tannins [107]. The gastroprotective effect exhibited by Ethanolic extract beta vulgaris is speculated to be attributed to its antioxidant property, which in turn could be linked to the presence of flavonoids and polyphenolic compounds, saponins and tannins [108]. These compounds most likely inhibit gastric mucosal injury.

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.

REFERENCES:

1.Poonam Dharmani , Vijay Kumar Kuchibhotla , Rakesh Maurya, Sudhir Srivastava, Sharad Sharma , Gautam Palit, Evaluation of anti-ulcerogenic and ulcer-healing properties of Ocimum sanctum Linn, Journal of Ethnopharmacology (2004), 93 , 197–206. 2.Jyoti Gupta1, Dinesh Kumar, Ankit Gupta, Evaluation of gastric anti-ulcer activity of methanolic extract of Cayratia trifolia in experimental animals, Asian Paicfic Journal of Tropical Disease (2012), 99-102.
3.Blaser, M.J. “Gastroenterology”. 1987, vol.93: 317-83.
4.Soll, A.H. and Berglindn, T. Physiology of isolated gastric glands and pareital cell; receptor and effectors regulating function “Physiology of the gastro Intestinal Tract, 2nd edition, 1987, 883-908.
5.Luis Bujanda, M.D., Ph.D.,The Effects of Alcohol Consumption upon the Gastrointestinal Tract, The American Journal Of Gastroenterology, 2000.Vol. 95, No. 12, 3374-3382.
6.Vikas Dhikav, Sindhu Singh, Swati Pande, Atul Chawla, Kuljeet Singh Anand, Non-Steroidal Drug-induced Gastrointestinal Toxicity Mechanisms And Management, Journal, Indian Academy of Clinical Medicine ,October-December 2003 , Vol. 4, No. 4 , 315-322.
7.Namiot, Kemona , Namiot Z, Smoking habit and gastritis histology, Advances in Medical Sciences · Vol. 52 · 2007,191-195.

8.Mitsuru Moriya1, Akira Uehara, Toshikatsu Okumura, Mitsuaki Miyamoto and Yutaka Kohgo ,Stress-induced hemorrhagic gastric ulcer after successful Helicobacter pylori eradication two case reports, , Journal of Medical Case Reports 2011, 5:252, pp1-3.
9.Mohammed Safwan Ali Khan, Syed Ahmed Hussain, Abdul Manan Mat Jais,Zainul Amiruddin Zakaria1 and Mohib Khan, Anti-ulcer activity of Ficus religiosa stem bark ethanolic extract in rats, Journal of Medicinal Plants Research February, 2011,Vol. 5(3), pp. 354-359.
10.http://www.zentiva.com/default.aspx/en/consumer s/glossary/group-indication
11.S.K.Gupta and I.J.Singhvi Herbal And Hepatoprotective Drugs Acting On Peptic Ulcer And Liver Disease:A Review, IJPT March-2011 ,Vol. 3 , Issue No.1 ,824-853
12.Marhuenda, E., Alarcon de la Lastra, C., Martin, M. J,Antisecretory and gastroprotective effects of aescine in rats. Gen.Pharmacol, (1994) ,25, 1213-1219.
13.http://www.botanical.com/botanical/mgmh/b/beetro28.html
14.Kanner, J.; Harel, S.; Granit, R. Betalains: a new class of dietary cationized antioxidants. J. Agric. Food Chem. 2001, 49, 5178–5185.
15.Kapadia, G. J.; Azuine, M. A.; Sridhar, R.; Okuda, Y.; Tsuruta, A.; Ichiishi, E.; Mukainake, T.; Takasaki, M.; Konoshima, T.;Nishino, H.; Tokuda,
H. Chemoprevention of DMBA-induced UV-B promoted, NOR-1-induced TPA promoted skin carcinogenesis, and DEN-induced phenobarbital promoted liver tumors in mice by extract of beetroot. Pharmacol. Res. 2003, 47, 141–148.
16.Ranju Pal1, Kundlik Girhepunje1, Hitesh Gevariya1, Thirumoorthy N. Hepatoprotective activity of Beta vulgaris against CCl4 induced acute hepatotoxicity in rats, Archives of Applied Science Research, 2010, 2 (1) 14-18
17.Swati Jain, Vipin Kumar Garg and Pramod Kumar Sharma, Anti-Inflammatory Activity of Aqueous Extract of Beta Vulgaris L. Journal of Basic and Clinical Pharmacy, Vol-002 Issue-002 May 2011 page no-83-86.
18.Anita Singh, Vipin Kumar Garg, Pramod Kumar Sharma, Surbhi Gupta, Wound Healing Activity OfEthanolic Extract Of Beta Vulgaris Pharmacologyonline 1: 1031-1038 (2011).
19.Jasna M. Canadanovic-Brunet, Sajama S. Savatovic, Gordana S. Cetkovic, Jelena J. Vulic, Sonja M. Dj ilas, Sinisa L. Markov and Dragoljub D. Cvetkovic Czech ,Antioxidant and Antimicrobial Activities of Beet Root Pomace Extracts J. Food Sci. 2011,Vol. 29, No. 6: 575–585.
20.ashok agarawal,sunil bavaskar,pankaj salunkhe,nadeem manyar,dnysheswar kale,pravin kawitkwar, Beta vulgaris: a dearth of anti depressant activity in mice. journal of pharmaceutical negative results,october 2010,vol 1,issue 2,55-57.
21.Aaishwarya Deshmukh1, Jayvadan Patel, Ashish
Prajapati, Mansi Vaghela, Hardik Goswami,Cerebroprotective effects of extract of Beta vulgaris (C.) in middle cerebral artery occlusion
(MCAO)-induced cerebral ischemia Phytopharmacology 2011, 1(5) 138-147.