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Research Article

ASSESSMENT OF ANTIULCER ACTIVITY OF *MOLLUGO PENTAPHYLLA* LINN. IN SOME EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

To investigate the gastroprotective activity of ethyl acetate fraction and chloroform fraction of the methanolic extract of aerial parts of *Mollugo pentaphylla* in different experimental models of gastric ulcer in rats. The ethyl acetate fraction and chloroform fraction of the methanolic extract of aerial parts of *Mollugo pentaphylla* were studied at one dose level (200 mg/kg, oral) in rats against pyloric ligation induced gastric ulcer, ethanol induced gastric ulcer, sodium hydroxide induced gastric ulcer and stress induced ulcer. Ranitidine (100 mg/kg, oral) was used as a standard drug. Mean ulcer indices and oxidative stress were measured. Acute toxicity test was also carried out. Administration of *Mollugo pentaphylla* to rats significantly decreased the ulcer index value when compared with the control treated group. Ranitidine (100 mg/kg, oral) also produced a significant decrease the ulcer index value when compared with the control treated group. This antiulcer activity may have for the phytoconstituents such as flavonoids, saponin and glycoside present in the plant. The results suggest that the aerial part of the *Mollugo pentaphylla* possess significant anti ulcer activity.

Keywords: *Mollugo pentaphylla,* Ethyl acetate fraction and chloroform fraction, Ranitidine, Gastric ulcer, Histology.

INTRODUCTION

Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. The traditional heritage of India includes many true tested medicinal plants/drugs for various diseases and to which there is no answer in modern medicine till today. Peptic ulcer disease is ulceration of mucous membrane penetrating through the muscularis mucosa exposed to acid and pepsin in stomach and duodenum. If ulceration occurs in Stomach, it is known as Gastric ulcer and when it is in duodenum, it is known as duodenal Ulcer. Peptic ulcer disease is a common medical emergency with an annual incidence of approximately 100 per 100,000 adults and overall mortality of 10 to 15% in recent studies[1].

Mollugo pentaphylla Linn. (family- Aizoaceae) is commonly known as carpet weed (English), Pita-gohun (Oriya). It is an erect slender, much branched annual herb, up to 30 cm. high, commonly found in dry as well as moist areas. Leaves are falsely whorled or opposite, linear-lanceolate to obovate. Flowers are white, greenish, orange or pink, in terminal compound cymes. Capsules are globose with many dark reddish brown seeds. Roots are creaper and adventitious[2]. The plant contains carotene, traces of vitamin C, saponin and potassium nitrate. It is also having numerous applications in traditional medicine as stomachic, aperient, antiseptic, emmenagogue and is also used in poultices for sore legs. An infusion of the plant is given to women to promote the menstrual discharge. Leaves are bitter and antiperiodic; they are warmed after smearing with oil and applied to the ear to relieve earache[3]. It has been reported that the plant possesses antimicrobial[4], whooping cough[5], hepatitis[6], anticancer[7], spermicidal[8], antibacterial[9] and antifungal activity[10]. Therefore, this present study was under taken to evaluate anti-ulcer activity potential of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of *Mollugo pentaphylla* (MEMP) on various animal models.

MATERIALS AND METHODS

Collection of the plant

The plant was collected from rural belt of West Bengal, Purbamedinipur district, during the month of July and August 2010 in the early morning. The plant was identified, confirmed and authenticated by taxonomist in the Department of Botany, Utkal University, and Bhubaneswar. After authentication the plant was collected in bulk and washed under running tap water to remove adhering dirt and soil particles. The aerial part of plants was dried under shade at room temperature, after washing. The dried materials were made into coarse powder by grinding in mechanical grinder and passed in sieve no 40 and used for further study.

Preparation of extracts

The coarse powder of the plant was taken in soxhlet apparatus and extracted with methanol and Water. The extraction with each solvent is done for 72 hours and the temperature was maintained in between 37-40ºc to prevent the loss of thermo sensitive constituent of the plant. The methanolic extract is mixed with water (1:1) and used for fractionation with chloroform and ethyl acetate by the use of separating funnel. The liquid extracts and fractionation were concentrated separately under vacuum and resulting dried extracts were preserved in desiccators until further use.

Treatment of animals

Wistar albino mice of either sex weighing between 25 and 30 g were selected for acute toxicity studies and healthy adult male Wistar albino rats weighing between 150 and 200 g were selected for the antiulcer studies taken from the Laboratory Animal bread center, School of pharmaceutical sciences, SOA University. The animals were acclimatized to standard laboratory conditions of temperature (22±3ºC) and maintained on 12:12 h light: dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai) and distilled water *ad libitum.* The animal care and experimental protocols were in accordance with CPCSEA/ IAEC.

Acute toxicity study

Acute toxicity study was performed as per OECD guidelines 423. (Acute toxicity class method) [11]. Eight groups of two mice each,of mixed sex fasted overnight were kept under loboratory conditions and allowed free access to water.The Ethylacetate fraction and Chloroform fraction at concentration of 1000, 2000, 4000, 5000mg/kg dissolved in distilled water were administered orally via a gastric catheter.

