Investigation On The Pharmacological Properties Of Hibiscus Radiatus Leaf Extracts For Anti-Ulcer Activities

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Abstract
The shrub Hibiscus radiatus is a member of the Malvaceae family. Known by another name, monarch rose mallow, it's a crop that grows easily in multi-cropping systems and is useful for both food and fibre, making it a perfect crop for impoverished nations. All of the plant's components are chopped, boiled, and used to make liquid concoctions since it is thought to be a blood purifier and fever cure5. Alkaloids, glycosides, phenolic compounds, tannins, sterols, steroids, saponins, flavones, flavonoids, proteins, amino acids, and carbohydrates are among the chemical elements that may be in charge of the biological functions.
For acute toxicity investigations, CPCSEA guideline No. 425 was adopted by the OECD. Two models were used to study the antiulcer activity of several Hibiscus radiatus extracts: the pyloric ligation method and the ethanol-induced ulcer model using rats. Omeprazole was used as the standard medication. Significant action has been demonstrated by methanol and aqueous extracts; among these, the aqueous extract of Hibiscus radiatus has demonstrated a highly significant antiulcer activity. According to the current study, Hibiscus radiatus leaf extracts have demonstrated strong antiulcer properties.

INTRODUCTION

1.1: INTRODUCTION TO MEDICINAL PLANTS:

Traditional medicine is "The knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness".[1]
Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments, and even original plant medicines. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants. Contemporary science has acknowledged their active action, and it has included in modern pharmacotherapy a range of drugs of plant origin, known by ancient civilizations and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants as well as the evolution of awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life[-2].
Plants serve as an important source of medicine and play a key role in world health. Medicinal plants or herbs have been known to be an important potential source of therapeutics or curative aids. The utilization of medicinal plants has attained a commanding role in health system all over the world. This involves the use of medicinal plants not only for the treatment of disorders and diseases but also as potential material for maintaining good health and conditions. Many countries in the world, that is, twothird of the world's population rely on herbal medicine for primary health care. The reason for this is because of their great cultural acceptability, better compatibility and adaptability with the human body system and pose lesser harmful side effects. From the records, most of the used drugs contain plant extracts.
Some of them contain chief active ingredients (bioactive components or substances) obtained from plants. Through recent research, plant derived drugs were discovered from the study of therapeutic, curative traditional cures and most especially the folk knowledge of indigenous people and some of these claims and believe of people are irreplaceable in spite of the

recent advancements in science and technology. Some of the drugs believed to be obtained from plants are aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine and vinblastine.

Natural products derived from plants for the treatment of diseases have proved that nature stands a golden mark to show the relationship between the interrelationship between man and his environment. The researches and utilization of herbal medicine in the treatment of diseases increases every day. Before the development and civilization by the British in Nigeria, medicinal plants are believed traditionally to be value of the therapeutic agent for the treatment of diseases such as typhoid, cholera, measles, and gonorrhea.

However, the knowledge of herbal medicines for treatment of diseases is confined to mostly the practicing herbalists or plant scientists with the belief that herbal medicines will lose their potency if revealed to other people. Although some herbs may have medicinal values, sometimes the medicinal preparation inflicts certain side effects[3]. Plant components are characterized by their capability to prevent the development of certain disorders. The adverse effect of conventional and allopathic medicine has also been important factors in the sudden increased population demands and total increase in the number of the herbal drug manufactures as well as a reduction in the use of chemical drugs.

Knowing the history of any science is as effective as understanding and using that science thus, the historical significance of the past, present and future to medicinal herbs will be continued to be addressed. In view of this, the present study focuses on the knowledge on medicinal uses of plants and the scientific investigation to confirm their medicinal values, and thus among such plants *Hibiscus radiatus* is one traditionally used plant, which has reported to have traditional uses and was used to cure many disorders.[4]

1.2 : PLANT PROFILE:

Hibiscus radiatus belongs to the family malvaceae. The plant is believed to be a cure for fever and is considered a blood purifier and all its parts are cut and boiled, and the liquid preparations used. In view of this, the present study focuses on the knowledge on medicinal uses of plants and the scientific investigation to confirm their medicinal values, and thus among such plants *Hibiscus radiatus*is one traditionally used plant, which has reported to have medicinal properties and was used to cure disorders[5].

1.2.1 : SCIENTIFIC CLASSIFICATION:

Botanical name **:** *Hibiscus radiatus* Cav.

Synonym	**:** Hibiscus lindleyi, Canhamobraziliensis, Monarch rosemallow
Family	**:** Malvaceae
Kingdom	**:** Plantae
Class	**:** Magnoliopsida
Order	**:** Malvales
Genus	**:** Hibiscus
Species	**:** *Hibiscus radiatus*[6]

1.2.2 : MORPHOLOGY:-

Hibiscus radiatus Cav. also known as monarch rose mallow, is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi- cropping systems and can be used as food and fiber. The genus *Hibiscus* (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees. The plant is about 3m tall and has a deep penetrating taproot. It has a smooth or nearly smooth, cylindrical, typically dark green to red stems. Leaves are alternate, 7 to 11cm long, green with reddish veins and long and short petioles. Leaves of young seedlings and upper leaves of older plants as simple; lower leaves are deeply 3 to 5 or even 7 – lobed and the margins are toothed.

1.2.3 : CHEMICAL CONSTITUENTS:

Alkaloids, glycosides, Phenolic compounds, steroids, sterols, saponins, flavones, flavonoids, proteins, amino acids and carbohydrates[7].

1.2.4 : MEDICINAL USES:

No reports were found on *Hibiscus radiatus,* other *Hibiscus* species are used, to treat cardiac conditions, and as a diuretic, spasmolytic, antibacterial, cholagogue, diuretic, and anthelmintic, for the treatment of high blood pressure, liver diseases and fevers.

