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ANTIULCER POTENTIAL OF FICUS DALHOUSIAE STEM BARK METHANOLIC EXTRACT IN ALBINO RATS

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Abstract:
The aim of the present study was to evaluate the antiulcer potential of Ficus Dalhousiae stem bark methanolic extract by excision and incision experimental models. Aalbino rats weighing 180 - 200 gm were utilised for the study. The preliminary phytochemical screening of the methanolic extract of stem bark showed the presence of various phytoconstituents namely alkaloids, flavanoids glycosides, saponins, sterols and tannins. Aspirin induced and ethanol induced gastric ulcers has been widely used for the experimental evaluation of antiulcer activity. Aspirin induced and ethanol induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury, the research justifies that the herbal extract can be effectively used in treatment of ulcers.

Key words: *Albino rats, methanolic extract, ulcers.*

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INTRODUCTION:

India is regarded as a herbarium of medicinal plants due to extensive research in herbal medicine.A herb is a plant valued for its medicinal, aromatic or savory qualities. Herbal plants produce and contain a variety of chemical substances that act upon the body.[1]Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value[2].Substances derived from the plants remain basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems[3].The plant products are more popular than synthetic drugs. It is mainly attributed to their low toxicity and also low cost.Traditionally leaf, bark, fruit and seed extracts of many plants are used for the treatment of different ailments.

Modern medicine has provided many breakthrough treatments for serious diseases. Some conditions, however, have eluded the healing grasp of contemporary western medicine, which emphasizes rigorous scientific investigation of therapies[4]. In addition, rising costs of some treatments have placed modern healthcare beyond the reach of many. The drugs that routinely fill pharmacy shelves of post-industrialized nations remain inaccessible to the majority of the people in the world. Instead, populations in many areas of the globe use herbal medicine, also called botanical medicine or phytotherapy, as that of healthcare.

In the present investigation a medicinal plant Ficus dalhousiae miq is evaluated for its ulcer protective effect.Much of the scientific research work and extensive literature has been reported on various pharmacological actions of the plant[5].But there is no information on antiulcer activity of the plant. Hence in the present investigation an initiative has been taken to carry out phytochemical screening and evaluation of antiulcer activity of stem bark methanolic extract of the plant.

MATERIALS AND METHODS:

Swiss Albino rats adult of either sex were obtained from Mahaveer enterprises, Hyd(169/CPCSEA/1999). The rats were divided randomly into 5 groups of 6 rats each for each model. Each rat that weighed between 180-200 gm was housed separately (Four rats per cage). The animals were left for 48 hrs to acclimatize to the animal room conditions. They were maintained in standard laboratory conditions of temperature 22±2°c, humidity, 12 hours light and dark cycles fed with standard pellet diet (Hindustan lever, Bangalore) and adequate tap water.

Methods:

Aspirin induced ulcer model

Experimental design: Rats were divided into 4groups, each group consisting of 6 animals.

Group I: Aspirin (500 mg/kg/body weight, p.o.),
Group II: Ranitidine (20 mg/kg/body weight, p.o.) and Aspirin (200 mg/kg/body weight, p.o.),
Group III: FDSBME (200mg/kg/body weight, p.o) and Aspirin (200 mg/kg/body weight, p.o.),
Group IV: FDSBME (400mg/kg/body Weight,p.o.) and Aspirin (200 mg/kg/body weight, p.o.),

Aspirin induced gastric ulcers in rats[6]

Aspirin was suspended in 1% carboxy methyl cellulose in water and administered orally in the dose of 500mg/kg in 36h fasted rats.Six hours later , the animals were sacrificed,stomachs were removed and opened along greater curvature for determination of ulcer index.Ulcer Index was measured .The acid secretory parameters like total acidity,total acid output and volume of gastric secretion were also measured

Ehanol induced ulcer model

The rats were randomly divided into 4 groups of 6 rats per group.

