IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
January 2020 Vol.:14, Issue:3

Gastroprotective Effect of Hibiscus against Experimentally Induced Ulcer in Albino Rat Model

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

V. RAVI KUMAR[1], P.SAILAJA RAO*[2]

[1]V.Ravi Kumar, Associate Professor, M.N.R. College of Pharmacy, Sangareddy, Telangana State, India.

[2]Dr. P Sailaja Rao, Associate Professor, Department of Pharmacology, S V College of Pharmacy, Hyderabad, Telangana State, India.*

Submission: 23 December 2019
Accepted: 29 December 2019
Published: 30 January 2020

Keywords: Anti-ulcer, ethanolic, *Hibiscus tiliaceus*, Ranitidine, gastric lesion

ABSTRACT

The present study was designed to investigate the anti ulcer activity of ethanolic extract of whole *Hibiscus tiliaceus* plant on animals. Ethanolic extract of *Hibiscus tiliaceus* was tested for acute toxicity studies on female rats at the dose of 2000 mg/kg body weight *p.o.* The working dose was decided as 1/10th of acute dose i.e., 200 mg/kg body weight, second dose selected was double dose of 1/10th i.e. 400 mg/kg body weight. The animals were divided into five groups in which each group contain six animals (n=6). First group was served as normal control group, second group as a disease control (ulcer induced by aspirin at a dose of 200 mg/kg), third group as a standard treated (Ranitidine, 150 mg/kg), fourth and fifth groups were of 200 mg/kg and 400mg/kg respectively. In the phytochemical constituent screening, Ethanolic extract of *Hibiscus tiliaceus* showed the presence of alkaloids, steroids, flavonoids, triterpenoids, tannins and phenolic compounds, saponins, glycosides. The data obtained were analyzed by One-way ANOVA followed by Dunnett Multiple Comparisons. The results obtained from anti-ulcer activity explained that the ethanolic extract of *Hibiscus tiliaceus* showed a significant anti-ulcer effect for dose 400 mg/kg body weight. It was concluded that the test drug possesses better and significant effect in alleviating the ulcer.

INTRODUCTION

Globally, peptic ulcer is serious public health problem that affects about 5-10% of population, with high rate of morbidity and mortality; need to be focused on experimental and clinical investigations [1]. Usually, peptic ulcers are aggravated by an imbalance between destructive and defensive factors in the stomach [2]. The etiological factors responsible for gastric ulcers are damage to the gastrointestinal mucosa which may be due to smoking, stress, alcohol consumption, prolonged ingestion of Non-steroidal anti-inflammatory drugs (NSAIDs), hypersecretion of gastric acid, ischemia of gastric mucosa, gastric secretions and especially infection by *Helicobacter pylori*. In the treatment of peptic ulcers, control over the acidic hypersecretion is critical, and the conventional drugs chosen have direct effects on the gastric mucosa [3]. The two main classes of drugs used to treat acid-related disorders include proton pump inhibitors (PPI) that inhibit the hydrogen pump in the parietal cell directly, independently of any membrane receptor stimulation, and histamine type 2 receptor antagonists (H2RAs), which block the histamine receptor on parietal cells thereby reducing hydrogen ion release. Long-term use of H2RAs is associated with the development of undesirable effects such as gynecomastia and galactorrhea as well as alteration of the bacterial flora of the gastrointestinal tract [4].

The target of treating peptic ulcer disease is to relieve pain, heal and prevent recurrence of ulcer. Currently, there is no cost-effective treatment that meets the target; hence, efforts are compelled to find a suitable treatment from natural product sources. In pre-clinical investigation, ulcers are induced by NSAIDs, ethanol, cold-restraint stress, pylorus ligation, as well as erosive agents. In each animal model, the therapeutic efficacy was found to be distinct which depended on the preparation and utilization of herbal medicines. Aspirin is a potent nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases [5]. Gastric ulcer associated with the use of aspirin is a major problem. Amongst different models, Aspirin-induced gastric ulcer model have been used widely to evaluate the efficacy of anti-ulcer agents.

