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RESEARCH ARTICLE

A Study on Anti-Ulcer Activity of *Diospyros Malabarica* Bark in Ulcer Induced Rats

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ABSTRACT

Peptic ulcer is a common gastrointestinal disease and it affects particularly the working years of a patient's life and its social implications are considerable. In the indigenous system of medicine, the DM bark is reported to be useful in the treatment of gastric ulceration. Hence, in the present study, the DM bark has been selected for its anti-ulcer potency on experimentally induced ulcer in rats. The authenticated DM bark, were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of DM bark was Soxlet extracted with the solvents with increasing order of polarity i.e. petroleum ether (60-80^{o}C), chloroform (59.5-61.5^{o}C), ethanol (64.5-65.5^{o}C), and distilled water. The extracts obtained were concentrated under reduced pressure. In the pharmacological screening, the effect of different extracts of DM bark was evaluated for their anti-ulcer profile by using Pylorus ligation, and ethanolic induced models using albino rats. Preliminary phytochemical investigation revealed the presence of flavonoids, carbohydrates, proteins, steroids, tannins and glycosides in chlorofom extract. LD_{50} cut-off dose was found to be 2000 mg/kg b.w. for the extracts of DM bark. Hence therapeutic dose was taken as 500 mg/kg b.w. for all extracts. Treatment with chlorofom extract of DM bark significantly showed the anti-ulcer activity as compared to control. The results were comparable to that of standard drug (Lansoprazole). From the literature survey and the work carried out, it may be confirmed that DM bark does possesses anti-ulcer property. Phytochemical investigation suggests the presence of flavonoids, tannins which may be responsible for the anti-ulcer activity.

KEYWORDS

Ulcer, *Diospyros Malabarica*, Extraction, Flavonoids, Pylorus Ligation, Chlorofom, Lansoprazole

INTRODUCTION

Nature

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The plants are indispensable to man for his life. The three important necessities of life –food, clothing, and Shelter and a host of other useful products supplied to him by the plant kingdom. Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring health care.[1] Nature's beauty in terms of health care is appreciated by developing various systems of traditional system (Ethnomedicine). Ethnomedicine may be defined broadly as the use of plants by human as medicine, but this use could be called ethno botanic medicine.

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Ayurveda –Indian System of Medicine

Ayurveda consider human being as a whole. It believes that imbalance in the Dosha, Dhatus and Malas generated disease and the restoration of balance in these eliminates disease. The aim of treatment is not only to cure the disease but also to root out the cause so that it may not take place in future. The aim of treatment is also to improve the vitality and to strengthen the immune system.[2]

Peptic Ulcer Disease (PUD) is a serious gastrointestinal disorder that requires a well-targeted therapeutic strategy. A number of drugs including proton pump inhibitors and H_2 receptors antagonists are available for the treatment of peptic ulcer, but clinical evaluation of these drugs has shown incidence of relapse, side effects, and drug interactions. This has been the rational for the development of new antiulcer drugs and the search for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse.

The objectives of the present study are

The number of pharmacological investigations on *Diospyros malabarica bark* has been reported so far. However, there is a need to evaluate the gastroprotective activity of *Diospyros malabarica bark* in experimental animals.

The present study is therefore, undertaken with the following objectives.

1. Extraction of *Diospyros malabarica bark* with suitable solvents.
2. Preliminary phytochemical screening of crude extract.
3. Determination of LD_{50} on mice.
4. Evaluation of various solvent extracts of *Diospyros malabarica bark* for anti-ulcer activity by following models in rats.

A. Pylorus ligation induced ulcer model.

B. Alcohol induced ulcer model.

I. Pylorus ligation induced ulcer model.

Parameters to study: Ulcer index

: Volume of gastric juice.

: pH of gastric juice.

: % protection.

II. Alcohol induced ulcer model.

Parameters to study: Ulcer index

: % protection

METHODOLOGY

Extraction[3]

The authenticated *Diospyros malabarica bark* were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the *Diospyros malabarica bark* was Sox let extracted with the solvents with increasing order of polarity i.e. petroleum ether (60-80^{o}C), chloroform (59.5-61.5^{o}C), ethanol (64.5-65.5^{o}C), and distilled water. The extracts obtained were concentrated under reduced pressure.

