An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANTI ULCER AND ANTI OXIDANT ACTIVITY OF *BAUHINIA PURPUREA* LINN. STEM BARK IN RATS

R.Radha*[1], A. Lakshmi Devi[1], B.Sitaram[2], R. Bhanu Murthy[1], D. Ranganayakulu[1], V.Shankarananth[1]

[1]*Department of Pharmaceutical chemistry, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati-517503, Andhra Pradesh, India.*
[2]*Sri Venkateswara Ayurvedic College, Tirupati, Andhra Pradesh, India.*

*Corresponding author E-Mail: radharayisree@gmail.com

ARTICLE INFO

Key words:

Antiulcer;Antioxidant; Lipid peroxidation; Superoxide dismutase; Catalase; Reduced glutathione

ABSTRACT

Objective: The aim of the present study was to determine the antiulcer and antioxidant activity of a methanolic extract of *Bauhinia purpurea* stem bark.

Materials and Methods: BPSME was administered at doses of 5, 50, 300 and 2,000 mg/kg and its effects on acute toxicity and absolute ethanol-induced gastric ulceration in rats was investigated.

Results: At a dose of 2,000 mg/kg, BPSME did not cause any signs of toxicity in rats when given orally. Oral administration of BPSME (250mg/kg & 500mg/kg) exerted anti-ulcer and antioxidant activity ($p < 0.05$) in ethanol-induced gastric ulcer model. The increase in the levels of superoxide dismutase, catalase and reduced glutathione and decrease in lipid peroxidation in the antioxidant activity of *B.purpurea.*

Conclusion: Thus it can be concluded that *B. purpurea* possesses antiulcer activity which can be attributed to its antioxidant mechanism.

INTRODUCTION:

Peptic ulcer is one of the most common gastrointestinal diseases. In recent years, a widespread search has been launched to identify new antiulcer drugs from natural sources. Peptic ulcer disease (PUD) is caused by disruption of gastric mucosal defense and repair system. The recurrence rates of PUD are high and have been associated with several factors, including persistent *Helicobacter pylori* infection, sustained presence of mucosal damaging factors (e.g. use of NSAIDs) and diminished mucosal defense ability.[1,2] Reactive oxygen species (ROS) which include superoxide anions and hydroxyl radicals have been implicated in several degenerative diseases including hypercholesterolemia, atherosclerosis, carcinogenesis, diabetes mellitus, ischemic reperfusion cardiac injury and digestive system disorders such as hypersecretion and gastric mucosal damage[3]. It has been shown that there is alteration in the antioxidant status following ulceration, indicating that free radicals seem to be associated with ethanol induced ulceration in rats[4]. Drugs with multiple mechanisms of protective action, including antioxidant properties may be one way forward in minimizing tissue injury in

human disease[5]. Herbal extracts and chemical constituents have a long history of traditional use for treating ulcers and animal studies have supported the efficacy of many herbs for the prevention and treatment of PUD [6]. *Bauhinia purpurea* is an ornamental plant of subtropical regions like India, Nepal and North and South America. In India, the habitat is the sub-Himalayan forest, northern India, Assam and Khasi hills. It belongs to the family Caesalpiniaceae and is commonly known as Raktakanchan and Khairwaal in folk and purple orchid tree in English[7]. The *B.purpurea* has been extensively used in Indian traditional and folklore medicine to cure various human ailments such as dropsy, pain, rheumatism, convulsions, wound healing, delirium and septicemia. The bark of the plant is used as an astringent and its decoctions are recommended for ulcers as a useful wash solution. Various pharmacological activities of *B. purpurea* have been reported like analgesic and anti-inflammatory[8], anti malarial, anti mycobacterial, antifungal and cytotoxic[9], cardiotonic[10,] hypo-lipidemic[11,] antioxidant [12], hepatoprotective[13], antidiabetic[14] and Nephro protective activities[15]. However, systematic and scientific reports on the investigation of methanol extract of stem bark of *B.purpurea* for its effects on protection against ethanol induced gastric ulceration model is scarce. In the present study, an effort has been made to evaluate the effect of the methanol extract of stem bark of this plant on ethanol induced gastric ulcer in rats and determined the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) , reduced glutathione (GSH) and the levels of lipid peroxidation (LPO) in the stomach tissues of all treated groups to check whether *B. purpurea* exerts anti ulcer action by means of its antioxidant activity.

