ANTIULCER EFFECT OF ETHNOLIC LEAF EXTRACT OF *AVICENNIA OFFICINALIS*.

SUMAN SURA*, DR.ANBU J, SULTAN ALI BASHA. MD, UMA MAHESH.B

Department of Pharmacology, Dhanvanthri College of Pharmaceutical sciences, Mahabub Nagar-509002, Andhrapradesh, (INDIA)
E-mail:suman.sura@live.com

***Author for correspondence:**
Mr.Suman Sura,
Asst.Professor,
Dhanvanthri college of Pharmaceutical Sciences,
Mahabub Nagar-509002.
Mobile No: 09885037642.
E-mail Id:suman.sura@live.com

Summary

The leaves of *Avicennia officinalis* L. (Family: Acanthaceae) is used in folk medicine for treating ulcerative stomatitis, skin diseases, ulcers, wounds, etc. Anti ulcer activity of ethanolic extract of leaves of *Avicennia officinalis* L. was evaluated by employing aspirin + pylorus ligation (APL) and indomethacine induced acute gastric ulcer models in albino rats. The effect of ethanolic extract of leaves of *Avicennia officinalis* L. (250 and 500 mg/kg, b.w., orally) on the free acid, total acid and ulcer index in the aspirin + pyloric legated and indomethacine induced rats was studied for the assessment of antiulcer activity. There was a significant ($P < 0.01$) dose-dependent decrease in the ulcerative lesion index produced by aspirin + pylorus ligation (APL) and indomethacine induced acute gastric ulcer models in albino rats as compared to the standard drug Omeprazole (30 mg/kg, b.w. orally). The reduction in free acid, total acid in APL and indomethacine treated rats proved the antisecretory and potential antiulcer activity of leaves of *Avicennia officinalis* L

Key words: *Avicennia officinalis*, Ethanolic Extract, Aspirin +Pylorus Ligation, Indomethacine, omeprazole, Antiulcer Activity.

Introduction

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors[1]. Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitor to H antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, impotence, gynaecomastia, enterochromaffin-like cell (ECL), hyperplasia and haemopoeitic changes[2]. There are evidences for the participation of reactive oxygen species in the etiology and pathophysiology of human disease, such as neurodegenerative disorders, inflammation, viral

infections, autoimmune gastrointestinal inflammation and gastric ulcer[3]. Drugs with multiple mechanism of protective action, including antioxidant activity, may be highly effective in minimizing tissue injury in human diseases. It has been demonstrated that many drugs and formulations possess potent antioxidant action and are effective in healing experimentally induced gastric ulcers[4,5,6].

The genus *Avicennia* forms part of mangrove vegetation in the form of halophytic shrubs or small trees. This genus is known for the presence of iridoids having c-11 carboxylic acid group. In India three species of *Avicennia* are found. Of these *Avicennia officinalis* is of wide occurrence[7]. Coasts of southern Asia to Australia and oceania. From east Pakistan, Andaman island, and Srilanka through coasts of Vietnam, Thailand, Sumatra, Madura, Java, New guinea. It was showed the presence of alkaloids, phenolic acids, flavonoids, sterols and pentacyclic triterpenoids. There not much more data was found on its leaf, stem and root phytochemical analysis. Therefore *Avicennia officinalis* were investigated for its antiulcer activity.

Method

Plant material

The whole plant was collected near the sea area of Tamilnadu and was identified pharmacognostically by a botanist. The fresh leaves were collected and dried in shade, powdered, weighed and stored in a clean, dry and air tight container. The powder was subjected for successive extraction with ethanol.

Preparation of extract:

The Ethnolic extract of *Avicennia officinalis* (*ELEAO*) of leaf was prepared by using ethanol, by Soxhlet method at 70^0 C temperatures. The extract was concentrated by simple evaporation at room temperature. A suspension of *ELEAO* in 1%(w/v) Carboxy methyl cellulose was prepared for oral administration.