Group I administered 1ml 80% ethanol
Groups II rats were orally dosed with Ranitidine (20mg/kg/bodyWeight, p.o.), 1ml 80% ethanol
Groups III – FDSBME (200mg/kg/bodyweight, p.o), 1ml 80% ethanol
Groups IV FDSBME (400 mg/kg P.o.), 1ml 80% ethanol

Ethanol induced gastric ulcers in rats[7]

In this model, animals were fasted for 36h before the experiment.1 ml of 80% ethanol was administered p.o in those animals. In the treated group, drug was administered p.o in the dose of 200 and 500 mg/kg,twice a day,1h before the administration of ethanol. After 2h of ethanol administration, animals were sacrificed and stomachs were removed,opened along the greater curvature and subjected to measurement of ulcer index

Free acidity and Total acidity

Centrifuge the gastric contents at 1000 rpm for 10 min, note the volume. Pippete out 1 ml of supernatant liquid and dilute it to 10 ml with distilled water. Note the P^H of the solution with the help of P^H meter. Titrate the solution against 0.01N NaOH using topfers reagent as an indicator.(It is Dimethyl-amino-azo-benzene with phenolphthalein and used for detection and estimation of hydrochloric acid and total acidity in gastric fluids) Titrate to end point when the solution turns to orange colour. Note the volume of NaOH which corresponds to free acidity. Titrate further till the solution regains its pink colour. Note the total volume of NaOH which corresponds to the total acidity. Acidity (mEq/1/100 g) can be expressed as:

$$\textbf{Acidity} = \frac{Vol.of\ NaOH \times Normality \times 100}{0.1}\ \textbf{mEq/l/100 g}$$

Estimation of Glutathione and tissue Malanoldialdehyde was done by the method described by moron et al.

Statistical analysis of data

Results were expressed as mean ± S.E.M. The difference was considered significant if P< 0.05 .The statistical analysis was done by using graph pad prism software.

RESULTS AND DISCUSSION:

Effect on Gastric Volume

Administration of the FDSBME significantly decreased the gastric volume in comparison with rats treated with Ranitidine. Comparing the gastric volume and gastric acidity, the gastric volume gets decreased, simultaneously the gastric acidity also decreased significantly.(tab 1&2)

Effect on Free Acidity and Total Acidity

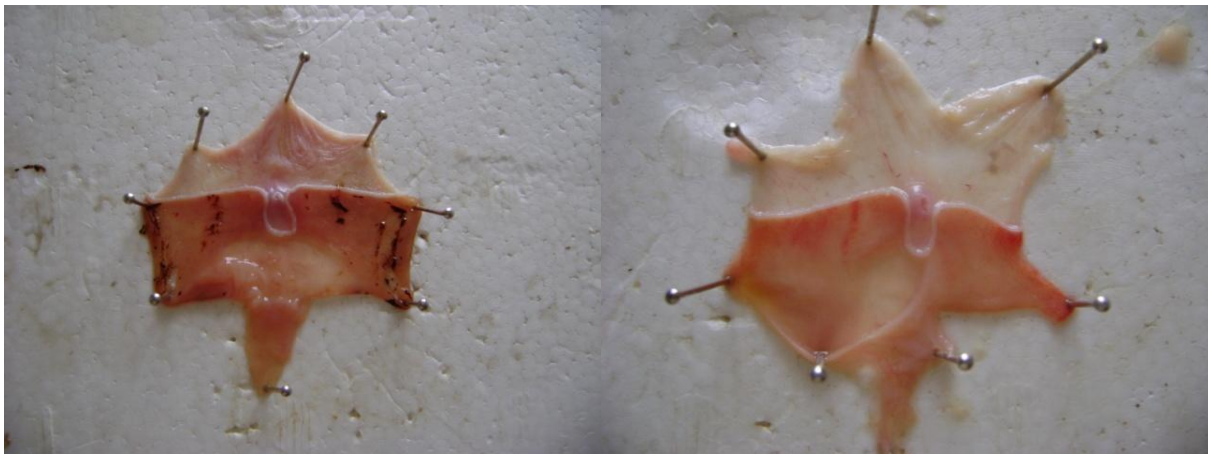
The free acidity and total acidity was determined based on the titre values. The free acidity and total acidity of extract on albino rats decreased significantly in comparison with the standard group treated with Ranitidine (tab 3&4)

Ulcer index

The ulcer index was calculated by taking the mean ulcer score of each groups.Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-axis. The histograms of different groups were then interpolated by comparing the ulcer index of group I with group II, III and IV. It was noticed that the ulcer index of Dose group (Dose-III&IV) was significantly less when compared to the standard group (Group-II) treated with Ranitidine. (tab 5&6)

Macroscopical View of Rat Stomach

After mounting the rat stomach on glass slide and observed in 10x magnification, different scores were noted in each groups. The mean ulcer score represnts the ulcer index. The scores on each group were compared, ie group I with group II, III and IV. It was noticed that Group III and IV shows similarity in the score as that of group II.