2.MATERIALS AND METHODS

2.1. Plant collection

Bauhinia purpurea stembark was collected from the surroundings of Tirumala hills,Tirupati during the month of October. It was identified and authenticated by Dr.B.Sitaram, Professor, Sri Venkateswara Ayurvedic College, Tirupati. A voucher specimen (SPSP/2010/625) has been deposited in Pharmacognosy Dept, Sri Padmavathi school of Pharmacy, Tirupati for future reference.

2.2. Preparation of Extract

The plant material was washed, shade dried ,coarsely powdered and extracted with petroleum ether to remove the oily and fatty substances present in the drug. Then the drug was dried and was extracted with methanol. The extract was filtered and the filtrate was concentrated over water bath and dried in vacuum desiccators for further use. The yield of powdered stembark of *B.purpurea* was found to be 25% w/w.

2.3. Preliminary phytochemical screening

The methanol extract of *B. purpurea* was subjected to preliminary phytochemical investigation for the presence of secondary metabolites by using standard methods of analysis [16].

2.4. Drugs and Chemicals

All the reagents used were of analytical grade obtained from S.D. Fine chemicals Ltd.

2.5. Experimental Animals

Female albino rats of Wistar strain weighing 150-200g body weight were obtained from Raghavendra enterprises, Bangalore. Animals were maintained under standard laboratory conditions with normal dark and light cycles and room temperature. All procedures used in the study were reviewed and approved by the Institutional Animal Ethical Committee (Application approval No.1016/a/06/CPCSEA-015/2009).

2.6. Acute toxicity studies (LD_{50})

Healthy Female adult albino rats were divided into five groups, each consisting of 6 animals for assessment of acute toxicity studies for the plant extracts individually. The animals were fasted overnight and the plant extract in the doses of 5,50, 300 and 2000 mg/kg body weight were administered orally to the first 4 groups. One group which received vehicle (water) served as control. The animlas were observed continuously for 2 hours, then intermittently at the end of 24 hours to note the number of deaths, to calculate LD_{50} of the selected plant extracts. The parameters were observed in the acute toxicity studies included changes in skin and fur, eyes and

mucus membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration,urinary incontinence and defecation) and central nervous system (drowsiness, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks according to OECD 420 [17].

2.7. EXPERIMENTAL DESIGN

The animals were divided into five groups, each containing of six rats. Group I represented the normal group, which received distilled water orally. Group II received alcohol alone (1ml of absolute alcohol, *p.o*), Group III received Ranitidine 20mg/Kg body weight orally which is a reference standard drug. Group IV and V received the extracts of BPSME at the dose of 250mg/kg and 500mg/kg (*p.o)* body weight.

2.8. Ethanol-induced ulcer model

The method described by Robert (1979) was adopted [18]. Ethanol was administered 1hr after administration of single dose of plant extracts and Ranitidine (20mg/kg). The rats were euthanized by cervical dislocation 60 minutes later under an overdose of diethylether and the contents of the gastric juice in the stomach were aspirated. Later the stomachs were removed and kept immersed in saline for 5 min. Incisions of the stomach were performed along the greater curvature and linear haemorrhagic lesions in the glandular regions were observed. A pair of dividers was used to measure the length of all the lesions in the stomach. The length (mm) of each lesion was determined at 10x magnification and summed up per stomach. Ulcer index was the sum of length of all lesions for each stomach.The percentage ulcer inhibition was calculated by the following formula and the results were tabulated.

$$\% \text{ Ulcer protection} = \frac{\text{Ulcer Index in Control} - \text{Ulcer Index in Test}}{\text{Ulcer Index in Control}} \times 100$$

The stomach was then weighed and processed for antioxidant estimations.

2.9. Biochemical parameters

Superoxide dismutase (SOD) was determined by the method Mishra and Fridovich [19]. Catalase was estimated by Hugo Aebi [20]. Reduced glutathione was determined by Moron[21]. Lipid peroxidation / MDA formation was estimated by Slater and Sawyer [22].