Pharmacological screening for antiulcer activity

Animal selection:

For acute toxicity studies, Wistar albino mice of either sex weighing between 25 and 30 g were selected and healthy adult male Wistar rats weighing 150-120 gms were selected for the antiurolithiatic study. The animals were acclimatized to standard laboratory condition with temperature 25±2ºC and fed with standard animal pellet feed (Hindustan lever limited) and water *ad libitum.* The protocol was approved by animal ethics committee constituted for the purpose of animal experimentation as per CPCSEA guidelines. (IAEC.Ref.No: 290/ CPCSEA/ 2009-PH/PCOL-08)

Acute toxicity studies:

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development-423 (OECD-423)[8] received from committee for the purpose of control and supervision of experimens on animals (CPCSEA). One-tenth of the median lethal dose (LD_{50}) was taken as effective dose[9].

Aspirin +Pyloric ligation model:

Ethanolic extract of *Aviciennia officinalis leafs(ELEAO),* aspirin and standard antiulcer drug, Omeprazole were prepared in 1% sodium Carboxy methyl cellulose (CMC) suspension as vehicle and administered orally once daily at a volume of 10 ml/kg body weight.

Procedure: In the present study albino wistar rats weighing about 150-200 gms were selected and divided into 6 groups containing 6 animals in each group. **Group-I** animals were treated with normal saline orally for 7 days[10]. **Group-II** animals were treated with omeprazole 30mg/kg for 7 days. Similarly **Group-III** and **Group-IV** animals were treated with 250 mg and 500mg of *ELEAO* for 7 days respectively. From days 5 to 7, animals of all the groups received aspirin orally as an aqueous suspension at a dose of 200 mg/kg, 2 h after the administration of respective drug treatment[11]. Animals in all the groups were fasted for 18h after the respective assigned treatment and were anaesthetized with anaesthic ether. Pyloric ligation was performed and pyloric ligation was done by ligating the pyloric end of stomach[12].After four hours of pyloric ligation the animals of all the groups were sacrificed and gastric contents were collected. The free acidity and total acidity was determined[13]. In addition, the ulcer index was determined by opening the stomach on greater curvature and the scores were given 0 to 3 depending upon the severity of ulcers.

Indomethacine induced ulcers:

Non-steroidal anti- inflammatory agents, like indomethacin and acetylsalicylic acid, induce gastric lesions in men and in experimental animals by inhibition of gastric cyclooxygenase resulting in less formation of prostacyclin, the predominant prostanoid produced in the gastric mucosa[14].

Procedure: In the present study albino wistar rats weighing about 150-200 gms were selected and divided into 6 groups containing 6 animals in each group. **Group-I** animals were treated with normal saline orally for 7 days. **Group-II** animals were treated with Omeprezole 30mg/kg for 7 days. Similarly **Group-III** and **Group-IV** animals were treated with 250 mg and 500mg of *ELEAO* for 7 days respectively. On 7th day thirty minutes after the administration of respective drug treatment animals of all the groups were administered indomethacine orally as an aqueous suspension at a dose of 40 mg/kg except Group-I animals[11] . Eight hours later, the rats were sacrificed and gastric contents were collected, and their stomachs isolated. The stomach were excised, cut along the greater curvature and gently rinsed under tap water. The stomachs were stretched on a corkboard and a magnifying glass (10X Magnification) used to spot and count ulcers.

Statistical Analysis:

The results are expressed as mean ± standard deviation (SD) differences in groups for gastric juice estimations. Statistical analysis was determined by one way analysis of variance (ANOVA), individual groups were compared using Dunnett`s test. ***P*** value **<0.001** has been considered as statistical significance level.

Results

Preparation of extracts:

Extract of *Avicennia officinalis* leaf was prepared by using ethanol, by Soxhlet method at 70^0 C temperatures. The extract was dried on water bath at 100^{0}C to get a solid mass. The dried and purified extracts were weighed and stored in air tight container. The percentage yield of extract was calculated as 8%.

Preliminary Phytochemical Analysis[14]:

Qualitative phytochemical studies were performed on extract using suitable chemicals and reagents to confirm the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, proteins, amino acids, phenolic compounds, flavonoids, triterpenoids and phytosterols. The result of qualitative phytochemical studies indicates the presence of Flavonoids, triterpenoids, tannins, sterols, carbohydrates, saponnins.