GROUP I (ASPIRIN500mgkg) **GROUP II (Ranitidine+Aspirin500mg/kg)**

GROUP III (FDSBME 200mg/kg) **GROUP IV (FDSBME 400mg/kg)**

Table 1: Results of gastric volume in aspirin induced ulcer model

Groups	Drugs given	Gastric volume
GROUP I	Aspirin(500mg/kg) +1% CMC	1 ± 0.04
GROUP II	Ranitidine(20mg/kg) + Aspirin(500mg/kg)	0.5 ± 0.05**
GROUP III	FDMLE(200mg/kg) + Aspirin(500mg/kg)	0.8 ± 0.03*
GROUP IV	FDMLE (400mg/kg) + Aspirin(500mg/kg)	0.6 ± 0.04**

Table 2: Results of Gastric Volume in Ethanol Induced Ulcer Model

Groups	Drugs given	Gastric volume
GROUP I	Ethanol(5 mL/kg)	1.5 ± 0.06
GROUP II	Ranitidine(20mg/kg) + Ethanol 1ml	0.6 ± 0.07**
GROUP III	FDSBME(200mg/kg) + Ethanol 1ml	0.9 ± 0.02**
GROUP IV	FDSBME (400mg/kg) + Ethanol 1ml	0.67 ± 0.03***

Table 3: Results of Free Acidity and Total Acidity in Aspirin Induced Ulcer Model

Groups	Drugs given	Free Acidity	Total Acidity
GROUP I	Aspirin(500mg/kg) + 1% CMC	14.70 ± 0.29	29.6 ± 0.69
GROUP II	Ranitidine(20mg/kg) + Aspirin(500mg/kg)	4.6 ± 0.31*	9.5 ± 0.69*
GROUP III	FDSBME(200mg/kg) + Aspirin(500mg/kg)	8.2 ± 0.32*	11.56 ± 0.68*
GROUP IV	FDSBME (400mg/kg) + Aspirin(500mg/kg)	6.3± 0.42**	9.8 ± 0.59**

Table 4: Results of Free Acidity and Total Acidity in Ethanol Induced Ulcer Model

Groups	Drugs given	Free Acidity	Total Acidity
GROUP I	Ethanol(5 mL/kg)	18.70 ± 0.25	39.4 ± 0.63
GROUP II	Ranitidine(20mg/kg) + Ethanol 1ml	5.9± 0.33**	10.23 ± 0.68*
GROUP III	FDSBME(200mg/kg) + Ethanol 1ml	8.9 ± 0.32*	12.56 ± 0.74**
GROUP IV	FDSBME (400mg/kg) + Ethanol 1ml	6.25 ± 0.42**	10.5 ± 0.51***

Table 5: Results of Ulcer Index in Aspirin Induced Ulcer Model

Groups	Drugs given	Ulcer index
GROUP I	Aspirin(500mg/kg) + 1% CMC	3.7 ± 0.14
GROUP II	Ranitidine(20mg/kg) + Aspirin(500mg/kg)	1.5± 0.10***
GROUP III	FDSBME(200mg/kg) + Aspirin(500mg/kg)	2.2± 0.18*
GROUP IV	FDSBME (400mg/kg) + Aspirin(500mg/kg)	1.8 ± 0.9**

Table 6: Results of Ulcer Index in Aspirin Induced Ulcer Model

Groups	Drugs given	Ulcer index
GROUP I	Ethanol(5 mL/kg)	4.2± 0.16
GROUP II	Ranitidine(20mg/kg) + Ethanol 1ml	2.1± 0.14**
GROUP III	FDSBME(200mg/kg) + Ethanol 1ml	2.9± 0.13**
GROUP IV	FDSBME (400mg/kg) + Ethanol 1ml	2.25 ± 0.25**