Anti-ulcer Study

Anti Secretory Studies

Pylorus Ligation induced gastric ulcers

Group I- Received Solvent (2ml/kg)

Group II- Received Ranitidine (100mg/kg)

Group III- Received Ethyl Acetate fraction (200mg/kg)

Group IV -Received Chloroform fraction (200mg/kg)

Albino rats were divided into four groups of six animals each and fasted for 48 h with free access to water. Pyloric ligation was performed under light ether anesthesia to each animal. Animals were given 1% CMC solution or fraction solutions 200mg/ kg or 100 mg/ kg Ranitidine orally immediately after pylorus ligation. Animals were sacrificed 4 h later. The stomach was carefully removed and gastric contents were collected. The gastric juice was centrifuged at 3000 rpm for 30 min and the volume of the gastric juice was measured. Free and total acidities in the supernatant were determined by titration with 0.1 N NaOH and expressed as mEq/L/100 g. The stomach was cut open along the greater curvature and pinned on a soft board for evaluating gastric ulcers and ulcer index was calculated.[12]

Determination of free acidity and total acidity

The gastric contents were centrifuged at 1000 rpm for 10min. 1ml of supernatant was diluted with 9 ml of distilled water. A volume of 2 ml diluted gastric juice was titrated with 0.1N Sodium hydroxide run from a microburette using 3-4 drops of Toper's reagent as indicator until canary yellow colour was observed. Volume of NaOH required was noted. This corresponds to free acidity. Further 2-3 drops of phenolphthalein was added and titrated with NaOH until pink colour was restored. This gives total acidity, free acidity and total acidity is expressed in terms of ml of 0.1N HCl per 100 gm of gastric contents. This is the same as mEq/lit. To obtain this figure multiply the burette reading obtained from titration by 10.[13]

Ulcer Scoring & Ulcer Index Determination:

Each stomach was examined by10×magnification for ulcers [14]. Score the ulcers as below:

0 - Normal coloured stomach

0.5 - Red coloration

1.0 - Spot ulcers

1.5 - Hemorrhagic streaks

2 - Ulcers ≥3 but ≤5

3 - Ulcer >5

Pylorus Ligation induced by aspirin gastric ulcer

Group I- Received aspirin Solvent (200mg/kg)

Group II- Received Ranitidine (100mg/kg)

Group III- Received Ethyl Acetate fraction (200mg/kg)

Group IV -Received Chloroform fraction (200mg/kg)

Out of four groups of six rats each, six were used as treatment groups and six each for Aspirin and Ranitidine treated group. The modified method of Goal et al (1985) was used for the production of experimental gastric ulceration. Aspirin (200 mg/kg) suspended in carboxy methylcellulose 1% was administered orally using a round trip stainless steel stomach tube. Ethyl acetate and Chloroform each extract (200 mg/kg) was given 3 hours prior to and after aspirin administration. Treatment was continued for 3 days and the pylorus was ligated on the fourth day under ether anaesthesia. The abdomen was closed and the animals were left to recover. Drinking water was withheld. After 4 hours rats were killed with an overdose of chloroform, the oesophagus was ligated and the stomach was removed. The gastric mucosa was washed with 3 ml distilled water. The gastric juice and the washings were homogenized and centrifuged at 5000 rpm for 5 min. The volume of gastric juice was measured and expressed as ml/100g body weight. The stomach was then cut along the great curvature and the glandular portion was examined under dissecting microscope. The number of ulcers was counted and the total length was measured. The curative ratio was calculated as mentioned before. [15]

Cytoprotective Studies

Ethanol induced gastric ulcer

Group I- Received solvent (10ml/kg).

Group II- Received Ranitidine (100mg/kg).

Group III- Received Ethyl acetate Extract (200mg/kg).

Group IV- Received Chloroform Extract (200mg/kg).

Twenty six Wister rats (150-200g body wt) were kept under standard conditions before their use. Rats were randomly divided into 4 equal groups. Animals were starved for 48 hour before use to ensure an empty stomach and were kept in cages with raised floors of wide wire mesh to prevent coprophagy. To prevent excessive dehydration during the fasting period, rats were supplied with sucrose (BDH) 8% (w/v) solution in NaCl (BDH) 0.2% (w/v) which ware moved 1 hour before experiments [16]. In the first day, rats of 2 groups were orally given two doses of 400 mg/kg of each extract 6 hours apart. The 400 mg/kg dose was chosen depending on the normal daily consumption of an adult man of the dried plants relatively to their yield of extract[15]. A third dose was given on the 2nd day 1.5 hours before oral administration of ethanol (Merck) 50% (v/v) in distilled water in a dose of 10 ml/kg.The control group was given equal volume of distilled water instead of the plant extracts but received ethanol in the same dose and route. In addition a group was given Ranitidine as a reference drug in a dose of 100 mg/kg by the same route and at the same time intervals. One hour after ethanol administration, all rats were euthanized by an over dose of chloroform and the abdomen was opened and the stomach were rapidly removed, opened along their greater curvature and gently rinsed under running water. Lesions in the glandular part of the stomach were measured under illuminated magnifying microscope (10).Long lesions were (v/v) in distilled water in a dose of 10 ml/kg. The control group was given equal volume of distilled water instead of the curative ratio was determined by the following formula:

Curative ratio =(control ulcer index –Test ulcer index)/(Control ulcer index)×100.

0.2M Sodium Hydroxide Induced Ulcer in Rats

Wister albino rats of either sex weighing 180-250 gm were divided into four groups of six animals each.Animals were fasted for 72 hours with water ad libitum.

Group I-Received control (1ml/kg)

Group II-Received Ranitidine (100mg/kg)

Group III -Received Ethyl acetate fraction (200mg/kg)

Group IV-Received Chloroform acetate fraction (200mg/kg)

The dosage to be tested were administered 30 min before the administration of necrotizing agent (i.e. 0.2 M sodium hydroxide) 1ml of 0.2M sodium hydroxide was administered by oral to each group[17]. Animals were sacrificed after 1hour, after the administered of necrotizing agent and the sum of length of erosion was expressed as lesion index.

Stress Induced Ulcer (Cold resistance stress induce ulcer)

Wister albino rats of either sex weighing 180 -250 gms were divided into four groups f six animals each. Animals were fasted for 12 hours with free access of water.