In large amounts, *hibiscus* tea acts as a mild laxative.[8]

1.2.5 : TRADITIONAL USES:

The plant is believed to be a cure for fever and is considered as blood purifier. All plants parts are cut and boiled and the liquid preparations are used.[5]

Figure No. 1.1: Photograph showing/ *Hibiscus radiatus* whole plant part

Figure No. 1.2: Photograph showing *Hibiscus radiatus flower and leaves part*

ULCER

1.3: INTRODUCTION:

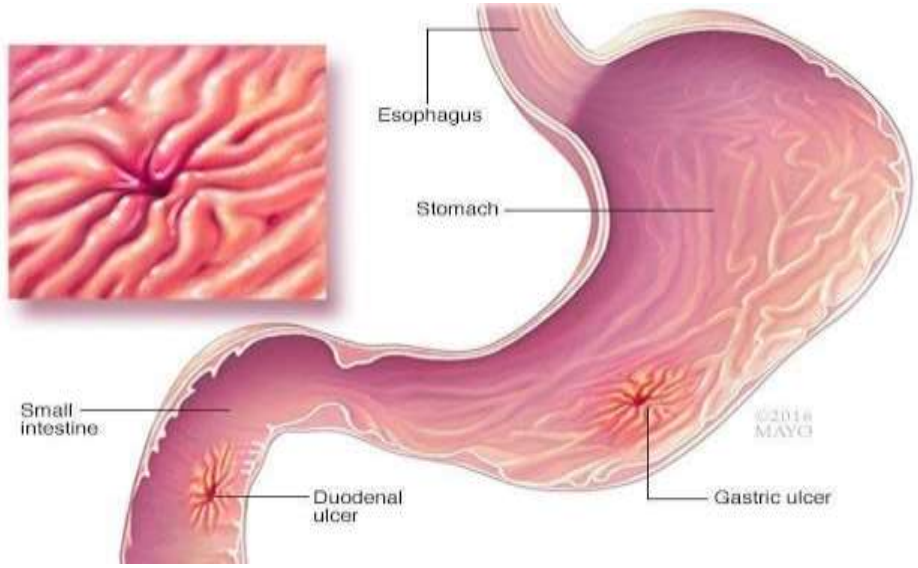
Peptic ulcer is chronic disease of multi factorial etiology and is the most prevalent disease among the gastro intestinal tract diseases. It is one of the most common chronic illnesses among working age adults. An imbalance between aggressive factors (acid and pepsin, H. Pylori, bile) and cytoprotective (defensive) factors of gastric mucous membrane

(mucus and bicarbonate secretion prostaglandins blood flow and the process of restitution and regeneration after cellular injury) results in peptic ulcer[9].

Signs and symptoms of peptic ulcer:

Abdominal pain with burning or gnawing sensation (pain 2-3 hours after eating pain is often made worse by an empty stomach, night time pain is common), indigestion (dyspepsia) belching, nausea vomiting poor appetite and weight loss[10].

Figure No.1.3: Photograph showing anatomy of stomach, duodenum, and common locations of gastric and duodenal ulcers.



Etiology and risk factors:

The etiology of gastric ulcer involves the following factors:

H. pylori.

NSAIDS

Genetic factors

Cigarette smoking

Psychological stress

Dietary factors like coffee, tea, cola, beverages, beer, and spices may cause dyspepsia but do not increase the risk for peptic ulcer disease. Caffeine is gastric acid stimulant, beer and wine may also increase gastric acid secretion.

- Excessive ingestion of alcohol may result in gastritis, characterized by mucous membrane edema at sub epithelial hemorrhages[11].

1.3.1 : PATHOPHYSIOLOGY:

There pathogenesis of duodenal ulcers and gastric ulcers is multifactorial and most likely reflects a combination pathophysiologic abnormality and environmental and genetic factors.

Most peptic ulcers occur in the presence of acid and pepsin in H. pylori, NSAIDs, or other factors disturb normal mucosal defense and healing mechanism.

Mucosal defense and repair mechanism include mucus and bicarbonate secretion, intrinsic epithelial cell defense and mucosal blood flow. Maintenance of this mechanism (mucosal defense and repair) is mediated production of endogenous prostaglandin. H. pylori and NSAIDs are the most important co factors which cause alteration of mucosal defense in peptic ulcer.

H. pylori infection causes gastritis in all infected individuals and is casually linked to peptic ulcer disease. Most non NSAIDs ulcers are infected with H. pylori.

H. pylori may cause ulcers by direct mucosal damage, altering the immune or inflammatory response, and by hypergastrinemia leading to increase acid production. Non-selective NSAIDs (including Aspirin) cause gastric mucosal damage by two Mechanisms.

a. Direct or topical irritation of gastric epithelium.

b. Systemic inhibition of Cox-1 enzyme which results in reduced protective prostaglandins synthesis.

Use of corticosteroids alone does not increase the complication or risk of ulcers, but the ulcer risk become double when the corticosteroid user taking NSAIDs concurrently.

Epidemiological evidence links cigarette smoking to peptic ulcer disease[12].

1.3.2 : ANTIULCER DRUGS:

These are drugs which can elevate mood in depressive illness. Practically all antidepressant affects monoaminergic transmission in the brain in one way or the other and many of them has other associated properties. Particularly over the past two decades, a large number of antidepressants with an assortment of effects on reuptake/ metabolism of biogenic amines and on pre/post-junctional aminergic/cholinergic receptors have become available so that a cogent classification is difficult. The following working classification may be adopted.