Hibiscus tiliaceus Linn is a plant which belongs to the family Malvaceae, commonly known as “bola” exists as herb, shrub and around 250 species of same genus were found in tropical and subtropical regions of the world, out of which out 40 species occur in India. Since ancient times Hibiscus spps were used as folk remedies for various disorders [6]. In folk medicine,

the leaves of this plant used to treat fevers, soothe coughs, ulcer, wounds and various skin diseases. The various phytochemicals isolated from plant are hibiscus, hibiscus amide, vanillic acid, Phydroxybenzoic acid, syringic acid, P-hydroxybenzaldehyde, scopoletin, N-transferuloyltyramine, N-cis-feruloyltyramine, β-sitosterol, stigmasterol, β-stigmasteronone, hibiscolactone, hibiscones, hibiscoqinones, lapachol, gossypol, gossypetin, manosononones, hyperoside, kaempferol, quercetin, gossypetin, gossytrine, para-coumaric and fumaric acid [7].

Considering the use of this species in ethnomedicine, as an analgesic, anti-inflammatory and antioxidant agent, the present study investigated the anti-ulcerogenic activity of the whole plant of *Hibiscus tiliaceus*.

MATERIALS AND METHODS

Collection of Plant Material:

The whole plant of *Hibiscus tiliaceus* was collected from the local market in Kukatpally, Telangana, India which was identified and authenticated by Prof. Suresh babu, Dept. of Botany, Govt. Degree College, Kukatpally.

Preparation of Extract: [8]

The whole plant species were collected and then dried under shade for a period of three weeks. The dried plant material was milled to a fine powder using commercial laboratory blender and were stored in airtight containers until extraction.

Maceration was a simple widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container. Simple maceration was performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio 1:5 or 1:10), ethanol was used as a solvent. It was mixed for several days with occasional shaking or stirring. The extract is then repeated from the plant particles by straining. The process is repeated for once or twice with fresh solvent. Finally, the last residue of extract is pressed out of the plant particles using a mechanical press or a centrifuge. Kinetic maceration differs from simple one by continuous stirring. The method is suitable for both initial and bulk extraction. Thus, the pulverized plant was soaked in ethanol in a separate container and was kept at room temperature for about 5 days. The residue of the extract is pressed out of the

plant particles by vacuum filtration using a Buchner funnel. Vacuum filtration is mainly used in order to dry the product in less time. The ethanol from the extract is removed by distillation and is recovered. Then the extract is stirred on a hot plate magnetic stirrer.

Drugs obtained from USV limited, Govandhi, Mumbai (Aspirin Delayed-Release tablets USP {Ecospirin-325}) and Glaxo Smith Kline (Zantac {ranitidine tablets}), Pfizer limited (Sertraline {Daxid}) and chemical used in the study were obtained commercially from Bros Scientifics, Tirupati, India.

Experimental Animals:

Wistar rats of either sex (180-200 g) were used for the pharmacological activities. They were kept in polypropylene cages at 25 ± 2 °C, with relative humidity 45-55 % under 12 h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were feed with standard animal feed and water *ad libitum.*

Preliminary Phytochemical Screening [9]

Ethanolic extract of *Hibiscus tiliaceus* (EEHT) was screened for the presence of various phytoconstituents like alkaloids, flavonoids, steroids, tannins, glycosides, triterpenoids and saponins.

Tests carried out for different phytochemical constituents:

Ethanolic extract of *Hibiscus tiliaceus* (EEHT) was subjected to preliminary phytochemical investigations to identify various phytoconstituents present in whole plant according to the method.

1) Test for alkaloids: A small portion of crude extract was dissolved in 5 ml of 1% hydrochloric acid, filtered and tested with Dragendorff's reagent and Mayer's reagent separately. Any precipitate or turbidity with the reagents suggested the presence of alkaloids.