Statistical analysis

The values were reported as mean ± SEM. The data were analysed by one way ANOVA followed by Dunnett's test and the significance was set at $P<0.05$.

3. RESULTS

3.1. Preliminary phytochemical screening

The preliminary phytochemical screening carried out on methanol extract of ***B.purpurea*** revealed the presence of phytoconstituents such as alkaloids, flavanoids, carbohydrates, glycosides, amino acids,saponins and tannins (Table - 1).

3.2. Acute toxicity studies

In acute toxicity studies, it was found that the animals were safe up to a maximum dose of 2000mg/kg body weight. There were no changes in normal behavioral pattern and no signs and symptoms of toxicity and mortality were observed.Hence the biological evaluation was carried out at the two doses i.e. 250 and 500 mg/kg body weight.

3.3. Effect of *B.purpurea* methanolic extract in ethanol induced ulcer model

Ethanol administration in rats (1ml/200g, *p.o*) for induced ulceration of the gastric mucosa of the control group, characterised by haemorrhagic gastric lesions. The methanolic extract of *B.purpurea* caused a reduction in the severity of these lesions induced by ethanol which was evident by a significant ($p<0.01$) reduction in the ulcer index and an increase in the percentage protection of ulcers when compared with the control group. The methanolic extract of *B.purpurea* showed a protection index of 59% at 250mg/kg and at 500mg/kg showed protection index of 64.5% respectively. The results were comparable to Ranitidine (20mg/kg) which reduced the ulcer index significantly (Table - 2).

3.4. Effect of *B.purpurea* methanolic extract on *in vivo* antioxidant studies in ethanol induced ulcer model

There is considerable evidence that oxidative stress has a challenging role in causing peptic ulcer due to generation of reactive oxygen species. Free radicals are generated as a result of cellular metabolism

and also due to imbalance between aggressive and defensive factors in the GIT.

Table 1: Preliminary phytochemical screening of the stem bark of *B.purpurea*

Phytoconstituents	Methanol Extract
Alkaloids	+
Amino Acids	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Gums & Mucilages	-
Proteins	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	-

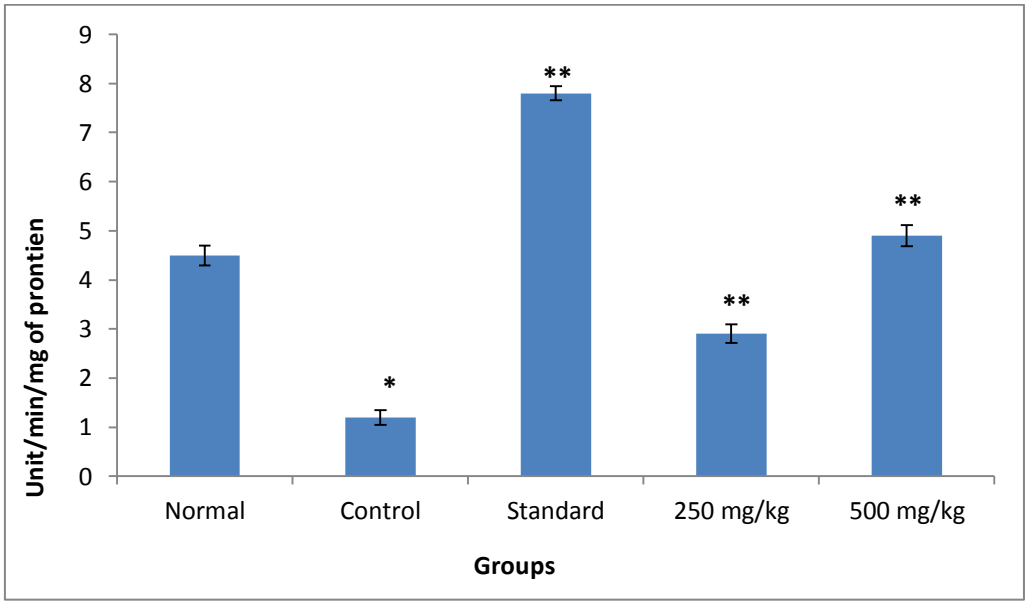
+ = present - = Absent

Table - 2: Effect of *B.purpurea* methanolic extract on ethanol induced ulcer model