Group I - Received Solvent (2ml/kg)

Group II- Received Ranitidine (100mg/kg)

Group III- Received Ethyl acetate fraction (200mg/kg)

Group IV- Received Chloroform acetate fraction (200mg/kg)

One hour after test substance treatment, the experimental rats were immobilized by strapping all the four limbs on a wooden plank and kept for 4 h, at 4º±1ºC[35]. After 4 h, the animals were sacrificed, gastric juice was collected, ulcers were examined on the dissected stomachs and ulcer index was measured. The pH of gastric juice was measured using *p*H paper [1].

RESULTS

Acute toxicity study

After administration of test sample, the animals were observed carefully for first 4 h for no behavioral changes. It was found that, after 48hours, the fractions were non toxic in the group treated with 5000mg/kg. **(Table 1 and 2).**

Anti-ulcer Study

Anti-Secretory Studies

Table 3 showed the status of gastric volume, Ulcer index, pH, free acidity and total acidity content of the groups treated with the EAF and CFF of *Mollugo pentaphylla* along with groups treated with standard drug ranitidine and the solvent control, in pylorus ligated gastric rat ulcer model.

1. Effect on Gastric volume: The data revealed that EAF and the standard drug ranitidine reduced the gastric volume significantly ($p<0.001$) when compared to that of the solvent control, while CFF registered no significant reduction in the same. However, the F-value showed no significant difference among the treated groups.

2. Effect on Ulcer index: The data represented that, EAF and the standard drug ranitidine showed a significant ($p<0.001$) reduction in the ulcer index i.e. 0.91 and 0.16 respectively as compared to that of solvent control group which registered the ulcer index of 6.0, while CFF showed no significant reduction in the ulcer index (4.2) profile when compared with that of solvent control; however the various treated groups showed a significant difference ($p<0.01$) among themselves.

3. Effect on pH: The data depicted that EAF and the standard drug ranitidine registered a significant ($p<0.001$) reduction in the pH of the gastric contents as compared to that of solvent control group, while CFF showed no significant change in the pH when compared with that of solvent control; but the F-value showed significant ($p<0.01$) difference among the treated groups.

4. Effect on free acidity: The data revealed that EAF and the standard drug ranitidine registered a significant ($p<0.001$ & $p<0.01$ respectively) reduction in the free acidity content i.e. 3.78 and 4.73 Meq/L/100g respectively, when compared to that of solvent control group which registered the free acidity content of 7.17 Meq/L/100g,, while CFF showed no significant change in the same (5.14 Meq/L/100g) when compared to that of solvent control. F-value showed significant ($p<0.01$) difference among the treated groups.

5. Effect on total acidity: The data showed that EAF and the standard drug ranitidine registered a significant ($p<0.001$ & $p<0.01$ respectively) reduction in the total acidity content i.e. 4.63 and 6.24 Meq/L/100g respectively, when compared to that of solvent control group which registered the total acidity content of 8.40 Meq/L/100g, while CFF showed no significant change in the same (5.25 Meq/L/100g) when compared to that of solvent control. F-value showed significant ($p<0.01$) difference among the treated groups.

B) Table 4 showed the status of gastric volume, Ulcer index, pH, free acidity and total acidity content of the groups treated with the ethyl acetate fraction (EAF) and chloroform fractions (CFF) of *Mollugo pentaphylla* along with groups treated with standard drug ranitidine and the control(Aspirin), in aspirin induce pylorus ligated rat ulcer model.

1. Effect on Gastric volume: The data revealed that EAF, CFF and the standard drug ranitidine reduced the gastric volume significantly ($p<0.001$) when compared to that of the control (Aspirin). However, the F-value showed significant difference among the treated groups.

2. Effect on Ulcer index: The data represented that, EAF and the standard drug ranitidine showed a significant ($p<0.001$) reduction in the ulcer index i.e. 0.00 and 0.5 respectively as compared to that of control (ethanol) group which registered the ulcer index of 5.66, while CFF showed no significant reduction in the ulcer index (5.5) profile when compared with that of control (Aspirin); Also the F-value ($p<0.01$) showed significant difference the ranitidine, EAF, treated groups.

3. Effect on pH: The data depicted that EAF and the standard drug ranitidine registered a significant ($p<0.001$) reduction in the pH of the gastric contents as compared to that of control (ethanol) group, while CFF showed no significant change in the pH when compared with that of control (Aspirin); but the F-value showed significant ($p<0.01$) difference among the treated groups.

4. Effect on free acidity: The data revealed that EAF and the standard drug ranitidine registered a significant ($p<0.001$ & $p<0.01$ respectively) reduction in the free acidity content i.e. 4.55 and 4.73 Meq/L/100g respectively, when compared to that of control aspirin treated group which registered the free acidity content of 7.92 Meq/L/100g,, while CFF showed no significant change in the same (8.16 Meq/L/100g) when compared to that of control. F-value showed significant ($p<0.01$) difference among the treated groups.

6. Effect on total acidity: The data showed that EAF and the standard drug ranitidine registered a significant ($p<0.001$ & $p<0.01$ respectively) reduction in the total acidity content i.e.6.65and 6.28Meq/L/100g respectively, when compared to that of control Aspirin group which registered the total acidity content of 8.90Meq/L/100g, while CFF showed no significant change in the same (9.89 Meq/L/100g) when compared to that of solvent control. F-value showed significant ($p<0.01$) difference among the (ranitidine, EAF) treated groups.

Cytoprotective Studies

Table 5 showed the status of Ulcer index, curative ratio%, content of the groups treated with the ethyl acetate fraction (EAF) and chloroform fractions (CFF) of *Mollugo pentaphylla* along with groups treated with standard drug ranitidine and the control (Ethanol) treated group, in ethanol induced rat ulcer model.