Qualitative Chemical Test[4]

Acute (Oral) Toxicity Study (Fixed Dose Procedure)[5]

Method: Acute toxicity studies for chlorofom extract of *Diospyros malabarica bark* were conducted as per OECD guideline 420 (modified, adopted 23rd march 2006) using Albino Wister mice. Each animal was administered chlorofom extracts solution by oral route. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD_{50}, confidence intervals. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Anti-Ulcer Activity

Pylorus Ligation[6]

Method: Albino wistar rats of either sex weighing between (150-200gms) were divided into six groups of six animals in group.

1. Group-I – Control (2% gum acacia)

2. Group-II – Standard (Lansoprazole 8mg/kg in 2% gum acacia p.o).
3. Group-III – chlorofom extract of *DM* bark (250mg/kg p.o.).
4. Group-IV – chlorofom extract *DM* bark (500mg/kg p.o.).

In this method albino rats were fasted in individual cages for 24 hr. care was taken to avoid coprophagy. *DM* bark powder extracts or standard drug or control vehicle was administered 30min. prior to pyloric ligation. Under light ether anaesthesia, give an incision of 1cm long in the abdomen just below the sternum.

Expose the stomach pass a thread around the pyloric sphincter and apply a tight knot. While putting the knot care was taken so that no blood vessels are tied along the knot. The abdomen was sutured clean the skin from any blood spots and bleeding. Apply collodion over the wound. At the end of 4 hr. after ligation the animals were sacrificed with excess of anaesthetic ether.

Open the abdomen and tie the oesophageal end (cardiac end) of the stomach. Cut and removed the entire stomach from the body of the animal. Gastric juice was collected into graduated centrifugation tube and was centrifuged at 1000 rpm for 10 min. and gastric volume was noted. The p^H of the gastric juice was recorded by p^H meter. Then the centrifuged supernatant contents were subjected to analysis for free and total acidity. Open the stomach along the greater curvature and washed with running water to see for ulcers in glandular portion of the stomach.

The number of ulcers per stomach was noted and severity of the ulcers of the ulcers scored microscopically with the help of hand lens (10X) and scoring was done as following.

0 = normal stomach.

0.5 = red coloration.

1.0 = spot ulcers.

1.5 = hemorrhagic streaks.

2.0 = ulcer > 3 but < 5.

3.0 = ulcer > 5

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula,

Percentage protection = $100 - U_t/U_c \times 100$

Where, U_t = ulcer index of treated group.

U_c = ulcer index of control group.

In this method following parameters was studied-

1. pH of gastric juice.
2. Volume of gastric secretion.
3. Ulcer index.
4. % protection.

Ethanol Induced Ulcers[7]

Albino wistar rats of either sex weighing between (150-200gms) were divided into six groups of six animals in group.

1. Group-I – Control. (Absolute Ethanol 1ml/200gm p.o.)
2. Group-II – Standard (sucralfate 400mg/kg p.o).
3. Group-III – chlorofom extract *DM* bark (250mg/kg p.o.).
4. Group-IV – chlorofom extract *DM* bark (500mg/kg p.o.).

On day 5, the animals were fasted for 24 hrs with free access to water. Animals were giving the extract of *DM* bark and standard Lansoprazole as mentioned above. Absolute alcohol at the dose of 1ml/200 gms was administered to the animals on the day of the experiment one hour after the administration of extracts or standard.

The animals are sacrificed 1h after administration of ethanol and the stomach is removed and opened along the greater curvature. Lesions are examined with the help of hand lens (10X) and sample was sending to further Histopathological study. Scoring is done for all the models as given below

Normal coloration: 0

Red coloration: 0.5

Spot ulcer: 1.0

Hemorrhagic stress: 1.5

Ulcer >3 but <5

Ulcer >5

Mean ulcer score for each animal is expressed as ulcer index. The percentage protection was calculated using the formula,

$$\text{Percentage protection} = 100 - \frac{U_t}{U_C} \times 100$$

Where, U_t = ulcer index of treated group.