1.3.3: CLASSIFICATION OF ANTIULCER DRUG:[13]

I. Reduction of gastric acid secretion: ranitidine, omeprazole, etc

II. Antacids:

a) Systemic: sod. bicarbonate, sod. citrate

b) Non systemic: Magnesium hydroxide, Aluminum hydroxide gel

III. Ulcer protectives: sucralfate, colloidal bismuth sub citrate

IV. Anti H. pylori drugs: Amoxicillin, Clarithromycin, Metronidazole, etc.

1.3.4 : MECHANISM OF ACTION:

Most available drugs for treating ulcers enact in their own way thus there lies different mechanism for each class of drug, summoned together the mechanism is as follows:

H2 receptor antagonist like Ranitidine predominantly inhibit basal acid secretion which accounts for the efficacy in suppressing nocturnal acid secretion evening dose of H2 receptor antagonist is adequate therapy in most instances of duodenal ulcer. It also stimulates GI motility.

Proton pump inhibitors are prodrugs that require activation in acid environment. After absorption into the systemic circulation the prodrug diffusers in the parietal cells of the stomach and accumulates in the acidic secretory canaliculi. here it is activated by proton catalyzed formation of a tetracyclic sulfonamide, trapping the drug so that it cannot diffuse back across the canalicular membrane.

The activated form then binds covalently with the sulfhydryl groups of cysteines in the H+, K+, ATPase, irreversibly inactivating the pump molecule. Acid secretion resumes only after new pump molecules are synthesized and inserted into the luminal membrane, providing a prolonged suppression of acid up to 24 to 48 hour.

- Sodium bicarbonate effectively neutralizes acid, it is very water-soluble and rapidly absorbed from the stomach, and the alkali and sodium loads may pose a risk for patients with cardiac or renal failure. Depending on particle size and crystal structure, $CaCO_3$ rapidly and effectively neutralizes gastric H+, but the release of CO_2 from bicarbonate and carbonate containing antacids can cause belching, nausea, abdominal distention. Magaldrate is a hydroxy magnesium aluminate complex that is converted rapidly in gastric acid to $Mg(OH)_2$ and $Al(OH)_3$, which are absorbed poorly and thus provide a sustained antacid effect.
- Regarding the H. pylori infection five important considerations influence the selection of an eradication regimen. First, single-antibiotic regimens are ineffective in eradicating H. pylori infection and lead to microbial resistance. Combination therapy with two or three antibiotics (plus acid suppressive therapy) is associated with the highest rate of H. pylori eradication. Second, a proton pump inhibitor or H2-receptor antagonist significantly enhances the effectiveness of H. pylori antibiotic regimens containing Amoxicillin or Clarithromycin. Third, a 14day course of therapy generally is preferred. Fourth, poor patient compliance is linked to the medication related side effects experienced by as many as half of patients taking triple-agent regimens, and to the inconvenience of three- or four drug regimens administered several times per day. Packaging that combines the daily doses into one convenient unit is available and may improve patient compliance. In the presence of in vitro evidence of resistance to Metronidazole, Amoxicillin can be used instead[14]. Sucralfate binds to duodenal and gastric ulcers and to gastric erosions produced by ethanol and anti-inflammatory drugs. The affinity of sucralfate for defective mucosa is explained by the drug's viscous adhesiveness and the formation of

polyvalent bridges between the negatively charged sucralfate polyanions and positively charged proteins present in high concentrations in mucosal lesions.
Sucralfate also buffers acid, inhibits the action of pepsin, and adsorbs bile salts.
These properties of sucralfate enable the drug to act as an effective barrier to the penetration of acid, pepsin, and bile salts. Evidence to support such a comprehensive protective barrier effect is presented. Sucralfate also binds to uninjured mucosa and is believed to exert a similar "barrier" effect on regenerated and normal mucosa. Other possible mechanism for sucralfate's antiulcer effect include depletion of acid, pepsin, and bile salts from the gastric secretion. Animal data show that the action of sucralfate is sustained because of its viscous adhesiveness, slow reaction with acid and high affinity for defective mucosa[15].

OBJECTIVES

The objectives of the present study is as follows

1. **Collection and authentication of *Hibiscus radiatus***

Hibiscus radiatus plant was collected from the local areas of Shivamogga district and it has been authenticated by Ms. Soukya. N Botanist, Department
of Botany, SRNMC, Shivamogga.

2. **Drying and powdering of leaves parts of *Hibiscus radiatus***

Leaves of *Hibiscus radiatus* were cut in to small piece and were shade dried and powdered.

3. **Extraction of the plant using different solvents.**

Powdered leaves of the *Hibiscus radiatus* were subjected to extraction using different solvents i.e., aqueous, methanol and ethyl acetate.

4. **Phytochemical investigation of extracts.**

Different extracts of *Hibiscus radiatus* were investigated for the presence of Phytochemicals by using standard procedures.

5. **Evaluation of Toxicological studies.**

Toxicity studies were carried out as per OECD guidelines No 425 in albino mice using the different extract to find out the LD50 of the extracts.

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6. **Pharmacological screening of different extract of *Hibiscus radiatus.***

Different extract of *Hibiscus radiatus* were investigated for its antiulcer.

 Antiulcer Activity.

To investigate the antiulcer activity of *Hibiscus radiatus* leaf using pyloric ligation method and ethanol induced ulcer model.

METHODOLOGY

4.1 **: Preparation of *Hibiscus radiatus***

4.1.1 **: Collection of Authentication of *Hibiscus radiatus***

The leaves of *Hibiscus radiatus* was collected from in and around the Shivamogga
District of Karnataka and was authenticated by the botanist, Ms. Soukya. N, Department of Botany, SRNMC, Shivamogga.

4.1.2 **: Drying and powdering of leaves parts of *Hibiscus radiatus***

The leaves parts of *Hibiscus radiatus* were shade dried and reduced to a coarse Powder in a pulvirizer (Sunbeam, Munger, India) using mesh no. 3 and passed through a Sieve No. 40 to obtain about 1kg of powder.

4.1.3 : Extraction of the plant using different solvents.