1. Effect on Ulcer index: The data represented that, EAF and the standard drug ranitidine showed a significant ($p<0.001$) reduction in the ulcer index i.e. 0.83 and 0.18 respectively as compared to that of control (ethanol) group which registered the ulcer index of 5.5, while CFF showed no significant reduction in the ulcer index 6.0 profile when compared with that of control (Ethanol) treated group; Also the F-value ($p<0.01$) showed significant difference the ranitidine, EAF, treated groups.

2. Curative ratio: The data depicted that EAF and the standard drug ranitidine registered a significant ($p<0.001$) increased Curative ratio i.e. 84.90 and 80.36 as compared to that of control (ethanol) group which registered Curative ratio0.00 while CFF showed no significant change in the Curative ratio when compared with that of control (ethanol); but the F-value showed significant ($p<0.01$) difference among the treated groups.

B) Table 6. The effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of Mollugo pentaphylla treated in 2(M) Sodium hydroxide induced rat ulcer model:

The perusal of Table 4-B showed the status of Ulcer index, p^Hcontent of the groups treated with the ethyl acetate fraction (EAF) and chloroform fractions (CFF) of *Mollugo pentaphylla* along with groups treated with standard drug ranitidine and the control Sodium hydroxide treated group, in ethanol induced rat ulcer model.

1. Effect on Ulcer index: The data represented that, EAF and the standard drug ranitidine showed a significant ($p<0.001$) reduction in the ulcer index i.e. 0.25 and 0.33respectively as compared to that of control Sodium hydroxide treated group which registered the ulcer index of 5.5, while CFF showed no significant reduction in the ulcer index 5.5 profile when compared with that of control Sodium hydroxide treated group; However, the F-value showed significant difference among the treated groups.

2. Effect on p^H: The data depicted that EAF and the standard drug ranitidine registere a significant ($p<0.001$) reduction in the pH of the gastric contents as compared to that of control Sodium hydroxide treated group, while CFF showed no significant change in the pH

when compared with that of control Sodium hydroxide treated group; but the F-value showed significant (p<0.01) difference among the treated groups.

Stress Induced Ulcer

Table 7: showed the status of Ulcer index, p^H content of the groups treated with the ethyl acetate fraction (EAF) and chloroform fractions (CFF) of *Mollugo pentaphylla* along with groups treated with standard drug ranitidine and the solvent control treated in cold and restraint rat ulcer model.

1. Effect on Ulcer index: The data represented that, EAF and the standard drug ranitidine showed a significant (p<0.001) reduction in the ulcer index i.e. 0.33and 0.41respectively as compared to that of control solvent treated group which registered the ulcer index of 5.5, while CFF showed no significant reduction in the ulcer index 5.5 profile when compared with that of solvent control treated group; However, the F-value showed significant difference among the treated groups.

2. Effect on p^H: The data depicted that EAF and the standard drug ranitidine registered a significant (p<0.001) reduction in the pH of the gastric contents as compared to that of solvent control treated group, while CFF showed no significant change in the pH when compared with that of control treated group; but the F-value showed significant (p<0.01) difference among the treated groups.

Histopathological Study

Histological observations of Pylorus ligated ulceration, Aspirin-Pylorus ligated ulceration, Ethanol induced ulceration, Sodium hydroxide induced ulceration, Stress induced gastric lesions in ulcer control group pre-treated with only CMC, showed comparatively extensive damage to the gastric mucosa, edema and leucocytes infiltration of the submucosal layer. Rats that received pre-treatment with the plant extract had comparatively better protection of the gastric mucosa as proven by reduction in ulcer area, reduced or absence of submucosal edema and leucocytes infiltration [**Figure 1 to 5 (plate No.1)]**. *Mollugo pentaphylla* extract has been shown to exert the cytoprotective effects in a dose-dependent manner. Enhanced gastric ulcer healing in rats pretreated with Ranitidine and *Mollugo pentaphylla* are shown in **[Figures 1 to 5 (plate No.3 to 4)].**

Table 1: Acute toxicity study of Ethyl acetate fraction of MEMP of by oral administration.

Group and fraction	Dose (mg/kg)	Mortality after 48hr (dead/alive)	Vehicle
1. Ethyl acetate fraction	1000	0/2	Tween 80 + Distilled water
2. Ethyl acetate fraction	2000	0/2	Tween 80 + Distilled water
3. Ethyl acetate fraction	4000	0/2	Tween 80 + Distilled water
4. Ethyl acetate fraction	5000	0/2	Tween 80 + Distilled water

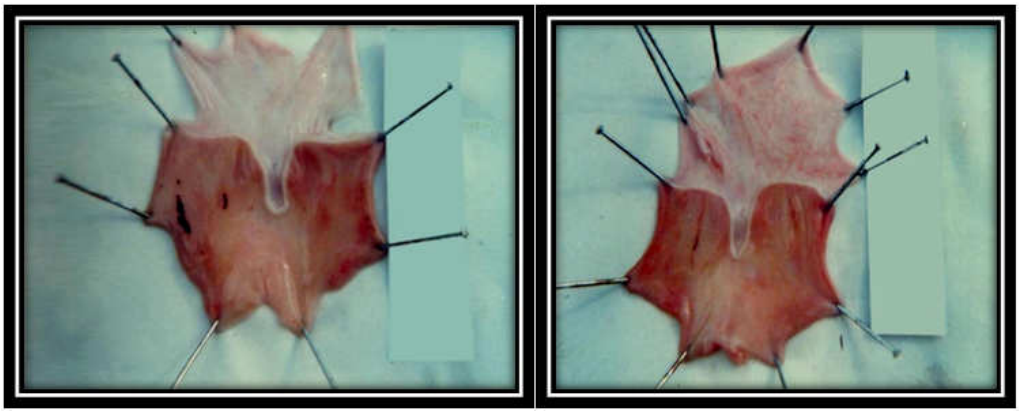
Table 2: Acute toxicity study of chloroform fraction of MEMP by oral administration.