S.no	Group	Ulcer index	% Ulcer Inhibition
I	Normal	---	---
II	Control (Ethanol)	52.8±5.02	----
III	Ranitidine 20mg/kg	10.4±0.135	80.3
IV	BPSME 250mg/kg	21.6± 0.187*	59.0
V	BPSME 500mg/kg	18.7±0.068**	64.5

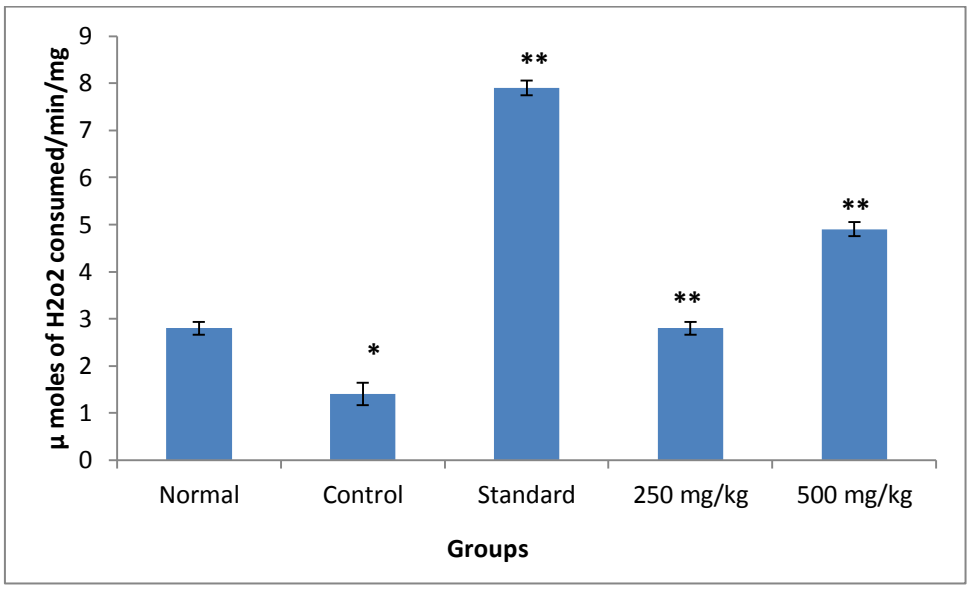
All values are shown as Mean ± SEM and n=6. *indicates p<0.05 when compared with control group, **indicates p<0.01 when compared with control group

Fig 3.4.1: Effect of BPSME on SOD levels in ethanol induced ulcer model



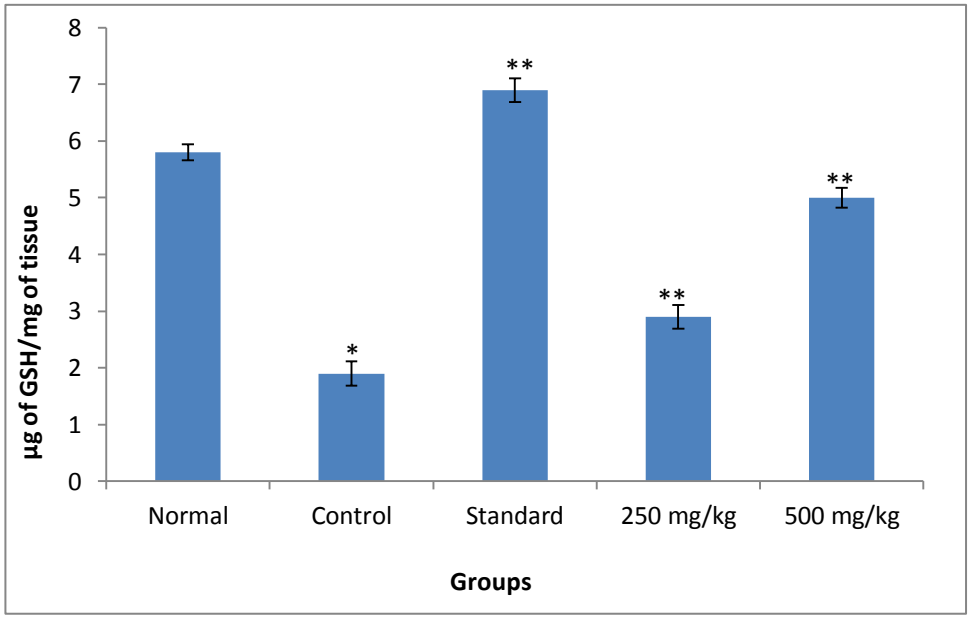
All values are shown as mean ± SEM and n=6. * indicates p<0.05 when compared with normal group, **indicates p<0.05 when compared with control group