2) Test for flavonoids: A few drops of conc. hydrochloric acid and 1-2 magnesium turnings were added to 1 ml of methanolic extract. The presence of flavonoids was indicated by the development of pink or magenta-red colour.

3) Test for phenols (Ferric chloride test): A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black color.

4) Test for amino acids and proteins (1 % ninhydrin solution in acetone): 2 ml of filtrate was treated with 2-5 drops of ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple color.

5) Test for carbohydrates (Molisch test): To a fraction of extract α-naphthol and alcohol was added. It was mixed well and conc. sulphuric acid was added drop by drop by keeping the test tube in inclined position. Violet ring is formed at the junction of two layers which shows the presence of carbohydrates.

6) Test for saponins (Foam test): To 2 ml of extract was added 6 ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirmed the presence of saponins.

7) Test for sterols (Liebermann-Burchard test): 2 ml of extract was treated with drops of chloroform, acetic anhydride and conc. H_2SO_4 and observed for the formation of dark pink or red colour.

8) Test for tannins (Braymer's test): 2 ml of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Acute Toxicity Studies of METS [10]

Acute toxicity studies were performed according to the OECD 425 guidelines.

Female Wister rats weighing 100-150g were selected and divided into four groups containing three animals in a group. Depending on the mortality or morbidity of animals a few steps may be necessary to judge the toxicity of the test substance. Minimal usage of animals while it allows for acceptable data is the main advantage of this method. The single dose of the ethanolic extract starting from 5mg/kg up to 2000mg/kg (5, 50, 300, 2000mg/kg) was administered orally. The starting dose of the ethanolic extract of *Hibiscus tiliaceus* L was 2000mg/kg (p.o). The drug treated animals were carefully observed individually for the toxicity signs and mortality up to 14 days.

Animals: Animal Protocol was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for Purpose of Control and Supervision of Experimentation on Animals) through its reference no: IAEC/SVCP/2018/002, Dated: 27/2/18. Male Wistar rats, weighing (180-250 gms) were obtained from NIN (National Institute of

Nutrition, Hyderabad. The animals were acclimatized to the experimental room at a temperature of 23±2^0 C, controlled humidity conditions (50-55%) and 12 hr light and 12 hr dark cycles. They were fed with standard food pellets (Hindustan Lever, Hyderabad) and water *ad libitum*.

Experimental protocol:

Albino rats of either sex weighing between 160-250 gms were divided into five groups of six rats each (n=6).

Group I: Control (Saline)

Group II: Aspirin 200 mg/kg

Group II: Standard (Ranitidine 150mg/kg)

Group III: Ethanolic extract of (200mg/kg)

Group IV: Ethanolic extract (400mg/kg)

Statistical Analysis:

The statistical analysis is carried out using analysis of variance (ANOVA), followed by Dunnett's test. P values < 0.05 considered as significant (Jayachandra Reddy P *et al.*, 2012).

Histopathological Studies:

One animal of each group was euthanized at the end of experiment. Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The specimens were then embedded in paraffin, sectioned and stained with hematoxylin and eosin, before being evaluated by light microscopy. Open excised stomach was also observed for histopathological study.

Antiulcer Activity -Aspirin Induced Ulcer:

Aspirin is a NSAID which inhibit the synthesis of prostaglandins. Prostaglandins protect the gastric mucosa by producing leukotrienes and bicarbonate ions. Aspirin also inhibit the gastric peroxidase and may increase mucosal hydrogen peroxide and hydroxyl ions levels ultimately leading to oxidative mucosal damage [11, 12].

The animals are fasted for 24 hrs. The test drug in varying concentrations based on the design of the experiment was administered orally in 2% gum acacia solution 30 minutes prior to aspirin (200 mg/kg) suspended in 3 mL of 1% carboxymethylcellulose in water. 4 hours later the rats are sacrificed by using anesthetic ether. Their stomachs dissected and they were opened along greater curvature for the determination of gastric lesions. Ulcer index was calculated by noting the number of ulcers per animal and severity scored was observed the ulcers microscopically with the help of 10X lens.