Group and fraction	Dose (mg/kg)	Mortality after 48hr (dead/alive)	Vehicle
1. Chloroform fraction	1000	0/2	Tween 80 + Distilled water
2. Chloroform fraction	2000	0/2	Tween 80 + Distilled water
3. Chloroform fraction	4000	0/2	Tween 80 + Distilled water
4 Chloroform fraction	5000	0/2	Tween 80 + Distilled water

Table 3: Effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of *Mollugo pentaphylla* in Pylorus Ligation gastric rat ulcer model

Group	Treatment and dose	Gastric Volume/100g	Ulcer index	p^H	Free acidity Meq/L/100g	Total acidity Meq/L/100g
Gr-I	Control 2ml/kg	4.18±0.21	6.0 ±0.63	2.72±0.21	7.17±0.34	8.40±0.36
Gr-II	Ranitidine100mg/kg	3.66±0.21^c	0.91±0.20^c	4.05±0.42^c	4.73±0.20^b	6.24±0.24^b
Gr-III	EFA 200mg/kg	4.31±0.14^c	0.16±0.10^c	5.89±0.28^c	3.78±0.32^c	4.63±0.26^c
Gr-IV	CFF 200mg/kg	4.18±0.28	4.2 ±0.63	2.74±0.23	5.14±0.35	5.25±0.40
	F(3.20)	1.68	47.09**	24.60**	30.12**	30.40**

Values are expressed as mean ± S.E.M; n= 6 animal, F-values denotes significance at *p<0.05, **p<0.01, t-value denotes significance at ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001



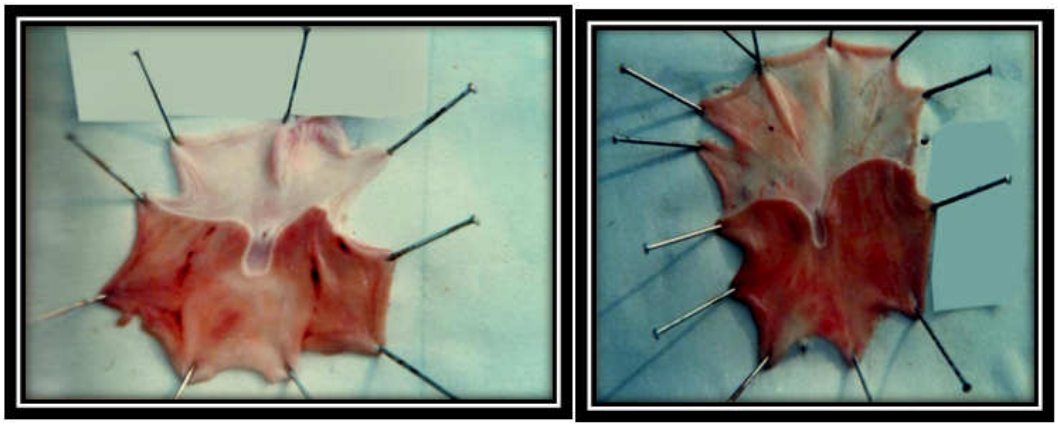


Fig. 1: Plate showing the effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of *Mollugo pentaphylla* in Pylorus Ligation gastric rat ulcer model, Plate No.1- Control (2ml/kg), Plate No.2- Ranitidine100 (mg/kg), Plate No.3- Chloroform fraction 200 (mg/kg), Plate No.4- Ethyl acetate fraction 200 (mg/kg)

Table 4: Effect of ethylacetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of *Mollugo pentaphylla* in Aspirin induce Pylorus Ligation rat ulcer model

Group	Treatment and dose	Ulcer Index	Gastric volume/100 g	p^H	Free acidity Meq/L/100g	Total acidity Meq/L/100g
Gr-I	Control 200mg/kg	5.66±0.80	2.60±0.06	2.21±0.06	7.92±0.14	8.90±0.18
Gr-II	Ranitidine 100mg/kg	0.5±0.12^c	2.32±0.05^b	3.13±0.08^c	4.73±0.16^c	6.28±0.05
Gr-III	Chloroform 200mg/kg	5.5±0.5	1.68±0.04^c	2.83±0.09^c	8.16±0.09	9.89±0.29
Gr-IV	Ethyl acetate 200mg/kg	0.00^c	2.12±0.01^c	3.75±0.09^c	4.55±0.18c	6.65±0.20^c
	F(3,20)0	41.82**	61.89**	59.90**	168.63**	72.57**

Values are expressed as mean ± S.E.M; n= 6 animal, F-values denotes significance at *p<0.05, **p<0.01., t-value denotes significance at ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001

