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Anti-Ulcer Activity of *Hibiscus sabdariffa* on Albino Rats

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ABSTRACT

To study the antiulcer activity of *Hibiscus sabdariffa* stems using different models of gastric ulceration in rats. Antiulcer activity of *Hibiscus sabdariffa* stem extract was studied in rats by administration of ethanol-induced method and by pyloric ligation method. Stem extract was administered in the dose of 250 mg/kg and 500 mg/kg orally 30 min prior to the ulcer induction. The antiulcer activity was assessed by determining and comparing the ulcer index in the test drug group with that of the ulcerated control group. Ranitidine was used as a reference drug. The ulcer index in the stem extract treated animals was found to be significantly less in all the models compared to ulcerated control animals. The antiulcer property was more prominent in animals in whom ulcers were induced by ethanol-induced and pyloric ligation. Ranitidine 50 mg/kg produced a significant gastric ulcer protection when compared with the control group. The antiulcer activity of *Hibiscus sabdariffa* was, however, less than that of ranitidine. Our results suggest that *Hibiscus sabdariffa* stem extract possess significant antiulcer property which could be either due to the cytoprotective action of the drug or by strengthing of gastric mucosa and thus enhancing mucosal defense.

INTRODUCTION:

Ulcers are deep lesions penetration through the entire thickness of the gastrointestinal tract (g.i.t) mucosa and muscularis mucosa. Peptic ulcer has unquestionably been a disease of the twentieth century. Epidemiological data for this disease and its complications have shown striking geographical variations in incidence and prevalence. There are different types of ulcers; most common are peptic ulcer, gastric ulcer which appeared to be due to damage to the lining of the stomach and duodenal ulcer, which was associated with excessive acid secretion by the stomach. The etiology of peptic ulcer was fiercely debated. It is believed that peptic ulcers developed due to an imbalance between aggressive factors (mucin, bicarbonate, prostaglandins) leading to an interruption in the mucosal integrity[1]. Various factors are implicated that play a pivotal role in the pathogenesis of ulceration like sedentary lifestyle, alcohol intake, spicy food, drugs and various bacterial infections. Moreover, several endogenous substances have been identified and are reported to be involved in the production of gastrointestinal lesions in animals. The more important ones include some of the bacterial infection, various drugs and chemicals, gastric secretion, lipid metabolites, neuropeptides, inflammatory mediators and reactive free radicals. Oxidative stress has emerged as one of the major pathogenic factors in the progression of ulcer that directly impaired the cellular functions and promotes cellular organelles damage in the cell, including mitochondria, lysosomes, and nucleus. Also, NO is accepted as the vital mediator of GIT mucosal defenses as decreased NO generation or synthesis contribute to the pathogenesis of ulceration. The present study summarises the ulcerogenic mechanism of these substances and the enable us to understand the better etiology of peptic ulcer[2].

MATERIALS AND METHODS:

The study was conducted on Wistar albino rats of weight 200±30 gm and maintained under standard conditions. The major chemicals used are ranitidine, anesthetic ether, and chloroform.

About 100 gm of powdered *Hibiscus sabdariffa* stems were packed in a thick paper and it was subject to extraction by using soxhlet extraction method for 72 hrs until the marc become colorless. Then the extract was concentrated under reduced pressure and dried in the vacuum condition to get a semi-solid mass[3].

A. PRELIMINARY PHYTOCHEMICAL SCREENING:

The preliminary phytochemical investigations were carried out for the qualitative detection of phytoconstituents. Qualitative tests were conducted for all the extracts to identify the various phytoconstituents. The various tests and reagents used are given below and the observations are recorded in table[4].

Alkaloids:

(a) Dragendorff's test: 1 ml of extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer's test: 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish or cream colored precipitate indicates the presence of alkaloids.