Evaluation of parameters: [13]

❖ **Collection of gastric juice:**

The stomach was excised carefully opened along the greater curvature and the gastric contents were removed. The gastric contents were collected in plain tubes and centrifuged at 3000 rpm for 5mins; the volume of the supernatant was expressed as ml/100gm body weight. The mucosa was flushed with the saline and observed for gastric lesions using a dissecting microscope, ulcer score was determined.

❖ **Ulcer scoring:**

After sacrificing the rat, stomach was removed and opened along the greater curvature and washed slowly under running tap water. Then keep it on the glass slide and observed under 10X magnification for ulcer. Score the ulcer as below.

- 0 - Normal stomach
- 0.5 - Red coloration
- 1 - Spot ulcers
- 1.5 - Hemorrhagic streaks
- 2 - Ulcer >3mm but <5mm
- 3 - Ulcers >5mm

Mean ulcer score for each animal was expressed as ulcer Index.

❖ **Free acidity and Total acidity:**

One ml of gastric juice was pipette in to a 100 ml conical flask and titrated with 0.01N NaOH using topfers reagent as an indicator. It is Dimethyl-amino-azo-benzene with phenolphthalein, used for the detection and estimation of hydrochloric acid and total acidity in gastric fluids. The endpoint was to orange color. Noted the volume of NaOH which responded, and continued with titration further till the solution regained its pink colour. Noted the volume of NaOH which corresponded to the free acidity. Acidity (mEq/L/100g) was be expressed as –

Acidity = Volume of NAOH × Normality of NAOH/0.1×100

RESULTS AND DISCUSSION:

Percentage yield of plant extract:

The yield obtained from the ethanolic extract of *Hibiscus tiliaceus* L. was about 50 g which was obtained from 1000 g stock powder.

% Yield =Actual yield/Theoretical yield X100

% Yield =50/1000 X 100

The percentage yield of extract=5%

Preliminary Phytochemical Studies:

The results of preliminary phytochemical screening of the ethanolic extract of *Hibiscus tiliaceus* L. were given in table no. 1. It showed the presence of Flavonoids, Glycosides, Tannins, Saponins and Triterpenoids.

Table No. 1: Preliminary screening for phytochemical constituents in ethanolic extract of *Hibiscus tiliaceus* L.

Sr. No.	Tests for different chemical constituents	Absent/Present
1.	Test for carbohydrate	Absent
2.	Test for Alkaloids	Absent
3.	Test for Flavanoids	Present
4.	Test for Glycosides	Present
5.	Test for Triterpenes	Present
6.	Test for Tannins	Present
7.	Test for Saponins	Present
8.	Test for Proteins	Absent

Table No. 2: Effect of test extracts on gastric mucosal factors in experimentally induced gastric ulcer in rats

Groups	**Treatment**	**Vol of gastric juice**	**pH**	**Free acidity**	**Total acidity**
I	Control	1.69±0.09	5.50±0.09	14.33±2.57	27.38±0.25
II	Control (Aspirin 200 mg/kg)	3.83±0.16*	2.17±0.21*	24.5±1.2*	41.5±1.8*
III	Aspirin+ Ranitidine	1.83±0.14**	5.83±0.24**	10.4±1.8**	18.71±2.1**
IV	Aspirin+Ethanolic extract of *Hibiscus tiliaceus* (200 mg/kg)	2.42±0.21**	4.61±0.31**	16.83±1.4**	25.47±1.6**
V	Aspirin+Ethanolic extract of *Hibiscus tiliaceus* (400 mg/kg)	2.13±0.17**	5.47±0.23**	13.3±1.6**	20.6±2.0**