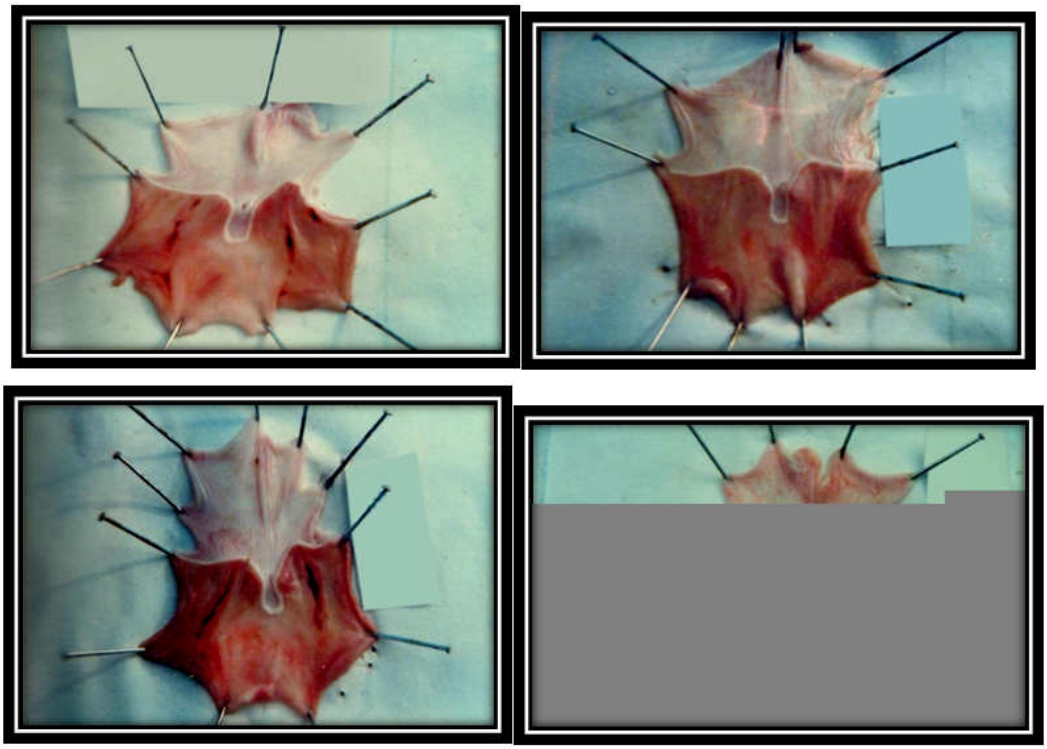


Fig. 2: Plate showing effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of *Mollugo pentaphylla* in Aspirin induce Pylorus Ligation rat ulcer model

Plate No. 1- Control Aspirin (200mg/kg), Plate No.2- Ranitidine100 (mg/kg), Plate No. 3- Chloroform fraction 200(mg/kg), Plate No. 4- Ethyl acetate fraction 200(mg/kg)

Table 5: Effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of *Mollugo pentaphylla* in Ethanol induced rat ulcer model

Group	Treatment and dose	Ulcer index	Curative ratio %
Gr-I	Control 10ml/kg	5.5±1.22	0.00
Gr-II	Ranitidine 100mg/kg	1.08±0.27[c]	80.36±4.93[b]
Gr-III	Chloroform 200mg/kg	6±0.63	9.09±11.49
Gr-IV	Ethyl acetate 200mg/kg	0.83±0.16[c]	84.90±3.03[c]
	F(3.20)	41.01**	61.47**

Values are expressed as mean ± S.E.M; n= 6 animal, F-values denotes significance at *p<0.05, **p<0.01., t-value denotes significance at [a]p<0.05, [b]p<0.01, [c]p<0.001

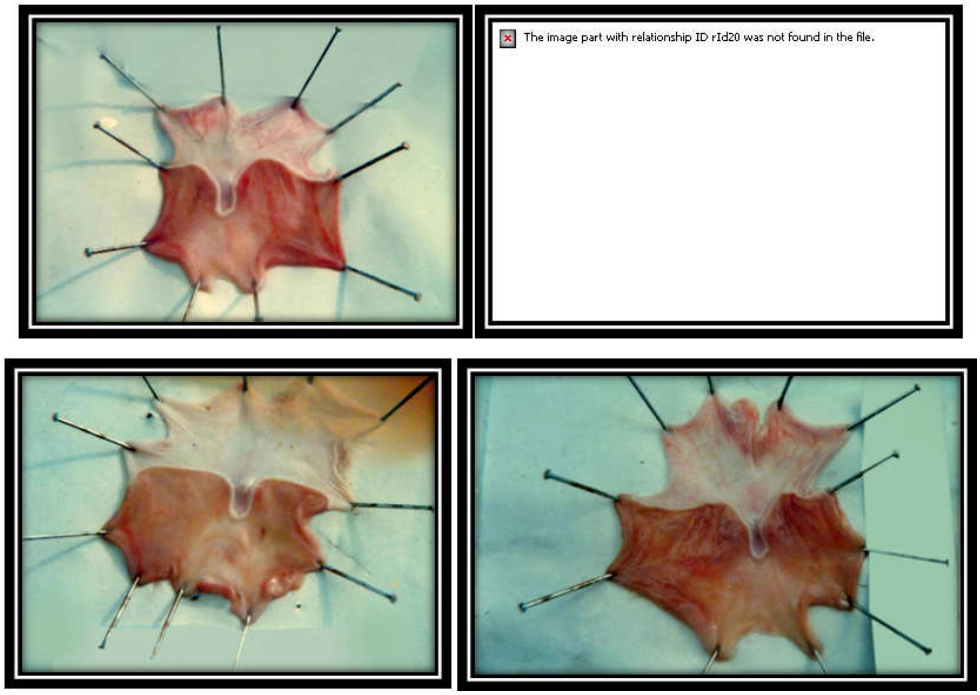


Fig. 3: Plate showing the effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of *Mollugo pentaphylla* in Ethanol induced rat ulcer model:

Plate No.1- Control Ethanol (10ml/kg), Plate No.2- Ranitidine100 (mg/kg), Plate No.3- Chloroform fraction 200(mg/kg), Plate No.4- Ethyl acetate fraction 200(mg/kg)

Table 6: The effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of Mollugo pentaphylla treated in 2(M) Sodium hydroxide induced rat ulcer model