(c) Hager's test: 1 ml of extract, add 3 ml of Hager's reagent (saturated aqueous solution of picric acid). Yellow colored precipitate indicates the presence of alkaloids

(d) Wagner's test: 1 ml of extract, add 2 ml of Wagner's reagent (iodine in potassium iodide). Reddish brown colored precipitate indicates the presence of alkaloids

Carbohydrates and Glycosides:

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(a) Molisch's test: Two ml of the prepared filtrate were mixed with 0.2 ml of an alcoholic solution of α-naphthol 10% in addition to 2 ml of sulphuric acid, a bluish violet zone is formed this indicates the presence of carbohydrates and /or glycosides.

(b) Fehling's test: In a test tube 5 ml of the filtrate were treated with 5 ml Fehling's solutions (A & B) and heated; the appearance of a red precipitate indicates the presence.

(c) Benedict's test: To 1 ml of the filtrate, 5 ml of Benedict's reagent were added. The mixture was heated; the appearance of red precipitate indicated the presence[5].

Flavones and flavonoids:

One ml of 10% ethanolic extract of the studied plant was mixed with 0.5 ml of hydrochloric acid (10%) and magnesium metal. A developed reddish color indicates the presence of flavonoids. Five ml of 1% hydrochloric acid extract were shaken with sodium hydroxide; a yellow color appeared indicating the presence of compound flavonoids.

Proteins and amino acids:

Five ml of extract, 2 drops of freshly prepared 0.2 percent ninhydrin reagent was added and heated. The appearance of the blue color indicates the presence of proteins, peptides or amino acids.

Saponins:

One g of the plant under investigation was boiled with 10 ml water for few minutes and filtrated. The filtrate was vigorously shaken. The persistent froth (1 cm height)was observed for 1 hr indicates the presence of saponins.

Steroids:

For testing the presence of unsaturated sterols and triterpenes, 1g of the air-dried powder of the studied plant was extracted with few ml of ethanol then filtrated and the filtrate was evaporated to dryness. The residue was dissolved in 10 ml chloroform, filtered and the filtrate was divided into two equal portions for preceding the following tests[4],

(a) Libermann-Burchard test:

To the first portion of chloroform filtrate 1 ml of acetic acid anhydride was added, followed by 2 ml of sulphuric acid down the wall of the test tube. The appearance of reddish-violet color at the junction of two layers and a bluish-green color in acetic acid layer indicates the presence.

To the second portion of chloroform filtrate, an equal volume of sulphuric acid was added. The appearance of a red color indicated the presence.

Tannins:

About 2 g of the air-dried powder of the plant was extracted with ethanol (50 %) and tested for the presence of tannins using the following tests.

One drop of ferric chloride was added to 2 ml of the extract, the appearance of bluish or greenish black coloration indicates the presence of pyrogallol or catechol tannins, respectively.

Five ml of the alcoholic extract of the studied plant were mixed with 2 ml vanillin Hydrochloric acid solution if a precipitate was formed. This indicates the presence of gallic acid[6].

B. METHODOLOGY:

Animal models used in the screening of antiulcer activity:

Various screening models are used for the screening of the antiulcer activity. It helps to understand the etiology of the ulcer and screening of antiulcer agents.

❖ Aspirin-induced ulcers

❖ Ethanol-induced ulcers

❖ Pylorus ligation induced ulcers

❖ Water immersion stress-induced ulcers

❖ Indomethacin-induced ulcers

❖ Histamine-induced ulcers

❖ Reserpine-induced ulcers

❖ Serotonin-induced ulcers[7]

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From this, majorly used models are explained below

1. Ethanol-induced ulcers method[7]:

Albino rats of either sex weighing between (150-200 gm) are divided into the group. The animals are fasted for 24 hours with free access water. Animals are given test drugs or standard drug. 1 hour later 1ml/200gm of 99.80% alcohol is administered orally to each animal. The animals were anesthetized 1 h later with ether and stomach was incised along the greater curvature and ulceration was scored. The number of ulcers and the length of each ulcer were measured. Ulcer index was calculated using severity scores and the average number of ulcers per animal. Severity scores as below.