U_c = ulcer index of control group

In this method following parameters was studied

1. Ulcer index.
2. % protection.

Statistical Analysis

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Dennett's 't' test P value less than <0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

RESULTS

Table 1: Preliminary Phytochemical Screening

S. no	Type of phyto chemical constituents	Pet. ether extract	Chloroform Extract	Ethanolic Extract	Aq. Extract
1	Carbohydrates	-	+	+	+
2	Proteins	-	-	+	+
3	Flavonoids	-	+	-	-
4	Steroids	+	+	+	-
5	Tannins	-	-	+	+
6	Saponin glycosides	-	-	+	+
7	glycosides	-	+	+	+
8	Alkaloids	-	-	+	-

[+ = present, - = absent]

Pylorus Ligation Ulcer Model

Effect of Chloroform Extracts of Diospyros malabarica Bark on pH of Gastric Secretion following Pylorus Ligation in Rats

At 250 mg/kg&500mg/kg the pH was remained unchanged when compared with control. The influence on the pH in pylorus ligation of Lansoprazole (8mg/kg); chloroform extracts of Diospyros malabarica bark (250, 500mg/kg) is mentioned in the following table.

Table 2: pH of Gastric Secretion

Group no.	Treatment	Dose	pH
1	Control	-	1.3±0.10
2	Lansoprazole	8 mg/kg	5.717±0.125***
3	Chlo. Extract 250mg	250 mg/kg	1.56±0.125
4	Chlo. Extract 500mg	500 mg/kg	2.10±0.125***

Data are expressed as Mean ± S.E.M

*** indicates significant difference when compared with control

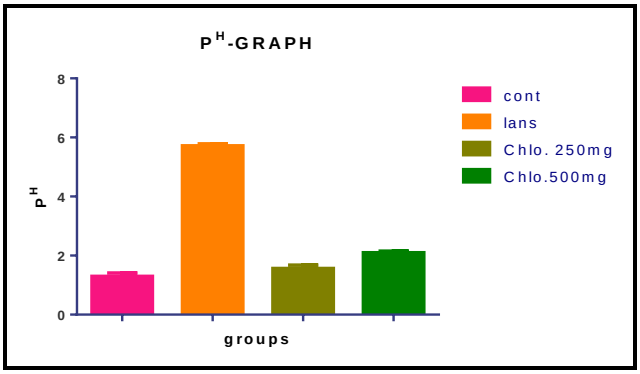

Figure 1: pH Graph

Effect of Chloroform Extracts of Diospyros malabarica Bark on Volume of Gastric Secretion following Pylorus Ligation in Rats

At 500mg/kg the volume of gastric juice secretion was significantly reduced by chloroform extract of DM bark in dose dependent manner when compared with control. The influence on the volume of gastric juice secretion in pylorus ligation of Lansoprazole (8mg/kg); chloroform extract of DM (250, 500mg/kg).

Table 3: Gastric Volume Table

Grp. no	Treatment	Dose	Volume of Gastric Juice
1	Control	-	6.35 ± 0.14
2	Lansoprazole	8 mg/kg	1.03±0.16** *
3	Chlo. Extract 250 mg	250 mg/kg	6.51±0.16
4	Chlo. Extract 500 mg	500 mg/kg	4.91±0.16 ***

Data are expressed as Mean ± S.E.M (n=6)

*** indicates significant difference when compared with control

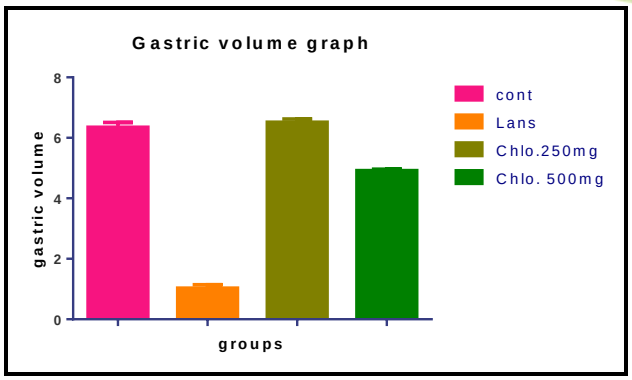

Figure 2: Gastric Volume Graph

Effect of Chloroform Extracts of Diospyros malabarica Bark on Ulcer Index and their % Protection in Pylorus Ligation induced Ulceration in Rats