Various extracts of the plant material were prepared by maceration and Soxhlet extraction method. The powdered material of *Hibiscus radiatus* was extracted with different solvents (aqueous, methanol, ethyl acetate) in a Soxhlet extractor. The extract was concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and finally desiccator dried.
The extract so obtained was labeled, weighed and the yield was calculated in terms of

1

grams percent of the weight of the powdered aerial part of the plant. These extracts are then used for the activities.

4.2 : Phytochemical investigation of extracts[40,41]

The extracts so obtained from each of the solvents were subjected to the following qualitative tests to detect the major chemical constituents.

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1. Test for carbohydrates

a. Molisch's test: To the test solution, few drops of Molisch's reagent and 2ml. of concentration sulphuric acid were added slowly through the sides of the test tube. A purple ring formed at the junction of the two liquids indicates the presence of Carbohydrates.

b. Barfoed's test: To the test solution, Barfoed's reagent was added, boiled on water bath, brick red precipitate was formed.
c. Benedict test: To the best solution, Benedict's reagent was added and boiled on water bath, reddish brown precipitate was formed.

2. Test for tannins:
a. Ferric chloride test: Test solution with few drops of ferric chloride solution gives dark red color.
b. Gelatin test: Test solution when treated with gelatin solution white precipitate.
3. Test for saponins
a. Foam test: Saponins when mixed with water and shaken, shows the formation of froth, which was stable at least for 15 minutes.
b. Haemolysis test: 2ml. each of 18% sodium chloride solution is taken in two test tubes test tubes. To one test tube 2ml of distilled water was added and to the other 2ml. of the test sample was added. A few drops of blood were added to both the test tubes, mixed and observed for haemolysis under microscope.
c.

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4. Test for flavonoids:
a. Ferric chloride test: Test solution with few drops of ferric chloride solution shows Intense green color.
b. Shinoda test: The solution with few fragments of magnesium ribbon and concentrated hydrochloric acid shows pink to magenta red color.
c. Zink-Hydrochloric acid reduction test: Test solution with zinc dust and few drops of hydrochloric acid shows magenta red color.
d. Alkaline reagent test: Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which becomes colorless on addition of few drops of dilute acid.
e. Lead acetate solution test: Test solution with few drops of lead acetate (10%) solution gives yellow precipitate.

6. Test for glycosides
a. Baljet test: The test solutions when with solution picrate give yellow to orange Color.
b. Keller- Killiani test: The test solution was tested with few drops of ferric chloride solution and mixed. When concentrated sulphuricacid acid containing ferric chloride solution was added, it forms two layers, lower layer reddish brown and upper acetic acid layer turns bluish green.
c. Raymond's test: The test solution when treated with dinitrobenzene in hot methanolic alkali gives violet color.
d. Bromine water test: The solution when dissolved in bromine water gives yellow precipitate.
e. Legal's test: The solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red color.
7. Test for alkaloids
a. Mayers's test: When Mayer's reagent (potassium mercuric iodide) was added to the test solution, it gives cream colored precipitate.
b. Wagner's test: The acidic test solution with Wagner's reagent (iodine in potassium iodide) gives brown colored precipitate.
c. Dragendroff's test: When Dragendroff's reagent (solution of potassium bismuthiodide) was added to the test solution, it gives orange brown colored precipitate.
d. Hager's test: When Hager's reagent (saturated picric acid solution) was added to the test solution, it gives yellow colored precipitate.

4.3 : Pharmacological activities:

Animals:

Healthy young adult mala and non-pregnant female albino rats (200-250g) of either sex and non- pregnant female albino mice (20-25gm) were used for the acute toxicity and pharmacological studies (antiulcer, anxiolytic and analgesic activity) using aqueous, methanol and ethyl acetate extracts of leave parts of *Hibiscus radiatus*. The animal was procured from Central Animal House, National College of Pharmacy, Shivamogga, and Karnataka. After randomization in to various groups, animals were acclimatized for period of 10 days under Standard husbandry conditions. Room temperature $27^0 \pm 2^0$C; Relative humidity 65±10%;12hours – light/dark cycle All the animals were fed with rodent pellet diet(Gold mohr, Lipton India Ltd.,) and water was allowed ad-libitum under strict hygienic condition.
Ethical clearance (Clearance number: NCP/IAEC/) for performing experiments on
animals was obtained from institutional animal Ethics committee (IAEC).

Statistical analysis:

All the values were expressed as mean ± S.E.M. Statistical analysis was carried out by performing One- way ANOVA followed by pair wise comparisons of Turkey's HSD (honestly significant difference) Test. A probability level of $P<0.05$ was considered moderately significant, $P<0.01$ is considered as Significant and $P<0.001$ is considered as highly significant.

4.3.1 **: Acute toxicity study:**[42] Acute oral toxicity study for the *Hibiscus radiatus* extracts was carried out using OECD Guideline-425 (modified, adopted March 23, 2006), the sequential test uses a maximum of five Animals. A test dose of 2000 or exceptionally 5000 mg/kg may be used in situation where experiment has information indication that the test material is likely to be nontoxic. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in
addition estimation of LD50 confidence intervals. The test also allows the observation

of sign of toxicity and can also be used to identify chemicals that are likely to have low toxicity. As suggested, after acclimatization of animals for 4-5 days, study was carried out as follows.
Healthy, young adult female albino mice (18-25gm) were used for this study. Food but not water, was withheld for 3-4 hours and further 1-2 hours after administration of sample under study. One animal was received test drug (plant extract) by oral route. Since this first test animal survived, four other animals were dosed (orally) at subsequent days, so that a total of five animals were tested. The acute toxicity studies of AEHR, MEHR and EEHR extracts was carried out according to above prescribed methods. At 2000mg/kg test sample did not produce any observable toxic Effects during entire duration of study. So, there was no mortality of the mice was found at 2000mg/kg