Table No: 1 Treatment schedule for Ethanol-induced ulcers method

Sr. No	Treatment	Purpose
Group 1	Normal control	Serve as normal physiology
Group 2	Ulcerated control	Disease control
Group 3	Ranitidine 40 mg/kg	Standard treatment
Group 4	AEHS 250 mg/kg	Serve as test-I group
Group 5	AEHS 500 mg/kg	Serve as test-II group

- 0 - Normal stomach
- 0.5 - Red coloration
- 1 - Spot ulcers
- 1.5 - Hemorrhagic streaks
- 2 - Ulcer $>$ 3 mm but $<$ 5 mm
- 3 - Ulcers $>$ 5 mm[7]

Calculation of ulcer Index

$$UI = UN + US + UP \times 10\text{-}1$$

UI = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP- Percentage of animal with ulcer

Percentage inhibition was calculated by the formula

$$\%\text{inhibition} = \frac{uic - uit}{uic} \times 100$$

Histopathological studies were conducted by fixing stomach tissues in 10% formalin for 24 h. The formalin-fixed specimens are embedded in paraffin and section (3-5µm) and stained with hematoxylin and eosin dye. The histochemical sections are evaluated by light microscopy[8].

2. Pylorus ligation induced ulcers[7]:

Albino Wister rats of either sex weighing between (150-200 gm) are divided into groups of animals. In this method, albino rats are fasted in individual cages for 24 hours. Test drug or standard drug or control vehicle is administered 30 minutes prior to pyloric ligation. Under light ether anesthesia, the abdomen is opened and the pylorus was ligated. The abdomen is then sutured. At the end of 4 hours after ligation, the animals are sacrificed with the excess of anesthetic ether, and the stomach is dissected out gastric juice is collected were drained into tubes and were centrifuged at 1000 rpm for 10 minutes and the volume is noted. The pH of gastric juice is recorded by pH meter. Then the contents are subjected to analysis for free and total acidity. The stomachs are then washed with running water to see for ulcers in the glandular portion of the stomach. The numbers of ulcers per stomach are noted and severity of the ulcers scored microscopically with the help of 10x lens.

Histopathological studies were conducted by fixing stomach tissues in 10% formalin for 24 h. The formalin-fixed specimens are embedded in paraffin and section (3-5µm) and stained with hematoxylin and eosin dye. The histochemical sections are evaluated by light microscopy.

Table No:2 Treatment schedule for Pylorus ligation induced ulcers

Sr. No	Treatment	Purpose
Group 1	Normal control	Serve as normal physiology
Group 2	Ulcerated control	Disease control
Group 3	Ranitidine 40 mg/kg	Standard treatment
Group 4	AEHS 250 mg/kg	Serve as test-I group
Group 5	AEHS 500 mg/kg	Serve as test-II group

- 0 = Normal stomach
- 0.5 = Red coloration
- 1 = Spot ulcers

- 1.5 = Hemorrhagic streaks
- 2 = Ulcer > 3 mm but > 5 mm
- 3 = Ulcers > 5 mm

Calculation of ulcer Index[9]

UI = UN + US + UP x 10-1

UI = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

$$\%\text{protection} = \frac{ulcerindexcontrol - textulcerindex}{ulcermean\ index} \times 100$$

Acute Toxicity Studies:

The Acute Toxicity studies were done as per OECD guidelines and compounds were administered orally in various doses, were 24 hrs toxicity was recorded to identify the toxic dose. No mortality and no signs of toxicity were founded at the dose of 2500mg/kg body wt of aqueous extract of *Hibiscus sabdariffa* so it might be considered that aqueous extract has lethal dose(LD) 50 values above the 2500 mg/kg. Two doses 250 mg/kg and 500 mg/kg were selected to conduct the studies[10].