At 250 & 500mg/kg the ulcer index had significantly reduced by Chlorofom extract of Diospyros malabarica in dose dependent manner when compared with control and percentage protection is comparable to lansoprazole.

The influence on the ulcer index in pylorus ligation of Lansoprazole (8mg/kg); Chlorofom extract of Diospyros malabarica 250, 500 mg/kg. Along with the percentage protection that had significant changes are summarized in Table.

Table 4: Ulcer Index

Grp. No	Treatment	Dose	Ulcer Index	% Protection
1	Control	-	7.33±0.14	0%
2	Lansopra-zole	8 mg/kg	1±0.42 ***	86.35%
3	Chlo. Extract 250 mg	250 mg/kg	2.31±0.36	68.48%
4	Chlo. Extract 500mg	500 mg/kg	1.81±0.35 ***	75.30%

Data are expressed as Mean ± S.E.M (n=6)

*** indicates significant difference when compared with control

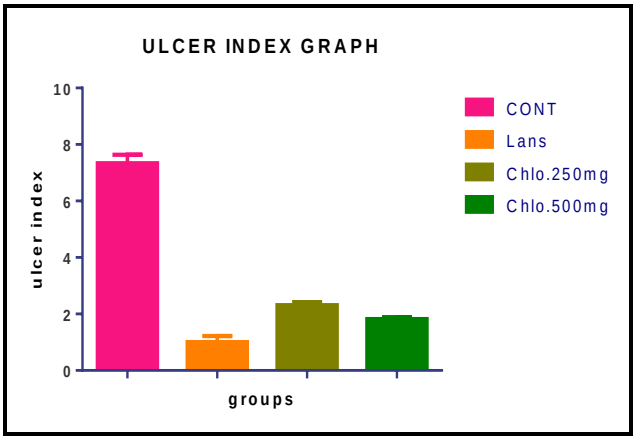

Figure 3: Ulcer Index Graph

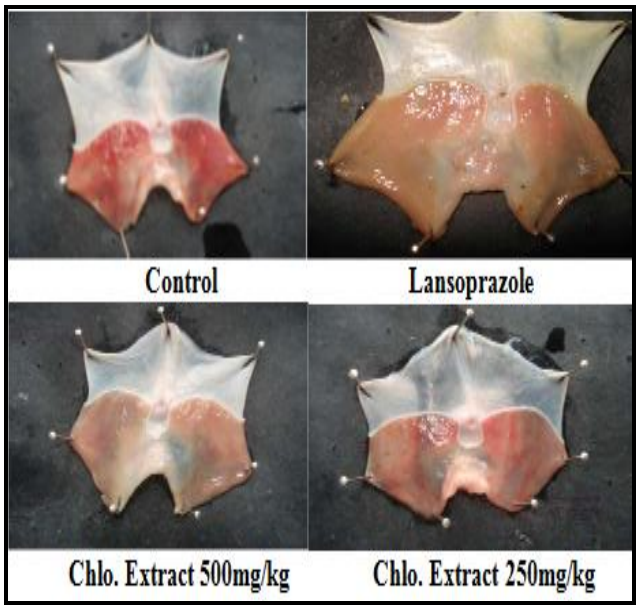

Figure 4: Effect of Chloroform Extracts of Diospyros Malabarica Bark on Ulcer Healing in Pylorus Ligation Model

Ethanol Induced Ulcer

Effect of Chloroform Extracts of Diospyros malabarica Bark on Ulcer Index and their % Protection in Ethanol induced Ulcer in Rats

At 250 & 500 mg/kg the ulcer index had significantly reduced by Chlorofom extract of *Diospyros malabarica* in dose dependent manner when compared with control, respectively and percentage protection is comparable to sucralfate.