Acute toxicity studies:

The purified and completely dried extract of *ELEAO* was subjected for the acute toxicity study to determine the therapeutic dose using albino mice in controlled environment. Acute toxicity studies were performed according to the OECD 423 guidelines[8]. The extract was administered through oral route to different groups of mice using oral feeding needle (22gauge). No deviation from normal behavioral pattern was observed. But only few animals showed mild behavioral changes like dyspnoea and mild writhings in higher dose. Observation was done continuously for 13 days and mortality was not observed in any of the drug treated group, hence it was conformed that the test drug *ELEAO* is practically non toxic in normal mice and fall under the category of class V drug, according to [9], $1/10^{th}$ of dose was considered as therapeutic dose and the 50% of therapeutic dose was considered as minimum dose for further pharmacological evaluation in animal model.

Antiulcer studies:

Aspirin + Pyloric ligation:

The effect of the ethanolic extract on Aspirin +pyloric ligation induced ulceration was studied and the results reduced the free acidity produced by Aspirin + pyloric ligation with a volume of 25.0 ±2.1 and 22.3 ±1.6 with the dose of 250 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was shown free acidity of 17.3 ±1.8.

The effect of the ethanolic extract on Aspirin + pyloric ligation induced ulceration was studied and the results reduced the total acidity produced by Aspirin + pyloric ligation with a volume of 59.7 ±2.58 and 47.3 ±4.93 with the dose of 250 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was shown total acidity of 43.5 ±2.88.

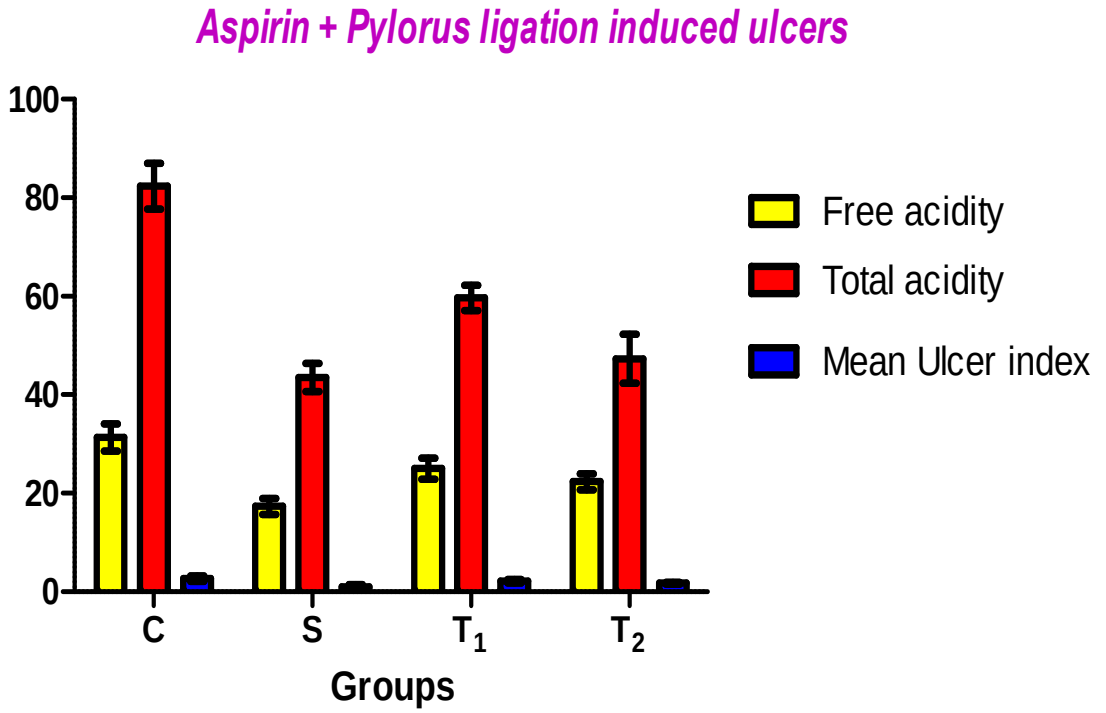
The effect of the ethanolic extract on Aspirin + pyloric ligation induced ulceration was studied and the results reduced the ulceration produced by Aspirin + pyloric ligation with a ulcer index of 18.7% and 34.4% with the dose of 250 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was shown reduction of ulcer 62.5%.

Indomethacine induced ulcers:

The effect of the ethanolic extract on Indomethacin induced ulceration was studied and the results reduced the free acidity produced by Indomethacin with a volume of 27.8 ±2.9 and 24.3 ±1.6 with the dose of 250 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was shown free acidity of 25.5 ±1.5.