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4.3.2 : Evaluation of Antiulcer Activity:

Antiulcer activity was evaluated in-vivo using total leaves extract of *Hibiscus radiatus* two models have been used to evaluate the antiulcer activity,

A. Pyloric ligation induced ulcer model
B. Ethanol induced ulcer model.

4.3.3. A. PYLORIC LIGATION INDUCED ULCER MODEL:[43]

Albino rats of either sex weighing between 200-250gms were selected and animals were divided into five groups each containing six animals. The method was carried out as per shay H et al., with slight modification. The rats were kept for fasting for 24 hours prior to the experiment but provided free access to water ad libitum. After 30 min of drugs administration, pylorus ligation was done to all the animals under light ether anesthesia by giving one-inch midline abdominal incision, without causing any damage to the blood supply of the stomach, and the abdominal wall was sutured. The animals were allowed to recover and stabilized in individual cages and were deprived of water during postoperative period.
After 6 hours of surgery, rats were sacrificed using high dose of anesthesia and abdomen opened and the stomach was lifted and cut, the stomach was opened along with greater curvature and ulcer scoring was calculated.

Rats were divided into 5 groups consisting of 6 animals each.
Group-I: Control group treated with only vehicle and pyloric ligation.
Group-II: Received Omeprazole (20mg/kg b.w. i.p.) and pyloric ligation.
Group-III: Received aqueous extract and pyloric ligation.
Group-IV: Received methanol extract and pyloric ligation.
-V: Received ethyl acetate extract and pyloric ligation.
Group Department of Pharmacology, NCP, Shivamogga.

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4.3.3. B. ETHANOL INDUCED ULCER MODEL:[44]

Albino rats of either sex weighing between 200-250gm were selected and divided into 5 groups of 6 animals each. The animals were fasted for 24 hours with free access of water, animals were given particular dose of *Hibiscus radiatus* extracts and standard drug mentioned above. Thirty minutes after treatment 1ml of alcohol was administered p.o to each animal. Animals were sacrificed 1hr after alcohol administration, stomach was dissected and cut open along the greater curvature and pinned on a soft board. The ulcer index and histological changes will be observed.
Rats were divided into 5 groups consisting of 6 animals each.

Group-I: Control group (1ml ethanol p.o)
Group-II: Received standard Omeprazole (20mg/kg b.w. i.p.)
Group-III: Received aqueous extract of *Hibiscus radiatus.*
Group-IV: Received methanol extract of *Hibiscus radiatus*.
Group-V: Received ethyl acetate extract of *Hibiscus radiatus*.

Ulcer score and Ulcer Index:

After stomach was opened along greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The number of ulcers was counted. Scoring of ulcer will be made as follows:

Normal colored stomach…… (0) Red coloration… ..... (0.5)

Spot ulcer…… (1) Hemorrhagic streak (1.5)

Deep ulcers……. (2) Perforation… ..... (3)

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows: Ulcer index (UI) was measured by using following formula:

UI = UN + US + UP X 10−1
Where,
UI= Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers. Percentage inhibition of ulceration was calculated as below:

% Inhibition of Ulceration = (Ulcer index Control -Ulcer index Test) × 100
Ulcer index control

RESULTS
In present investigation Phytochemical study was carried out for different extracts of *Hibiscus radiatus* leaves and antiulcer, anxiolytic and analgesic activities were carried Out.

5.1: Preparation of different extracts of *Hibiscus radiatus*:
The leaves of plant were shade dried and reduce to the coarse powder in a pulvilizer. The powdered material was extracted with different solvents (aqueous, methanol, ethyl acetate). The solvents of extracts were removed completely over the water bath and finally desiccator dried. Obtained extracts were labelled weighed and the yield was calculated in terms of the gram's percentage yield of the powdered leaves of the leaves *Hibiscus radiatus* (shown in the table No 5.1). The concentrated extract of *Hibiscus radiatus* were used for pharmacological activities.

Table 5.1: Table showing the percentage yield of various extracts of *Hibiscus radiatus.*

Sl. No.	Name of the extract	Quantity taken in grams	Yield in grams	% Yield
1.	Aqueous extract	100	5	5
2.	Methanol extract	100	6	6
3.	Ethyl acetate extract	100	6	6

5.2. Phytochemical analysis:

Table 5.2: Showing the Qualitative chemical investigation of extracts of *Hibiscus radiatus.*

Sl. No	Phytoconstituents	Aqueous extract	Methanol extract	Ethyl acetate extract
1.	Carbohydrates	+	+	–
2.	Tannins	+	+	+
3.	Saponins	+	–	–
4.	Phenolic Compounds	+	+	+

5.	Flavonoids	+	+	+
6.	Glycosides	+	+	+
7.	Alkaloids	+	+	–

(+: Present; -: absent)

The Phytochemical analysis reveals that Carbohydrates, Tannins, saponins, Phenolic compounds, Flavonoids, steroids, Glycosides and Alkaloids present in the aqueous extract. In Methanol extract Carbohydrates, Tannins, Phenolic compounds, Flavonoids,
Glycosides and Alkaloids are present. And in ethyl acetate extract
Tannins, Phenolic compounds, Flavonoids, Glycosides, are present (as shown in Table No: 5.2).

5.3. Pharmacological studies:

I. Acute toxicity study:

OECD guideline No. 425 of CPCSEA was adopted for toxicity studies. The experiments were initiated only after the approval of the institutional Animal Ethical Committee. Female albino mice weighing 20-25 gm and of 8-12 weeks of age were used to determine the dose. The animals were fasted overnight prior to the acute toxicity experimental procedure.