Table 5: Ulcer Index

Grp no	Treatment	Dose	Ulcer Index	% Protection
1	Control	-	6.08± 0.19	0%
2	Sucralfate	400 mg/kg	1.33± 0.18 ***	78.12%
3	Chlo. Extract 250mg	250 mg/kg	2.15± 0.18	64.63%
4	Chlo. Extract 500mg	500 mg/kg	1.83± 0.18 ***	69.90%

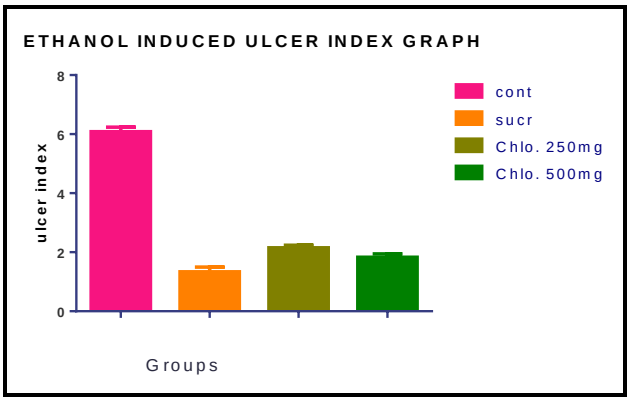

Figure 5: Ulcer Index Graph

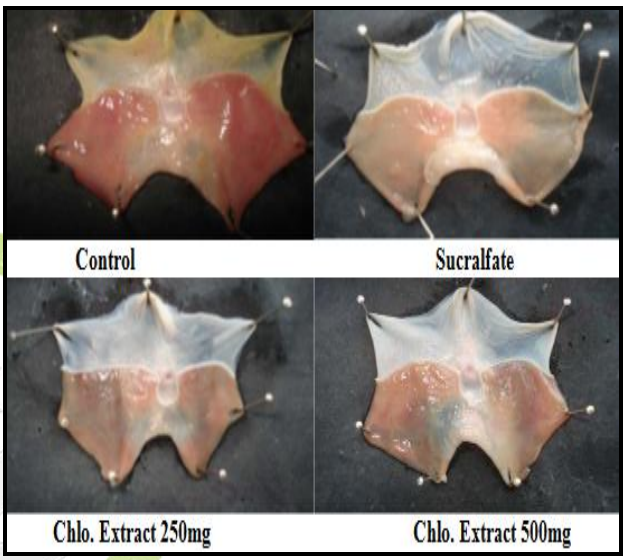

Figure 6: Effect of Chloroform Extracts of Diospyros Malabarica Bark on Ulcer Healing in Ethanol Induced Ulcer Model

CONCLUSION

- Anti ulcer activity of *Diaspyrous malabarica bark* was confirmed by using different rat models (pylorous ligation, ethanol induced ulcer models.) and different doses of Chloroform extracts i.e. 250 mg/kg and 500 mg/kg of body weights.
- 8 mg/kg body weight of lansoprazole (pylorus ligation model) treated group increased the pH of gastric fluid to slightly neutral and reduced the volume of gastric fluid when compared to control group.
- Chloroform extracts treated groups at the dose level of 250 mg/kg and 500mg/kg body weight showed nearly same results of pH as that of control group i.e. acidic .The

volume of gastric fluids in chloroform extracts treated groups at the dose level of 500mg/kg body weight has decreased significantly.

- Among all the treated groups, chloroform extract of *Diaspyrous malabarica* at the dose level 500 mg/kg body weight offered greater percentage protection by reducing ulcer index in all the two models(pylorus ligation, ethanol induced models) studied when compared with standards. Lansoprozole (pylorus ligation model) and sucralfate (ethanol induced model)are the respective standards used.
- Flavonoid is an anti-oxidant and its mucosal barrier protecting capacity may be responsible for the anti ulcer activity of *Diaspyrous malabarica* bark.

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