RESULTS:

1. Phytochemical screening of *Hibiscus sabdariffa*:

The water extract and other extracting reagents such as methanol, ethanol, ethyl acetate and petroleum ether on *Hibiscus sabdariffa* were subjected to preliminary phytochemical screening to identify the chemical constituents Quantitative determination of the phytochemical constituents of the plant was done using the gravimetric method described by with some modifications.

Table No: 3 Phytochemical screening of the stem extracts of *Hibiscus sabdariffa* [11]

Sr. No.	Test for	Result
1.	Carbohydrates	+
2.	Proteins	-
3.	Alkaloids	+
4.	Phenols	+
5.	Tannins	+
6.	Saponins	_
7.	Glycosides	+
8.	Gum	+

Pylorus ligation induced ulcers:

Albino Wistar rats were fasted in individual cages for 24 hours. Care was being taken to avoid coprophagy. Rats of either sex were randomly divided into five groups of 6 animals in each group

Group 2, group 3, group 4, group 5 were pylorus ligated and the abdomen was sutured, after 30 minutes of Pylorus ligation. Group 2 left untreated, group 3 treated with Ranitidine, group 4 and group 5 are treated with 250mg/kg and 500mg/kg body weight respectively.

After 4 hrs animals were sacrificed with anesthetic ether, stomach was isolated and opened along with greater curvature. Gastric juice was collected and PH was examined. The glandular portion was then exposed and examined for ulceration.