The effect of the ethanolic extract on Indomethacin induced ulceration was studied and the results reduced the total acidity produced by Indomethacin with a volume of 58.0 ±3.4 and 52.5 ± 2.1 with the dose of 250 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was shown total acidity of 52.3 ± 3.2.

The effect of the ethanolic extract on Indomethacin induced ulceration was studied and the results reduced the ulceration with an ulcer index of 11.6% and 25.3% with the dose of 250 and 500 mg/kg respectively in comparison to control, Omeprazole as reference standard drug was shown reduction of ulcer 51%.


Aspirin + Pylorus ligation induced ulcers
100
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Groups
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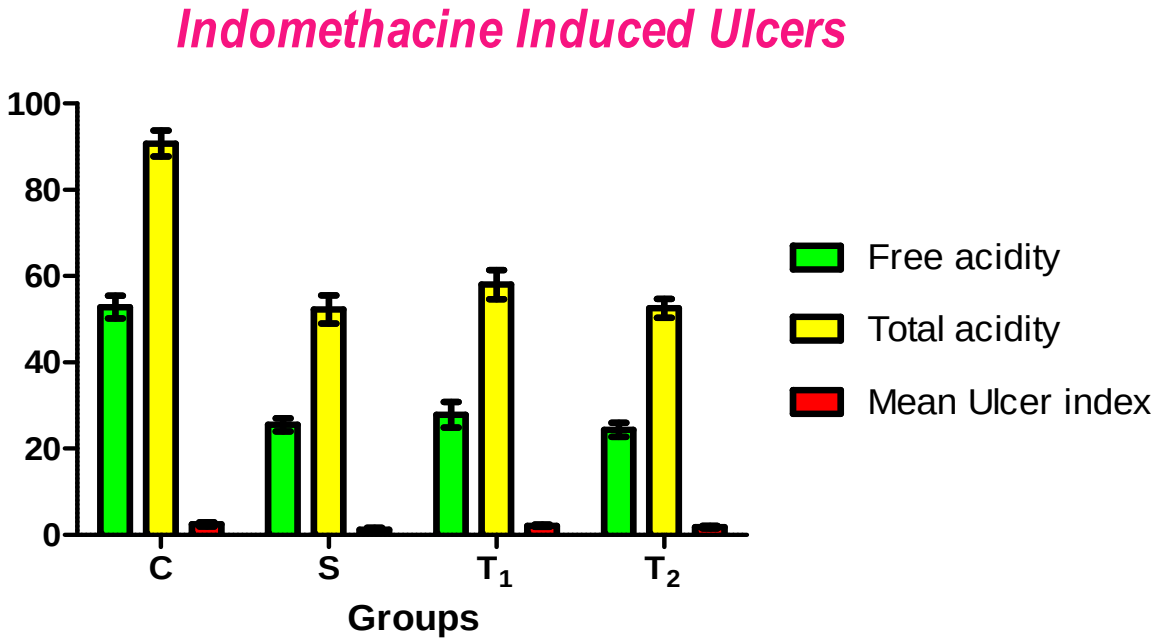
Mean Ulcer index


Indomethacine Induced Ulcers
100
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Groups
Free acidity
Total acidity
Mean Ulcer index

Table 1: Effect of *ELEAO* against Aspirin + Pyloric ligation induced gastric ulcers in rats.

S.No	Group & Drug Treatment	Free acidity	Total acidity	Mean ulcer index	% Inhibition
1	Control	31.3 ±2.8	82.3 ±4.63	2.67 ±0.51	__
2	Standard (Omeprazole 30mg/kg)	17.3 ±1.8***	43.5 ±2.88***	1.00 ±0.44***	62.5%
3	ELEAO (250mg/kg)	25.0 ±2.1***	59.7 ±2.58***	2.17 ±0.4	18.7%
4	ELEAO (500mg/kg)	22.3 ±1.6***	47.3 ±4.93***	1.83 ±0.25**	34.4%

All values are expressed as mean ±S.D for six rats in each group.

Comparisons made with control, Significant at *** P<0.001., One-way ANOVA followed by Dunnett's-t test.

Table 2: Effect of *ELEAO* against indomethacine induced gastric ulcers in rats.