Group	Treatment and dose	Ulcer index	pH
Gr-I	ControlNaoH 1ml/kg	5.5±0.5	2.95±0.17
Gr-II	Ranitidine 100mg/kg	0.33±0.10	5.18±0.40[c]
Gr-III	Chloroform 200mg/kg	5.5±0.5	2.92±0.16
GrIV	Ethyl acetate 200mg/kg	0.25±0.11	5.05±0.44[b]
	F(3.20)	69.08**	15.03**

Values are expressed as mean ± S.E.M; n= 6 animal, F-values denotes significance at *p<0.05, **p<0.01., t-value denotes significance at [a]p<0.05, [b]p<0.01, [c]p<0.00

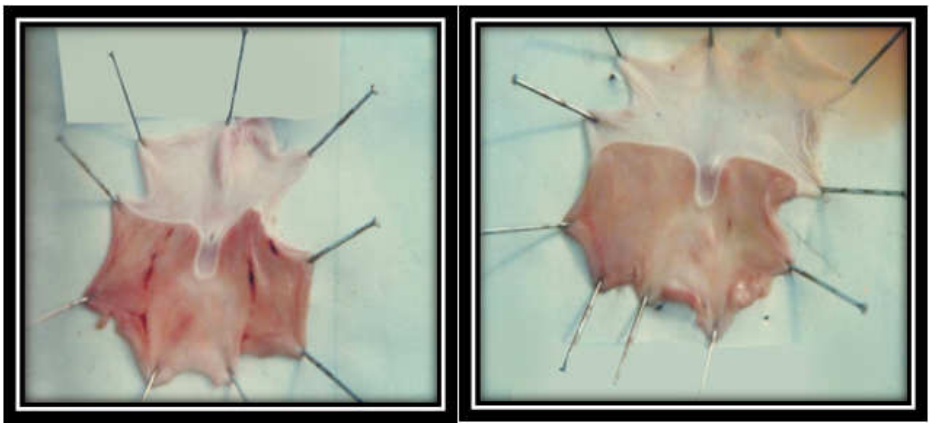


Fig. 4: Plate showing the effect of ethyl acetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of aerial parts of *Mollugo pentaphylla* treated in 2(M) Sodium hydroxide induced **rat ulcer model:**

Plate No.1- Control NaoH 1ml/kg, Plate No.2- Ranitidine100 (mg/kg), Plate No.3- Chloroform fraction200 (mg/kg), Plate No.4- Ethyl acetate fraction 200(mg/kg)

Table 7: The effect of ethylacetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of *Mollugo pentaphylla* treated in cold and restraint induce rat ulcer model

Group	Treatment and dose	Ulcer index	pH
Gr-I	Control 2ml/kg	5.5±0.5	2.15±0.04
Gr-II	Ranitidine 100mg/kg	0.41±0.15^{c}	4.82±0.45^{c}
Gr-III	Chloroform 200mg/kg	6±0.63	2.65±0.20
Gr-IV	Ethyl acetate 200mg/kg	0.33±0.16^{c}	5.26±0.50^{c}
	F(3.20)	55.16**	19.28**

Values are expressed as mean ± S.E.M; n= 6 animal, F-values denotes significance at *p<0.05, **p<0.01., t-value denotes significance at ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001

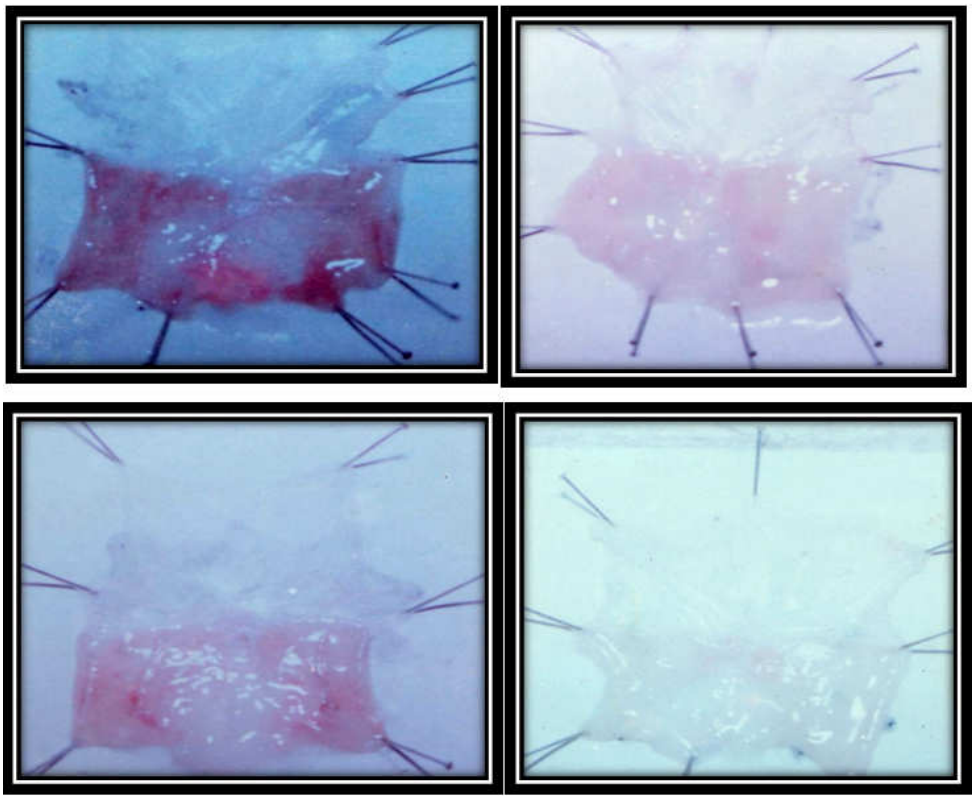


Fig. 5: Plate showing the effect of ethylacetate fraction (EAF) and chloroform fraction (CFF) of the methanolic extract of *Mollugo pentaphylla* treated in cold and restraint induce rat ulcer model