Result of MDA And GSH in Experimental Models

The MDA levels decreased in both the experimental models after administration of standard drug and test extracts,which were elevated in control group(tab 7&8).In contrast to this there was increase in the levels of GSH which was decreased in the control group(tab 7&8)

Table 7: Result of MDA And GSH in Aspirin Induced Ulcer Model

Groups	Drugs given	MDA	GSH
GROUP I	Aspirin(500mg/kg) + 2% Tween 80	18.57±0.491	15.56±1.159
GROUP II	Ranitidine(20mg/kg) + Aspirin(500mg/kg)	14.73±0.498^{ns}	27.31±0.936^{ns}
GROUP III	FDSBME(200mg/kg) + Aspirin(500mg/kg)	16.97±0.829**	31.88 ±0.96**
GROUP IV	FDSBME (400mg/kg) + Aspirin(500mg/kg)	14.97±0.679**	32.88 ±0.76**

Table 8: Results of MDA and GSH in Ethanol Induced Ulcer Model

Groups	Drugs given	MDA	GSH
GROUP I	Ethanol 1ml	20.57 ±0.491	20.56 ±1.159
GROUP II	Ranitidine(20mg/kg) + Ethanol 1ml	13.73 ±0.498^{ns}	28.31 ±0.936^{ns}
GROUP III	FDSBME(200mg/kg) + Ethanol 1ml	15.97 ±0.829**	27.88 ±0.96**
GROUP IV	FDSBME (400mg/kg) + Ethanol 1ml	13.97 ±0.679**	25.88 ±0.76**

DISCUSSION:

It is evident from the result of the present investigation that the formulation of *Ficusdalhousie* possesses antiulcer activity in aspirin induced and ethanol induced acute ulcer model. It has shown a significant reduction in the gastric lesions in both the models. Although the etiology of gastric ulcer is not known in most cases, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms[8]. To regain the balance, different therapeutic agents including plant extracts are used (in experimental animals) to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucus production, stabilizing the surface epithelial cells/or enhancing prostaglandin synthesis. Ranitidine the proton pump inhibitor play an important role in the reduction of gastric volume and total acidity and thus perform a cytoproective effect.

The present results demonstrate that the formulation of *Ficusdalhousiae* protects the rat gastric mucosa against hemorrhagic lesions produced by aspirin and ethanol. These inducing methods of gastric lesions are rapid and convenient way of screening plant extracts for antiulcer potency and cytoprotection in macroscopically and microscopically visible lesions[9]. Aspirin induced and ethanol induced gastric ulcers has been widely used for the experimental evaluation of antiulcer activity. Aspirin induced and ethanol induced gastric lesion formation may be due to stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspect of tissue injury[10]. It is of interest to note that administration of antioxidants inhibit aspirin induced gastric injury in the rats. And also that the estimation of Glutathione shows that the levels were increased and Estimation of Malanoldialdehyde showed that the levels were decreased when given FDSBME as compared to the standard. Ficusdalhousie possess significant antioxidant activity. In conclusion, the antiulcer effects of the above plants have been reported earlier, but there are no studies reporting the combination of these herbals and their activity in these models are quite impressive. The antiulcer activity of the formulation FDSBME can be compared to the activity of the standard drug Ranitidine.

CONCLUSION:

From the results discussed above it can be summarized that the FDSBME possess the gastro protective activity against the Aspirin and Ethanol induced gastric ulceration animal model of rats. And also the dose level tested does not show any signs of toxic effects in treated mice as well as rats.

In the present study the methanolic extract of Ficus dalhousiae shows better anti-ulcer activity by the decrease in gastric acid secretion, free acidity and also by reducing the levels of MDAand increasing the levels of glutathione i.e.,anti oxidant activity through decrease in free radicals. The phytochemicals test shows the presence of flavonoids, alkaloids, proteins amino acids and carbohydrates other polyphenolic compounds. Hence, the research justifies that the herbal extract can be effectively used in treatment of ulcers. Further studies are needed to isolate and characterize the active components responsible for the gastro protective of the test extract and findings should be confirmed by performing clinical studies.

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