The initial dose in the experiment was 2000mg/kg body weight caused no mortality during the observation time. Thus, the median lethal dose (LD50) of the plant extract is said to be greater than 2000mg/kg, indicating a good safety margin. To study various pharmacological activities the fraction was administered in the dose of 200mg/kg body weight which is equal to $1/10^{th}$ of 2000mg/kg body weight.

II. Anti-ulcer activity

Pyloric ligation induced ulcer model

The test sample of *Hibiscus radiatus* were evaluated for their anti-ulcer activity by pyloric ligation induced ulcer model and ethanol induced ulcer model in albino rat. It was observed that the aqueous, methanol, and ethyl acetate and extracts of *Hibiscus radiatus* shown highly significant anti-ulcer activity when Compared to control. Pyloric ligation induced ulcer model (Table 5.3) and Ethanol induced ulcer model Shown in
Table 5.4

A. PYLORIC LIGATION INDUCED ULER MODEL

Table 5.3: Table showing the effect of various extracts of leaves parts of *Hibiscus radiatus* on antiulcer activity by pyloric ligation induced ulcer.

Groups	Dose Mg/kg b.w	Ulcer index	Percentage of Inhibition
Control (Normal saline)	-	8.9±0.42	-
Standard (Omeprazole)	20	3.50±0.33***	60.67
Aqueous extract	200	4.4±0.11**	50.56

Methanol extract	200	5.5±0.34**	38.20
Ethyl acetate extract	200	6.9±0.43*	20.22

NOTE: Data was analysed using one-way ANOVA followed by Turkey's pair wise Comparison. Value are expressed as mean ± **S.E.M. n=6, ***P < 0.001 is Considered** as highly significant.

Figure No.5.1: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on ulcer index in pyloric ligation model

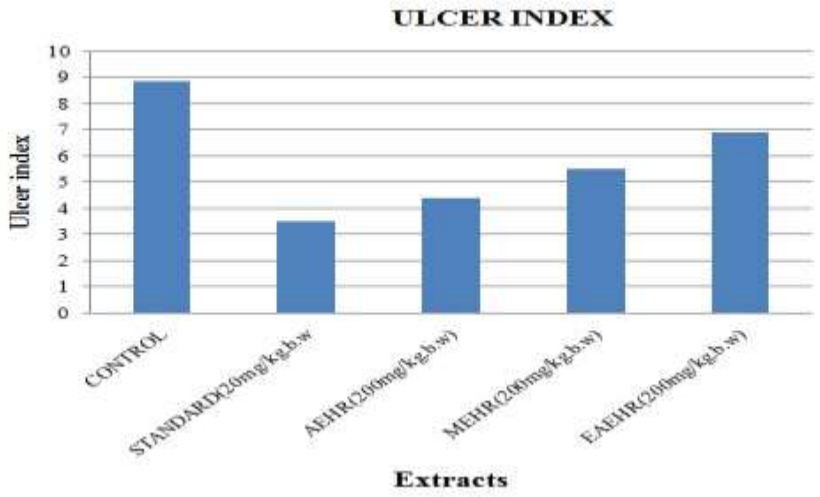


Figure No.5.2: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on percentage inhibition of ulcer in pyloric ligation model.

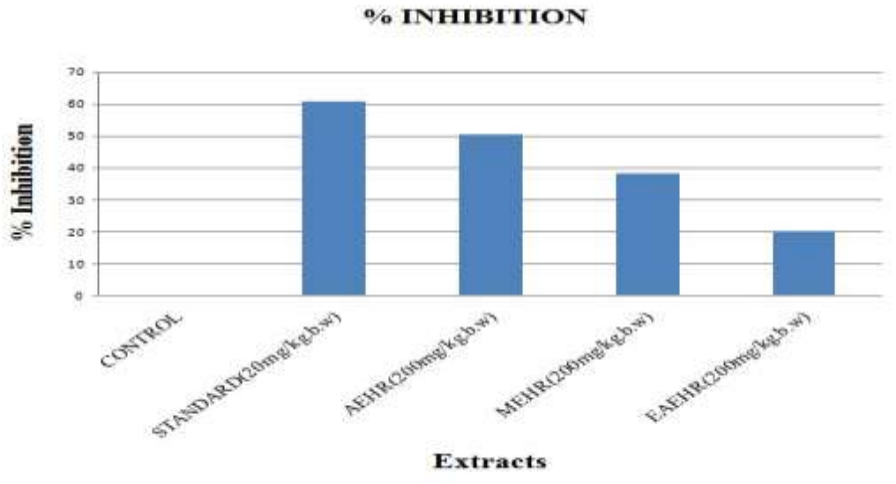
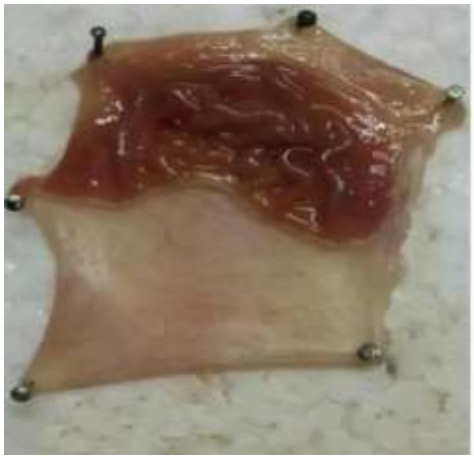


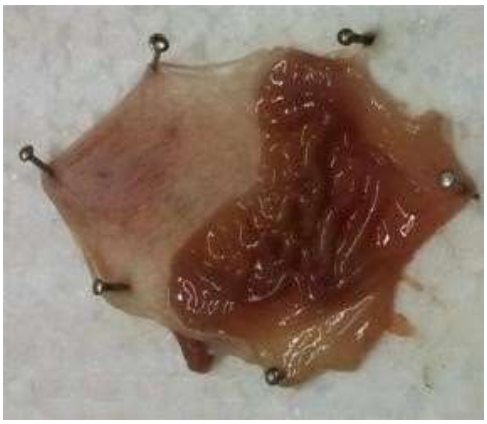
Figure No. 5.3 Photographs showing rat stomach of pylorus ligation induced ulcer

Control Standard drug



Aqueous extract

Methanol extract



Ethyl Acetate extract

B. Ethanol induced ulcer model:

In the locomotor activity study, it was found that *Hibiscus radiatus* extract significantly (P<0.01) depressed the locomotor activity in rat in a dose and time- dependent fashion. The activities increased as time approached to 120 mins.