S.No	Group & Drug Treatment	Free acidity	Total acidity	Mean ulcer index	% Inhibition
1	Control	52.8 ±2.6	90.7 ±2.9	2.41±0.49	__
2	Standard (Omeprazole 30mg/kg)	25.5 ±1.5***	52.3 ±3.2***	1.18 ±0.47***	51%
3	ELEAO (250mg/kg)	27.8 ±2.9***	58.0 ±3.4***	2.13 ±0.24	11.6%
4	ELEAO (500mg/kg)	24.3 ±1.6***	52.5 ±2.1***	1.8 ±0.3*	25.3%

All values are expressed as mean ±S.D for six rats in each group.

Comparisons made with control, Significant at *** P<0.001., One-way ANOVA followed by Dunnett's-t test.

Discussion

Results of this study shows a gastroprotection action of ELEAO as it was found effective against both models, i.e, ALP and indomethacine induced ulcers. Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, tannins, triterpenoids and steroids in *ELEAO*. Previous studies on tannins have been shown to possess antiulcer activity. The major active principles of the avicennia officinalis (mangrove) are polyphenols, represented in their majority by polymeric tannins and (80%) and hydrolysable tannins (20%) and catechin, chlorogenic, Gallic and elagic acids as well as cyclooxygenase 1 and 2. gallotannins, elagitannins and condensed tannins. These substances characterized by their polyphenolic nature, have shown cytoprotective properties[15] and have been. associated to antiulcerogenic activity in other plants[16,17]

The purified and completely dried yield of *ELEAO* was subjected for the acute toxicity study to determine the therapeutic dose using albino mice in controlled environment. Acute toxicity studies were performed according to the OECD 423 guidelines. The extract was administered through oral route to different groups of mice using oral feeding needle

(22gauge). No deviation from normal behavioural pattern was observed. But only few animals showed mild behavioural changes like dyspnoea and mild writhings in higher dose. Observation was done continuously for 13 days and mortality was not observed in any of the drug treated group, hence it was confirmed that the test drug *ELEAO* is practically non toxic in normal mice and fall under the category of class V drug, according to (Anupama and Handa.,1990). $1/10^{th}$ of dose was considered as therapeutic dose and the 50% of therapeutic dose was considered as minimum dose for further pharmacological evaluation in animal model.

Ulcers develop when the normal defense and repair mechanisms of the lining of the stomach or duodenum are weakened, making the lining more likely to be damaged by stomach acid. A peptic ulcer is a sore on the lining of the stomach, small intestine or esophagus. A peptic ulcer in the stomach is called a gastric ulcer. Different therapeutic agents including plant extracts are used to inhibit the gastric acid secretion, or to stimulate the mucosal defence mechanism by increasing the mucus production protecting the surface epithelial cells, or interfering with PG synthesis. Gastrointestinal injury is induced by various chemical agents. Thus the present investigation was carried out to evaluate the antiulcer activity of the ethanolic extract of *Avicennia officinalis* against different ulcer models.

Aspirin causes direct irritant effect and mucosal damage by interfering with prostaglandin synthesis[19], increasing acid secretion by increasing the H^+ion transport/backdiffusion of H^+ions, resulting overproduction of leukotrienes and other products of 5-lipoxygenase pathway[20]. The ethanolic extract significantly reduced the ulcer index and afforded significant protection against Aspirin induced ulcers could be due to prevention of direct irritation, increased mucus secretion and due to its 5-lipoxygenase pathway.

Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa[21]. A copious amount of mucus is secreted during superficial damage and provides favorable microenvironment in repair. Hence estimation of acid secretion is valuable part of the study to clarify the mechanism of action of drug under trial. In the present study, ethanolic extract of *Avicennia officinalis* has reduced the free acidity.

Indomethacin causes generation of reactive oxygen metabolites (such as superoxide anion, hydrogen peroxide and hydroxyl radical), which damages the gastric tissue and causes ulcer formation. The pathogenesis of gastric mucosal lesions by indomethacin is associated with increased lipid peroxidation. Reduced glutathione in the gastric mucosa acts as the major scavenger of the oxygen-derived free radicals[21]. *Avicennia officinalis* has preventive action on indomethacin induced ulcer in rats. It is possible that the antioxidant effect of *Avicennia officinalis* might also play a role in the mechanism of antiulcer activity.