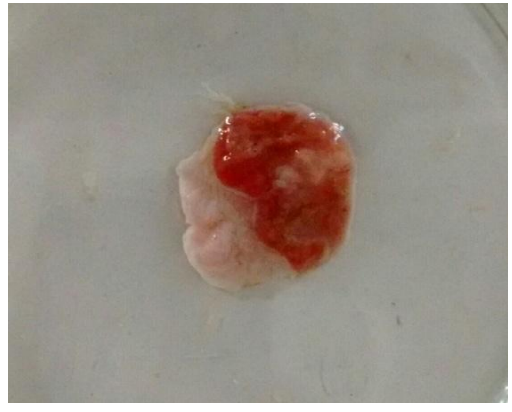

Fig 1: Normal stomach

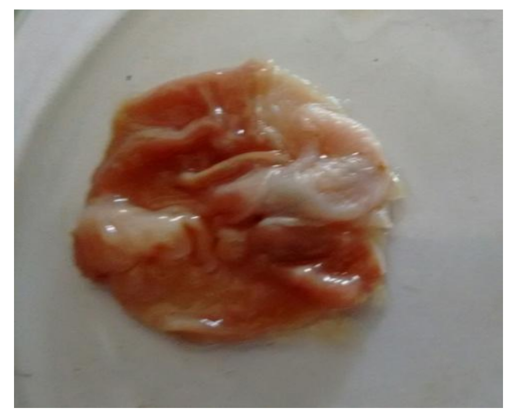

Fig 2: Pylorus ligation stomach

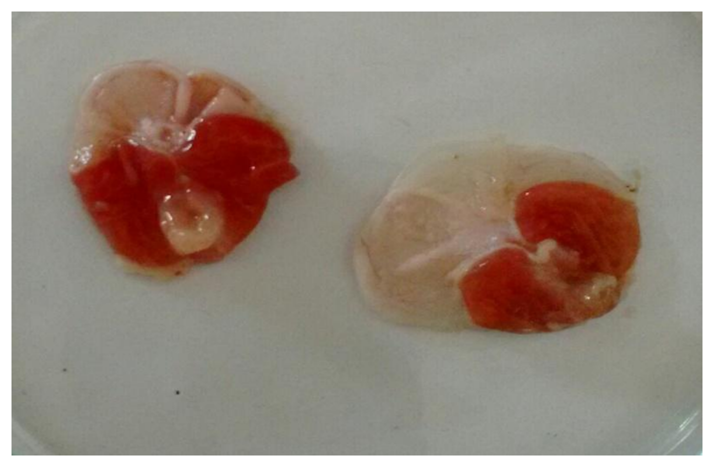

Fig 3: Ranitidine induced stomach

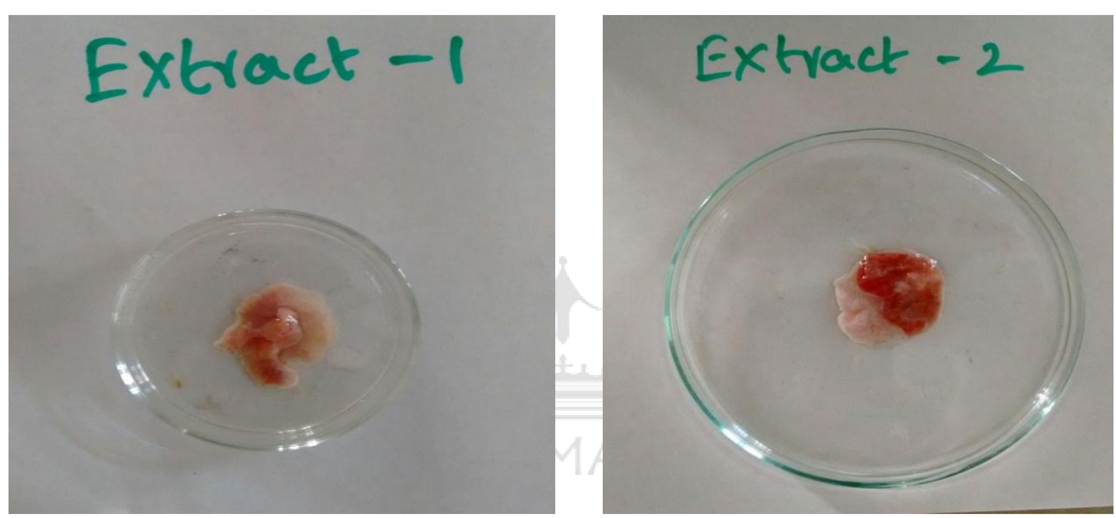

Fig 4: AEHS(250 mg/kg) treated Stomach

Fig 5: AEHS(500 mg/kg)treated stomach

Table No: 4 Effect of *Hibiscus sabdariffa* on various parameters in pylorus ligation method[12]

GROUP	TREATMENT	ULCER INDEX	% PROTECTION	pH
Group-1	Normal saline	-	-	3.1
Group-2	Pyloric ligation	8.1	-	1.8
Group-3	Pyloric ligation+ aq extract 250 mg/kg	5.8	28.39	2.0
Group-4	Pyloric ligation+ aq extract 500 mg/kg	4.6	43.20	2.63
Group-5	Pyloric ligation+ ranitidine	2.4	70.39	3.27

Ethanol-induced ulcers:

Albino rats of either sex weighing between (250±30 gm) were maintained under standard conditions (room temp 24-27^{0}C, humidity 60-65%) with 12 hours light and dark cycle. The food in the form of dry pellets (Amrut Lab, Pune) and water was available adlibidum.30 minutes after the test (AEHS 250 mg/kg and 500 mg/kg) and reference (Sucralfate) and control vehicle of the treatment, 1ml of ethanol was administered to each rat. After 1-hour rats were sacrificed by excess anesthetic ether and stomach was isolated and opened along with the greater curvature, washed the residual matter with saline and the inner surface was examined for ulceration.