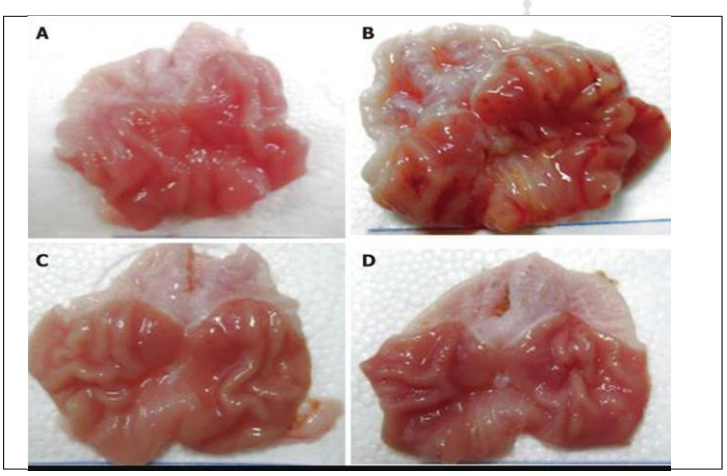
Values were showed as the mean ±SEM. *p < 0.05 compared with the control rats. *p < 0.001 compared with the aspirin group.

Table No. 3: Effect of test extracts on ulcer index in experimentally induced gastric ulcer in rats

Groups	Treatment	Ulcer Index	% protection
I	Control	--------	---------
II	Control (Aspirin 200 mg/kg)	6.17± 0.62	---------
III	Aspirin+ Ranitidine	1.72±0.23*	72.12
IV	Aspirin+Ethanolic extract of *Hibiscus tiliaceus* (200 mg/kg)	2.75±0.35*	55.35
V	Aspirin+Ethanolic extract of *Hibiscus tiliaceus* (400 mg/kg)	2.08±0.24*	66.30

Values were showed as the mean ±SEM. *p < 0.05 compared with the aspirin and aspirin + standard rats.

1

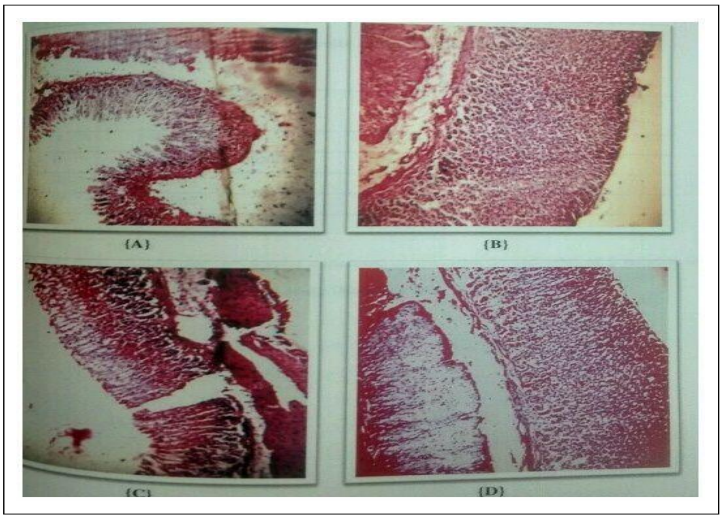


A: Gastric lesions induced by Aspirin (200 mg/kg)

B: Absence of gastric lesions in Ranitidine (150 mg/kg)

C: Fraction inhibition in gastric lesions at 200mg/kg of EEHT

D: Inhibition in gastric lesions at 400mg/kg of EEHT



A: Aspirin (200 mg/kg) damaged mucosal epithelium was observed

B: In Ranitidine (150 mg/kg) treated, no damaged to epithelium was observed

C: In EEHT (200mg/kg) treated, apparent epithelisation was observed

D: In EEHT (400mg/kg) treated, apparent

Figure No. 1: explains - I) Open excised stomach in aspirin induced gastric lesions model,

II) Histopathological examination of open excised stomach in aspirin induced ulcer model

Aspirin induced gastric ulcers:

Aspirin is a non-steroidal anti-inflammatory drug which induces ulcers by inhibiting prostaglandin synthesis in the stomach by blocking the cyclooxygenase enzymes [14, 15]. Non-steroidal anti-inflammatory drugs also cause an inflammatory response increasing the reactive oxygen species in the gastric mucosa. Previous studies have shown that the leaves of *H. tiliaceus* L possess reactive oxygen species scavenging activity, suggesting the role of anti-oxidation as one of the mechanisms responsible for its gastroprotective action [16, 17].