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References

1. Hoogerwerf, W.A., Pasricha, P.J., 2006. Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease. In: Brunton, L.L., Lazo, J.S., Parker, K.L. (Eds.), Goodman & Gilman's The Pharmacological basis of therapeutics, 11th ed. McGraw-Hill Medical Publishing Division, New York, pp. 967–981.

2. Akthar, M.S., Akthar, A.H., Khan, M.A. Antiulcerogenic effects of Ocimum basilicum extracts, volatile oils and flavanoids glycosides in albino rats. International Journal of Pharmacognosy, 1992 30, 97–104.
3. Repetto, M.G., Llesuy, S.F. Antioxidant properties of natural compounds used in popular medicines for gastric ulcers. Brazilian Journal of Medical and Biological Research, 2002. 35, 523–534.
4. Dhuley, J.N. Protective effect of Rhinax, a herbal formulation against physical and chemical factors induced gastric and duodenal ulcers in rats. Indian Journal of Pharmacology, 1999. 31, 128–132.
5. George, S.,Sathiamoorthy, A., Sathiamoorty, S.S.. Effect of alpha tocopherol on gastric ulcers induced by pylorus ligation in rats. Indian Journal of Pharmacology, 1999 31, 431–433.
6. Goel, R.K., Sairam, K.. Anti-ulcer drugs from indigenous sources with emphasis on Musa sapientum, Tamrabhasma. Asparagus racemosus and Zingiber officinale. Indian Journal of Pharmacology, 2002 34, 100–110.
7. http://www.en.wikipedia.org/wiki/Avicennia_officinalis
8. OECD 423 guidelines, pg no: 1-14
9. Anupama S, Handa S.S.. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against CCl_4. Indian Journal Of Medical Research, 1990, 92, 276.
10. Goel, R.K., Gupta,S., Shankar,R., Sanyal,A.K. Antiulcrogenic effects of banana powder (musa sapientum var. paradisiacal) and its effect on mucosal resistance. Journal of Ethno pharmacology, 1986 18, 33-44.
11. Shay et.al Anti-ulcer actions of the bark methanol extract of *Voacanga africana* in different experimental ulcer models in rats. Journal of Ethno pharmacology, 2000, 73(2) 422-428.
12. Kulkarni S. K., 1997. Handbook of Experimental Pharmacology, 2nd ed., (Vallabh Prakashan, Delhi), 69.
13. Urushidani T, Kashuya Y and Okabe S. The mechanism of aggravation of indomethacin induced gastric ulcer by adrenalectomy in rats. Journal of Pharmacology, 1979, 29, 715-780.
14. Khandelwal, K.R. Practical Pharmacognosy, 11th ed. Nirali Prakashan, Pune, 2004, pp. 149.156.
15. Gonzales, E., I. Iglesias, E. Carretero and A. Villar. Gastric cytoprotection of Bolivian medicinaplants. Journal of Ethano pharmacology, 2000, 70: 329-333.
16. Konig, M., E. Scholz, R. Hartmann, W. Lehmann and H. Rimpler. Ellagitannins and complex tannins from *Quercus petraea* bark. Journal of Natural Products, 1994, 57: 1411-1415.
17. Ramirez, R.O. and C.C. Roa. The gastroprotective effect of tannins extracted from duhat (Syzygium cumini skeels) bark of HCL/ethanol induced gastric mucosal injury in Sprague-Diwley rats. Clinical Hemorheology and Microcirculation,2003, 29: 253-261.
18. Rasika D.Bhalke,Mahendra A.Giri ,Sneha et al., antiulcer activity of the ethanolic extract of leaves of Sesbania grandiflora (LINN.) International journal of pharmacy and pharmaceutical sciences, vol 2, suppl4, 2010.
19. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for asprin like drugs. Nat New Biol 1971;231:232-5.
20. Rao, Ch.V.,Sairam, K., Goel, R.K., 2000. Experimental evaluation of *Bacopa monniera* on rat gastric ulceration and secretion. Indian Journal of Physiology and pharmacology, 44,35-41.
21. Goel RK, Bhattacharya SK. Gastroduodenal mucosal defense and mucosal protective agents. Indian Journal of Experimental Biology, 1991;29:701-14.