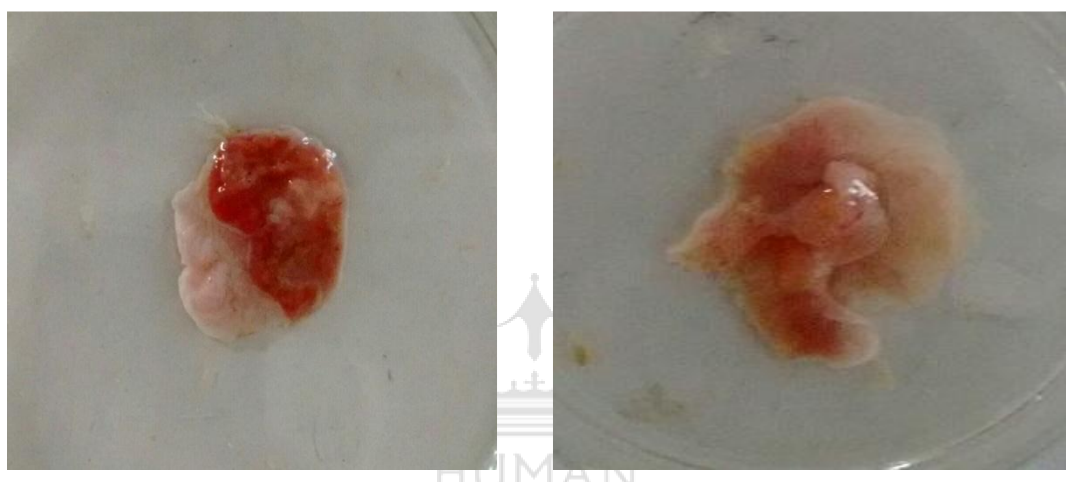

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Fig 6: Normal stomach **Fig 7: Ethanol-induced stomach**

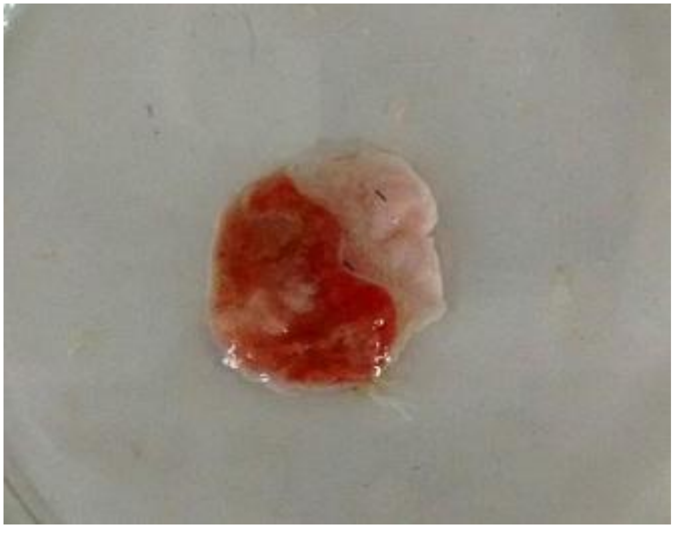

Fig 8: Ranitidine induced stomach

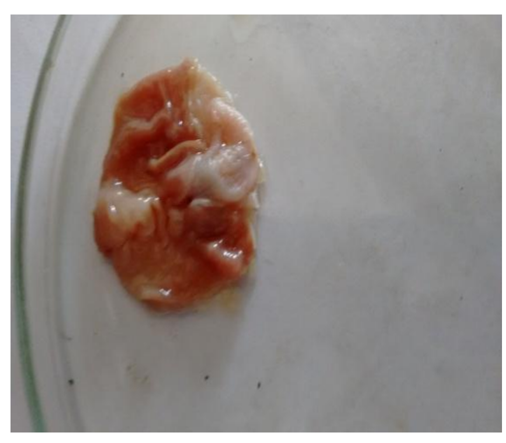

Fig 9:AEHS (250 mg/kg) Treated stomach

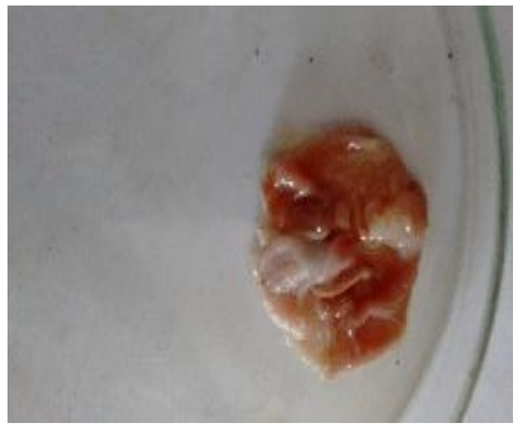

Fig 10:AEHS (500 mg/kg) treated stomach

Table No. 5: Extraction of *Hybiscus subdariffa* on various parameters in ethanolic induced method[13]