Fig 3.4.2: Effect of BPSME on CAT levels in ethanol induced ulcer model



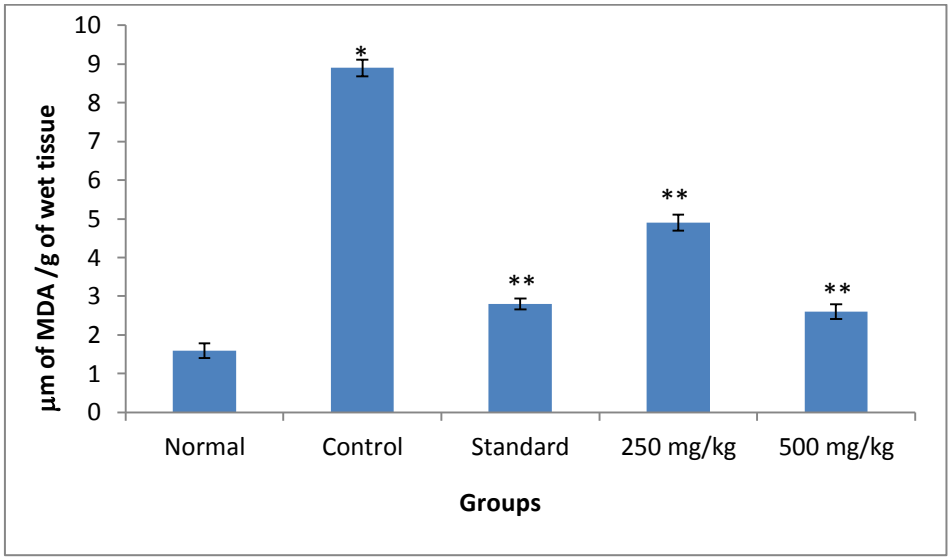
All values are shown as Mean ± SEM and n=6, * indicates p<0.05 when compared with normal group, **indicates p<0.05 when compared with control group

Fig 3.4.3: Effect of BPSME on reduced glutathione in ethanol induced ulcer model



All values are shown as Mean ± SEM and n=6.* indicates p<0.05 when compared with normal group, **indicates p<0.05 when compared with control group

Fig 3.4.4 : Effect of BPSME on lipid peroxidation in ethanol induced ulcer model



All values are shown as Mean ± SEM and n=6. * indicates p<0.05 when compared with normal group, **indicates p<0.05 when compared with control group.

As oxidative stress has a prominent role in the etiology of ulcer, the effect of plant extracts on the oxidative stress in EIU model was studied and the results are represented in the figures.

3.4.1. Effect of BPSME on SOD levels in ethanol induced ulcer model

Superoxide dismutase was estimated in rat stomach homogenate. The BPSME in 250mg/kg, 500mg/kg doses has shown significant (p<0.05) increase in the SOD (U/mg protein) content of the rat stomach when compared with the control group. The significant increase produced was in a dose dependent manner. The reference standard (standard SOD) also possessed significant (p<0.05) increase in SOD levels when compared with the control rats. The result also shown that the vehicle treated control group exhibited significant (p<0.05) decrease in the SOD levels when compared with the normal rats (Fig 3.4.1).