Table 5.4: Table showing the effect of aqueous, methanol, ethyl acetate leaf extract of *Hibiscus radiatus* on ethanol Induced ulcer model.

Groups	Dose Mg/kg b.w	Ulcer Index	Percentage of Inhibition
Control (Normal saline)	-	8.9±0.42	-
Standard (Omeprazole)	20	3.5±0.33***	60.66
Aqueous extract	200	5.5±0.41**	38.20
Methanol extract	200	7.4±0.23*	16.85
Ethyl acetate extract	200	7.2±0.34**	19.10

NOTE: Date was analysed using one way ANOVA followed by Turkey's pair wise **Comparison values are expressed as mean ±S.E.M. n=6, ***P<0.001 is considered as** highly significant.

Figure No.5.4: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on ulcer index in ethanol induced ulcer model.

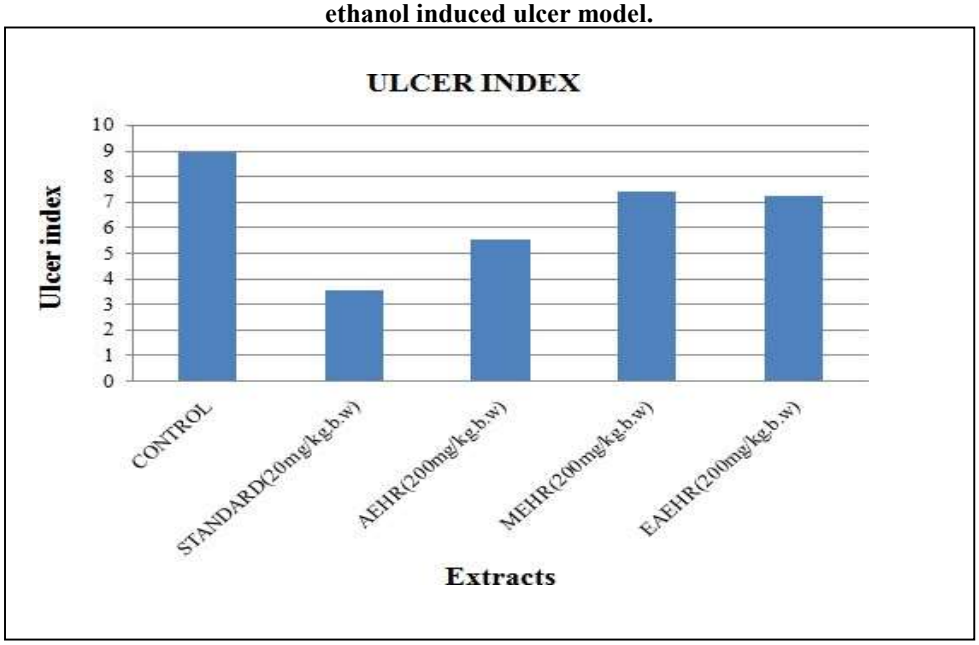


Figure No.5.5: Histogram showing the effects of various leaf extracts of *Hibiscus radiatus* on percentage inhibition in ethanol induced ulcer model.

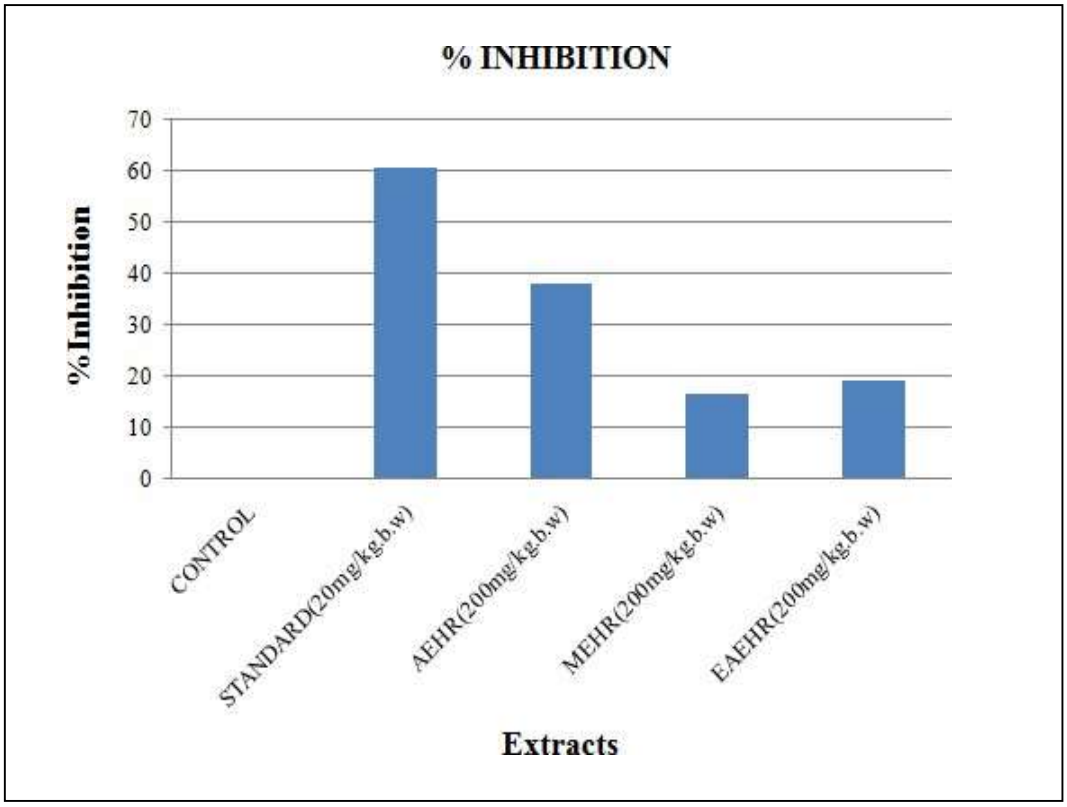
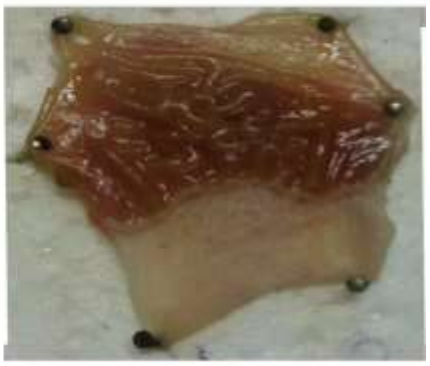