GROUP	TREATMENT	ULCER INDEX	% PROTECTION	pH
Group: 1	Normal saline	-	-	3.1
Group: 2	Ethanol/ alcohol	7.2	-	1.9
Group: 3	Ethanol+ aq extract 250 mg/kg	4.9	31.94	2.2
Group: 4	Ethanol + aq extract 500 mg/kg	3.7	48.61	2.75
Group: 5	Ethanol + ranitidine	2.1	70.83	3.62

DISCUSSION:

The ulcer is a major health hazard both in terms of morbidity and mortality. It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism. The role of free radicals is also reported in the indication of ulcers. Prostaglandins (PG) offer protection to duodenum through both increases in mucosal resistance as well as the decrease in aggressive factors, mainly acid and pepsin. Ethanol-induced gastric ulcers have been widely used for the evaluation of the gastroprotective activity. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals.

The incidence of ethanol-induced ulcers is predominant in the glandular part of the stomach. It was reported to stimulate the formation of leukotriene C_4 (LTC_4), mast cell secretory products and reactive oxygen species resulting in the damage of rat gastric mucosa. It has been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration in the gastric mucosa and scavenging these free radicals can play an appreciable role in healing these ulcers. When aspirin is in the lipid-soluble undissociated form it can damage the gastric mucosa. Aspirin causes a dose-dependent reduction in mucosal prostaglandins – PGE_2 and PGI_2 bio-synthesis accompanied by an increase in the mean area of gastric ulcerations.

Investigation of aqueous extract of *H. sabdariffa* (AEHS) in the present study provides sample indications of its strong gastric anti-ulcerogenic property. The observation in the present study, a significant decrease in the ulcer index in this model suggests the ability of Roselle extract is involved in decreasing the gastric acid secretion. Furthermore, AEHS also showed a significant effectiveness by inhibiting basal gastric acid secretion and ulcer formation in the pylorus-ligated rat model. The ulcer index in pylorus ligation method was found to be 8.1 at P^H 1.8, Our extract at concentration 250mg/kg, the ulcer index is 5.8 and percentage protection were found to be 28.39% at P^H 2.0. At 500mg/kg, the ulcer index is 4.6 &%protection is 43.20% at P^H 2.63. These results show less antiulcer activity compared with Salah Alqasoumi et. al. The present study compared with Ranitidine (Std, the UI 2.4 & % protection is 70.39%), our extract shows less activity.

AEHS significantly prevented gastric lesions induced by ethanol, the most commonly employed tests in the evaluation of anti-ulcer/cytoprotective activity. The ulcer index in ethanol-induced method was found to be 7.2. Our extract at concentration 250 mg/kg, the ulcer index is 4.9 and percentage protection was found to be 31.94%. At 500mg/kg the ulcer index is 3.7 &% protection is 48.61%. The present study in accordance with Rachhadiya Rakesh et.al, (ulcer index is 5.1&2.0 at 250,500mg/kg respectively). The present study compared with Ranitidine (Std, the UI 2.4 & %protection is 70.39%), our extract shows less activity.

CONCLUSION:

From the study, we concluded that aqueous extract of *Hibiscus sabdariffa* has potent antiulcer activity. From the result, it was proved that AEHS in the dose of 250 mg/kg shows 28.39% protection from ulcers, whereas 500 mg/kg shows almost double protection.

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