In the present study, the anti-ulcerative effects of ethanolic extract of *Hibiscus tiliaceus* was investigated in aspirin-induced gastric ulcer rat model.

- **Effect on gastric volume:**

Administration of the test extract at both the doses (200 and 400 mg/kg) significantly decreased (*$p < 0.05$, *$p < 0.001$) the gastric volume in comparison with control Aspirin rats and rats treated with ranitidine. Also, there was a significant increase in pH in both the extract

treated animals (200 and 400 mg/kg), and it was more prominent with the higher dose.

- **Effect of Free acidity and Total acidity:**

The free acidity and total acidity was determined based on the titer values. The free acidity and total acidity in extract treated rats was (*p < 0.001) decreased significantly in comparison with the standard group treated with ranitidine.

- **Ulcer index:**

The ulcer index was calculated by taking the mean ulcer score for each group. It was noticed that the ulcer index of dose groups treated with extract (200 and 400 mg/kg) was significantly less (*p < 0.05) when compared to the standard group treated with Ranitidine. *H. tiliaceus* in two doses caused a significant reduction in the UI and an improvement in the PI, indicating a possible involvement of the prostaglandin pathway.

Aspirin induced gastric hemorrhagic ulcer formation:

The macroscopic findings of the opened excised stomach were shown in figure No. 1 (I) which emphasizes that gastric ulcers covered with coagulated blood were more evident in the aspirin control group. There was inhibition of gastric ulcer with the extract treated group at the dose of 400 mg/kg. Secondly, the histopathological findings of gastric mucosa in aspirin treated rats revealed that there was an intact architecture, ulcers combined with distorted glands, a damaged epithelium, inflammatory exudates and cellular debris as showed in fig 1(II). In the test extract treated rats, there was an epithelialization which indicated a repair of wounded area. Epitheliazation occurred in proliferative phase of wound healing.

The etiology of peptic ulcer unknown in most of cases, yet it is generally accepted that it results from imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms. To regain the balance, phytomedicines are used (in experimental animals) to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucous production, stabilizing the surface epithelial cells or enhancing prostaglandin synthesis [16]. The present results demonstrated that the ethanolic extract of *Hibiscus tiliaceus* protected the rat gastric mucosa against hemorrhagic lesion produced by aspirin. This was a convenient way of screening plant extracts for anti-ulcer effect and cytoprotection in macroscopically visible lesions.

Hibiscus tiliaceus L. had significantly protected the gastric mucosa against aspirin challenge as shown by reduced values lesion as compared to control group suggesting its potent cytoprotective effect [18]. It showed a significant inhibition of gastric ulcer and also decreased mucosal parameters like acid concentration, gastric volume and increased the pH values. Hence, it was suggested that ethanolic extract of *Hibiscus tiliaceus* L. can suppress the gastric damaged induced by aggressive factors. The phytochemical screening of the extract of *Hibiscus tiliaceus* L. revealed the presence of flavonoids, tannins, glycosides, triterpenes and saponins [19]. Any of these metabolites may be responsible for the antiulcer activity of *hibiscus tiliaceus* L.

CONCLUSION

The extract showed protection against characteristic lesions produced by aspirin administration, and this antiulcer effect of ethanolic extract of *Hibiscus tiliaceus* was apparent from both reductions in gastric acid secretion and gastric cytoprotection. These findings thus prompt further necessary studies to elucidate the mechanism of action related to antiulcer activity at molecular level, by which more effective treatment for the disease can be achieved.

Conflicts of interest

The authors have no conflicts of interest with anyone.