Figure No. 5.6: Photographs showing rat stomach of Ethanol induced ulcer.

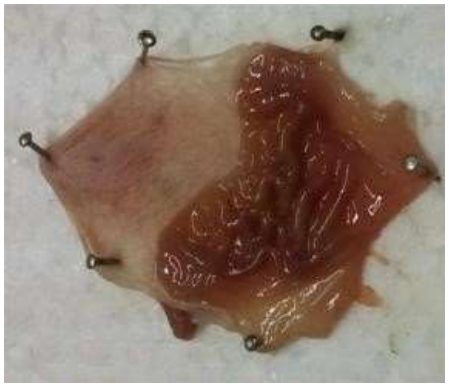
Control Standard

drug

Aqueous extract



Methanol extract



Ethyl acetate extract

DISCUSSION

Medicinal plants comprise the undefinable source of economic value in every parts of the world. Plants used in traditional medicine consist of wide range of constituents that can be used to treat chronic as well as communicable diseases. *Hibiscus radiatus* is one such medicinal plant being used in folk medicine which helps in treating various diseases and disorders. Reports suggest that the plant contains alkaloids, glycosides, flavonoids,
saponins, tannins, phenols and carbohydrates.

The present study was undertaken to evaluate anti-ulcer, anti-anxiety and analgesic activities of aqueous, methanol, and ethyl acetate extracts of leaves of *Hibiscus radiatus* using animal experimental models in rats and mice.

I. Antiulcer activity:

In this present study the test samples of leaves extracts of *Hibiscus radiatus* belonging to the family Malvaceae were tested for anti-ulcer activity. Several reports are available on many plant species belongs to the presently studied family Malvaceae
with antiulcer activity.

In this present study anti-ulcer activity was evaluated by pyloric ligation model and ethanol induced ulcer model. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by models employed in the present study involving the increase of gastric acid output, vascular injury, depletion of gastric wall mucin and mucosal damage induced by ethanol[48].

Gastric ulcers are due to imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin gastric acid make up the offensive factors whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostraglandins[49, 50].

The adrenergic system is involved in gastric secretion. Activation of presynaptic α-2
adreno receptors located on the vagus nerve inhibits gastric acid secretion[51].

In the present study all the test samples (. aqueous, methanol, ethyl acetate) of leaf extracts exhibited significant ($P < 0.001$) anti-ulcer activity. Among these test samples aqueous and methanol exhibited more significant activity when compare to control.

The phytochemical investigation has been shown the presence of flavonoids, tannins, glycosides, alkaloids and phenolic compounds in the presently tested samples.

The anti-ulcer activity may be due to the presence of flavonoids.

The mechanism of flavonoids as anti-ulcer:

Flavonoids are a group of about 4000 naturally occurring compounds with a wide range of biological effects, including anti-ulcer activity. They are important constituents of the human diet (a daily diet contains approximately 1 g of flavonoids per day) and are also found in several medicinal plants used in folk medicine around the world.

Several mechanisms have been proposed to explain the gastro protective effect of flavonoids; these include increase of mucosal prostaglandin content, decrease of histamine secretion from mast cells by inhibition of histidine decarboxylase and inhibition of Helicobacter pylori growth[62].

It has been also reported that flavonoids like quercetin seem to play a very important role in the prevention and treatment of peptic ulcer. It acts by promoting mucus secretion, thereby serves as a gastro protective agent. Among other flavonoids such as methyl-3-(+) catechin interferes with the formation of histamine in gastric mucosa and hence produces the protective effect.

However, further studies are necessary to find the exact mechanism of anti- ulcer activity and to isolate the active compound(s) responsible for this pharmacological activity.

CONCLUSION

The present study concludes that aqueous, methanol and ethyl acetate extract of the plant *Hibiscus radiatus* exhibited significant actions on antiulcer, anxiolytic and analgesic activities.

Anti-ulcer activity:

Anti-ulcer activity of various leaves extracts of *Hibiscus radiatus* was carried out by using two models namely, pyloric ligation induced ulcer model and ethanol induced ulcer model. In the present study all the test samples (aqueous, methanol and ethyl acetate) of leaves extracts exhibited significant anti-ulcer activity. Among these test samples aqueous and methanol exhibited more anti-ulcer activity ($P<0.001$) when compared to control.

It can be concluded that the active constituents are responsible for anti-ulcer activity might be present in the leaves extracts. However, further, studies are necessary to find the exact mechanism of anti-ulcer effect and to isolate the active compound(s) responsible for this pharmacological activity.

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