3.4.2. Effect of BPSME on CAT levels in ethanol induced ulcer model

The treatment with the BPSME in tested doses has significantly (p<0.05) increased the catalase (µmoles of H_2O_2 /min/mg protein) content in the rat stomach when compared with the control animals. The significance produced by the plant extracts was dose dependent manner whereas the reference standard (standard catalase) exhibited significant (p<0.05) increase in the catalase levels when compared with the control group.The vehicle treated control group exhibited significant (p<0.05) decrease in the catalase levels when compared with the normal animals (Fig 3.4.2).

3.4.3. Effect of BPSME on reduced glutathione in ethanol induced ulcer model

The enzyme reduced glutathione (µg/mg tissue) in the rat stomach was significantly (p<0.05) increased by the treatment of BPSME in tested doses when compared with the control animals. The significant (p<0.05) increase produced by the BPSME was dose dependent. The reference standard (standard glutathione) also possessed significant (p<0.05) increase in the reduced glutathione concentration when compared with the control group animals. The vehicle treated control group exhibited significant (p<0.05) decrease in the enzyme levels when compared with normal animals (Fig 3.4.3).

3.4.4. Effect of BPSME on lipid peroxidation in ethanol induced ulcer model

Lipid peroxidation (nm of MDA/mg tissue) was significantly ($p<0.05$) decreased in the stomach after treatment with the BPSME in tested doses when compared with the control animals. The reference standard, malondialdehyde also produced significant ($p<0.05$) decrease in the lipid peroxidation process when compared with the control group. The vehicle treated control animals exhibited significant ($p<0.05$) increase in the lipid peroxidation when compared with the normal animals (Fig 3.4.4).

4. DISCUSSION:

The present study showed that pre treatment with the methanolic extract of *B. purpurea* exhibited both gastroprotective and ulcer healing properties in rats, probably as a result of the antioxidant action of the drug. In most of the cases etiology of peptic ulcer is not clearly known, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanisms [23]. To regain the balance, different therapeutic agents including herbal preparations are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production.

The anti-ulcer effect of *B.purpurea* was tested against gastric lesions induced by ethanol, the experimental model related to lesion pathogenesis with production of reactive oxygen species. ROS are involved in the pathogenesis of ethanol – induced gastric mucosal injury *in vivo*. Results in the present study also indicate similar alterations in the anti-oxidant status after ethanol induced ulcers. Much attention has been recently focused on ROS contents, such as super oxide, hydroxyl radicals (OH•) and singlet oxygen[24]. ROS cause lipid peroxidation in membranes by attacking unsaturated fatty acids [25]. Therefore antioxidant defense system, including antioxidant enzymes, foods and drugs are important in the prevention of the toxic ROS effects [26,27]. Preventive antioxidants such as SOD and CAT enzymes are the first line of defense against reactive oxygen species. GSH is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation[28]. Administration of methanolic extract of stem bark of *B.purpurea* resulted in a significant increase in the SOD, CAT and GSH levels as compared to the control animals, which suggests its efficacy in preventing free radical induced damage. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease states. It involves the formation and propagation of lipid radicals, the uptake of oxygen and rearrangement of double bonds in unsaturated lipids. Biological membranes are often rich in unsaturated fatty acids. Therefore it is not surprising that membrane lipids are susceptible to peroxidative attack [29]. Similarly, the present study showed that there was a significant increase in lipid peroxidation in rat stomach tissues of control animals. However, significant decrease in lipid peroxidation was observed by the administration of the two doses of *B.purpurea* in ethanol – induced ulceration, which suggests its protective effect.

CONCLUSION

Thus the present study proved that stem bark of *B.purpurea* possess antiulcer activity, which may be due to its antioxidant mechanism of action. However, further work is warranted to identify the active constituent(s) responsible for therapeutic effect.

ACKNOWLEDGEMENTS

The authors are thankful to Dr**.** D. Ranganayakulu**,** the Principal and the management of Sri Padmavathi School of Pharmacy, Tiruchanoor, for providing necessary facilities to carry out the present research work.