REFERENCES

1) AlKofahi A, Atta AH., Pharmacological screening of the antiulcerogenic effects of some Jordanian Medicinal Plants in rats. J Ethnopharmacol. 1999; 65: 341-5.
2) Peskar BM, Maricic N. Role of prostaglandins in gastroprotection. Dig Dis Sci. 1998; 43:S23-9.
3) Toma W, Hiruma-Lima CA, Guerrer RO Souza AR., Preliminary studies of *Mammea Americana* L (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice, Phytomedicine. 2005; 12: 345-50.
4) Borelli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies, Phytother Res. 2000; 14: 581-91.
5) Zhongzhi Wang, Junichi Hasegawa, Xinhui Wang, Akiko Matsuda, Takahiro Tokuda, Norimasa Miura and Tatsuo Watanabe. Protective effects of Ginger against Aspirin induced gastric ulcers in rats. Yonaga Acta Medica. 2011; 54: 11-19.
6) Borhade PS, Dalal PS, Pachauri AD, Lone KD, Chaudhari NA, Rangari PK. Evaluation of anti-inflammatory activity of *Hibiscus tiliaceus* Linn wood extract. Int J Res Pharm Biomed Sci. 2012; 3(3):1246–1250
7) Sharma MC, Sharma S, Kohli D V. Phytochemical and anti-ulcer investigations of 95% ethanolic benzene-chloroform leaf extract of *Hibiscus tiliaceus* Linn. In albino rat model. Annals of Biological Research. 2010;1(1): 15-20.

8) Dhandapani R, Sabna B. Phytochemical constituents of some Indian medicinal plants. Anc Sci Life. 2008; 27(4):1–8

9) Kokate C K, Purohit AP, Gokhale S B. Text Book of Pharmacognosy. 33th Ed. *Nirali Prakashan*, 2001; Pune; 593-597.

10) Bruce. OECD guideline for testing of chemicals Acute Oral Toxicity- Up-and-Down Procedure, 2001: 1-26.

11) Al-Yahva MA, Rafatullah S, Mossa JS, Ageel AM, Parmar NS, Tariq M. Gastroprotective activity of Ginger, *Zingiber officinal* rocs, in albino rats. Am J Chin Med, 1989; 17:51–56.

12) Animal models in experimental gastric ulcer screening-a review. International Journal of Pharmacological Screening Methods. 2012; 2(2): 82-87.

13) Hollander D, Taranawski A, Krause WJ Gergely H. Protective effect of sucralfate against alcohol-induces gastric mucosal injury in the rat. Gastroenterology, 1985; 88: 366–374.

14) Maruthappan V, Sakthi Shree K. Antiulcer activity of aqueous suspension of Saraca indica flower against gastric ulcers in albino rats. Journal of Pharmacy Research. 2010; 3(1):17-20.

15) Kumar A, Dewan B, Rama T. Evaluation of anti-ulcerogenic properties from the root of *Flemingia strobilifera*. Journal of Basic and Clinical Pharmacy. 2011; 2(1); 33-39.

16) Thomas W, Hiruma-Lima CA, Guerrero RO, Brito AR. Preliminary studies of *Mammea americana* L. (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice. Phytomedicine. 2005; 12: 345-350.

17) Narender Kumar S, Kumar D, Kumar V. Antinociceptive and anti-inflammatory activity of Hibiscus tiliaceus leaves. Int J Pharmacogn Phytochem Res. 2009; 1(1):15–17.

18) Njoku OV, Obi C. Phytochemical constituents of some selected medicinal plants. Afr J Pure Appl Chem. 2009; 3(11):228–233.

19) Rosa RM, Melecchi MI, da Costa Halmenschlager R, Abad FC, Simoni CR, Caramao EB, Henriques JA, Saffi J, de Paula Ramos AL. Antioxidant and antimutagenic properties of *Hibiscus tiliaceus* L. methanolic extract. J Agric Food Chem. 2006; 54:7324–7330.