Plate No.1- Control Water (2ml/kg), Plate No.2- Ranitidine100 (mg/kg), Plate No.3- Chloroform fraction 200(mg/kg), Plate No.4- Ethyl acetate fraction 200(mg/kg)

DISCUSSION AND CONCLUSION

The present study data demonstrate the anti-ulcerogenic activity of ethylacetate and chloroform fraction of the methanolic extract of *Mollugo pentaphylla* against gastric ulceration induced by various models viz. Pylorus ligated ulceration, Aspirin-Pylorus ligated ulceration, Ethanol induced ulceration, Sodium hydroxide induced ulceration, Stress induced ulcer model[18].

In the present study two fractions i.e chloroform and Ethyl acetate fraction of methanolic extract from aerial parts of *Mollugo pentaphylla* at dose levels of 200mg/kg were used. It was found from the above study that, there was significant change registered by both the fractions in ulcer index, gastric volume, P^H, total acidity and free acidity. The ethyl acetate fraction showed more potent and significant anti-ulcer activity as compared to that of the chloroform fraction which shows no or very less significancy in the tested models, when compared with that of the solvent control group. Goyal et al, 1997 proposed that GABA and Baclofen a GABA mimetic agent exert marked anti-ulcerogenic effects against Pylorus ligated ulcer. It may not be due to attenuation of offensive acid factors since GABA has been shown to induce an increase in gastric acid output.This was observed with Pylorus ligated rat ulcer model using the plant fraction (Ethyl acetate fraction) of *Mollugo pentaphylla* [19].

In the aspirin-pylorus ligation model the initial damage by Aspirin aggravates the damage further with increase acid secretion and decreased mucus production with increase pepsin activity. In aspirin-pylorus ligation induced gastric ulcer model the Ethyl acetate fraction of *Mollugo pentaphylla* significantly attenuated the gastric volume, free acidity, total acidity and ulcer index thus showing the anti-secretory activity. Ranitidine is the standard control, used here to test anti-secretory mechanism. Ulcer index parameter was used for the evaluation of anti-ulcer activity since ulcer formation is directly related to factors such as reduction in gastric volume, decrease in free and total acidity. It is significant to note when the pH reached 3, the ulcer score appeared less. This is borne out by the decrease in free acid, which might have contributed to the anti-ulcer property of the plant extract [20]. In case of vehicle control, aspirin plus pylorus ligation aggravated the acid secretion, which in turn caused increase in gastric volume, increased free and total acidity, low pH and increased ulcers are thought to be due to increase in offensive factors like gastric acid, pepsin, H. pylori and bile salts but it has been observed that gastric ulcer patients have either normal or below normal acid level in the stomach. This indicates that other mechanisms are also involved in ulcer formation. More over the disturbance of defensive factors like mucus secretion, bicarbonate secretion and mucosal blood flow has been reported to cause ulcers[21].

Pylorus ligation and Cold restrain stress induced ulcer occurs due to auto digestion of mucosal cell in the stomach due to over production or excess accumulation of HCL in the stomach [22]. Ethyl acetate fraction *Mollugo pentaphylla* at dose level of 200mg/kg significantly reduced the volume of gastric juice, total acidity, ulcer index and increased the gastric juice pH where as no significant changes were observed in case of chloroform fraction in the same parameters. The ulcer protective activity of *Mollugo pentaphylla* may be due to the flavones such as Apigenin and Mollupentin.

Ethanol induced gastric ulcer formation may be due to stasis in gastric blood flow which contributes to the development if hemorrhage and necrotic aspect of tissue injury[23]. It is also reported that leukotriene antagonists and 5-lipoxygenase inhibitors are capable of inhibiting alcohol and NSAID'S induced gastric ulceration. In present study the Ethyl acetate fraction of *Mollugo pentaphylla* significantly increase the curative ratio when compared with control group, against ethanol induced gastric ulceration which may be due to inhibition 5-lipoxygenase pathway or leukotriene antagonistic activity and also by mucoprotective activity. Sodium hydroxide induces ulceration by causing lesions on the surface of gastric mucosa when it comes in contact with it.

In the present study, Ethyl acetate fraction of *Mollugo pentaphylla* showed a significant reduction in the ulcer index produced by pylorus ligation, aspirin plus pylorus ligation, alcohol or sodium hydroxide in rats.

The noxious chemical induced ulcer is commonly used for the screening of antiulcer agents. The possible mechanisms for antiulcer activities are anti-secretory activity on pepsin and acid, mucosal protection by increased mucus synthesis, prostaglandin level, and protective coating [24] treatments with Ethyl acetate fraction of *Mollugo pentaphylla* causes a significant decrease in ulcer index or other related factors.

Cold and restraint rat ulcer model is a mostly used model for screening of antiulcer activity of drugs. This causes both psychological and physical stress results in excess secretion of acid. In our study the ethyl acetate fraction of *Mollugo pentaphylla* shows a significant reduction in ulcer index and gastric pH, where as no such effects observed in case of chloroform extracts [25-29].

In conclusion, it may be concluded that the ethyl acetate and chloroform fraction from the methanolic extract from the aerial parts of *Mollugo pentaphylla* Linn. possess potential anti-gastric anti-ulcer activity. Such protection was shown to be dose dependent as ascertained by the reduction of ulcer areas in the gastric wall, reduction or inhibition of edema, and leucocytes infiltration of submucosal layers.

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