REFERENCES

1. Hawkey CJ. (2000) : Non-steroidal anti-inflammatory drug gastropathy. *Gastroenterology* 119, 521-535.
2. Crespo A, Suh B. (2001) : *Helicobacter pylori* infection: epidemiology, pathophysiology and therapy. *Arch.Pharm. Res*. 24, 485-498.
3. Dhuley. (1999): Anti-oxidant effect of cinnamon (Cinnamomum verum) bark and greater cardamom (Amomum subulatum) seeds in rats fed with high fat diet. *Indian J. Exp. Biol*. 37, 238-242.

4. Pihan G, (1987): Free radicals and lipid peroxidation in ethanol or aspirin induced gastric mucosal injury. *Dig.Dis.Scs,*32, 1395-1401.
5. Barry H. (1991) : Antioxidant effects a basis for drug selection. *Drugs* 42, 569.
6. Borrelli, (2001): The plant kingdom as a source of anti-ulcer remedies. *Phytother. Res*. 14,581-591.
7. Khare CP (2007). Indian Medicinal Plants: An Illustrated Dictionary. New York: Springer.
8. Zakaria, (2007): Antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of *Bauhinia purpurea* leaves in experimental animals. *Med Princ Pract* ; 16:443-9.
9. Boonphong, (2007). Bioactive compounds from *Bauhinia purpurea J. Nat. Prod*. 70: 795-801.
10. Muralikrishna, (2008): Effect of *Bauhinia purpurea* Linn. on alloxan-induced diabetic rats. *Int. J. Green Pharm*. 2: 83-86.
11. Lakshmi, (2013): Antihyperlipidemic activity of *Bauhinia purpurea* extracts in hypercholesterolemic albino rats. *Int J PharmTech Res*; 3:1265-9.
12. Shajiselvin, (2013): *In-vitro* antioxidant potential of various extracts of Bauhinia purpurea (Linn). *Int J PharmTech Res*; 3:919-24.
13. Veena Rani, (2011): Evaluation of Hepatoprotective Activity of *Bauhinia purpurea* Linn, *International Journal of Research in Pharmaceutical and Biomedical Sciences,* 2(3), 1389-1393.
14. Guptha, *In-vitro* Antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. *Der Pharmacia Lettr* 4(2):614-619.
15. Lakshmi, (2009): Protective effect of *Bauhinia purpurea* on gentamicin-induced nephrotoxicity in rats. *Indian J Pharm Sci*; 71:551-4.
16. Harbone, (1973): Phytochemical methods, a guide to modern technique of plant analysis (chapmann and hall, London). pp.1-271.
17. OECD (2000). Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No.24.
18. Robert A, (1979): Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol,HCl, NaOH, hypertonic NaCl and thermal injury. *Gastroenterology* 77, 433-443.
19. Mishra.(1972) : The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem*.247, 3170-3175.
20. Aebi H (1984): Catalase *in vitro*. *Methods in Enzymology* 105:121-126.
21. Moron MS, Depierre JW, Mannervik B. (1979): Levels of glutathione, glutathione reductase and glutathione -S-transferase activites in rat lung and liver. *Biochem.Biophys. Acta* 582, 67-78.
22. Slater , (1971) : The stimulatory effect of carbon tetrachloride and other halogenoalkanes or peroxidative reaction in rat liver fraction *in vitro.Biochem. J.* 123, 805-814.
23. Piper DW, Stiel D (1986): Pathogenesis of chronic peptic ulcer, current thinking and clinical implications. *Med. Prog.,* 2: 7-10.
24. Ames, (1993): Oxidants,antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci.* 90, 7915-922.
25. Takeuchi, (1991): Oxygen free radicals and lipid peroxidation in the pathogenesis of gastric mucosal lesions induced by indomethacin in rats Relation to gastric hypermotility. *Digestion* 49,175-184.
26. Smith, (1988): Gastric ulcers: Role of oxygen radicals. *Crit. Care Med*. 16, 892-898.
27. Bafna. (2004): Anti-ulcer and antioxidant activity of DHC-1, herbal formulation. J. *Ethnopharmacol*. 90, 123-127.
28. Halliwell. (1995) Antioxidant characterization, *Biochem. Pharmacol.*49, 1341-1348.
29. Cheesman. (1993): Lipid peroxidation in biological systems, edited by Halliwell B, Aruoma, Ellis Horwood, London, 12-17.