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GASTRIC ULCER CURATIVE POTENTIAL OF *MOLLUGO OPPOSITIFOLIA* LINN. EXTRACT- A PRECLINICAL STUDY.

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ABSTRACT

Peptic ulcer is a major health hazard both in terms of morbidity and mortality. It occurs due to imbalance between offensive factors such as acid and pepsin secretion, *Helicobacter pylori* infection (a stomach inhabiting pathogen), increased bile secretion, increased production of free radicals and decreased antioxidant activity versus defensive factors such as secretion of mucus and bicarbonate, blood flow and the process of restitution and regeneration of cellular injury. The plant products are proved as effective preventive as well as curative agents. They have been acquired a greater appreciation because they have lesser side effects and higher efficacy. **Objectives**: The present study was aimed to evaluate the gastro protective and antiulcer efficacy of *Mollugo oppositifolia* plant extract in the alcohol induced ulcerated rats. The activity was compared to the known antiulcer drug, Omeprazole. **Experimental design**: An animal model study was conducted by using albino Wistar rats. The rats were divided into four groups comprising of six rats in each group and treated as follow. Group I: Normal healthy control, Group II: Disease control (ulcer was induced by 50% alcohol), Group III: Ulcer induced rats treated with *Mollugo oppositifolia* extract, Group IV: Ulcer induced rats treated with Omeprazole. **Results obtained**: Ulcer induced rats showed increased level of ulcer index and reduction in the gastric output and total acidity, but they showed significant reduction in serum protein and carbohydrate levels. The level of lipid peroxides and activities of enzymatic antioxidant such as superoxide dismutase, catalase, glutathione peroxidase and non enzymatic antioxidant such as reduced glutathione were altered. There was a significant decrease in the level of hemoglobin and Red Blood Cells count and an increase in the White Blood Cells count. The treatment with *Mollugo*

oppositifolia extract significantly inhibit the alcohol induced ulcer congestion, hemorrhage and necrosis in diseased stomach which was evidenced from the resumption of various parameters. **Conclusion**: The results of the present study substantiate that *Mollugo oppositifolia* extract is a potential gastro protective and antiulcer herb. Its activity was comparable with standard drug, Omeprazole.

KEY WORDS: Peptic ulcer, *Mollugo oppositifolia* leaf extract, antiulcer activity, animal study.

INTRODUCTION

Stomach ulcer is defined as erosion in the lining of the stomach or duodenum and is caused by the disruption of the gastric mucosal defense systems. Ulcer in the stomach is called gastric ulcer and in the duodenum is called duodenal ulcer and together it is named as peptic ulcer. Ulcer incidence varies with the type of ulcer, gender and age. Peptic ulcer has initiated as open craters or sores in the inner lining (mucosa) of the stomach or the duodenum. A coating of mucus and other biochemicals normally shield the stomach and duodenum from digesting themselves. When these protective mechanisms are disturbed, powerful digestive acids can erode into the lining of these organs and cause ulcers. Ulceration is an imbalance between the rate of secretion of gastric juice and the degree of protection afforded by the gastro- stomach mucosal barrier as well as the neutralization of the gastric acid by stomach juice. Infection by the bacterial pathogen *Helicobacter pylori*, frequent usage of Non-Steroidal Antiinflammatory Drugs (NSAIDs) and high acid secretion are main reasons for induction of ulcer. Other causes of peptic ulcer are smoking, alcohol consumption, psychological stress and irregularity in diet. Reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches to cure peptic ulcer[1]. Due to any cause, an increase in aggressive factors or a decrease in defensive factors will lead to loss of mucosal integrity resulting in ulceration[2][3]**.** Gastric ulcers are located in the stomach, characterized by pain and common in older age group. Other symptoms may include nausea, vomiting and weight loss[4][5]**.**

Reduction of gastric acid production as well as re-enforcement of gastric mucosal production has been the major approaches to cure peptic ulcer disease. As a result, more and more synthetic drugs are introduced and offering newer options for treatment of peptic ulcer. The types of drugs vary from proton-pump inhibitor to H_2 antagonist or a cytoprotective agent. At the same time, each of these drugs confers simple or several side effects like arrhythmias,

impotence, and gynaecomastia, hyperplasia and haemopoetic changes. Because of several side effects of synthetic medicines, there is new thought of better natural alternative for the treatment of peptic ulcer.

Alternative approach in recent days is the research of medicaments from Ayurvedic and other traditional medicinal systems. The phytoconstituents available in the medicinal plants have proved to be clinically effective and relatively less toxic than the existing synthetic drugs and reducing the offensive factors and serving as tool in the prevention of peptic ulcers. Several herbal plants are reported to have antiulcer activity and several pre clinical (animal) studies are reported on the efficacy of herbal medicines such as *Pithecellobium dulce*[6], *Annona squamosa*[7], *Ocimum sanctum*[8], *Emblica officinalis*[9], *Pterospermum acerifolium* [10], *Bauhinia variegate* [11], *Terminalia chebula*[12]. Plant juices such as *Aloe vera*, banana stem juice and banana flower juice[13]and *Carica papaya* (papaya) fruit juice[1]and *Aloe vera* and Amla juice[3] are proved as antiulcer agents**.** *Ganoderma lucidum*, a medicinal mushroom extract is reported to have antiulcer efficacy[14]. Several reviews are published with the compilation of medicinal plants which have antiulcer activity [5][15][16].

Mollugo oppositifolia Linn. [syn. *Glinus oppositifolius* (Linn.) Aug. DC., *Mollugo spergula* Linn., *Mollugo subserrata* Blanco] belongs to the Botanical Family: Molluginaceae/ Aizoaceae. It is a prostrating annual herb growing in India, Sri Lanka, Pakistan and Bangladesh. The common names are: English- Bitter Leaf, Hindi- Jima and Tamil-Thora Poondu[17].

In India, *Mollugo oppositifolia* is traditionally used in the treatment of inflammation and various pains. The plant is used as an aperients, appetizer, uterus stimulant and antiseptic. It is used in itch and other skin diseases and for suppression of the lochia. Warmed herb with castor oil is a good cure for earache. Evaluation of the anti-inflammatory and analgesic activity of this plant is reported, but there is no scientific investigation so far been documented in literature about its antiulcer activity. Hence the plant is selected for its anti-ulcer activity in preclinical study in albino Wistar rats. In the present study 80% methanolic extract of *Mollugo oppositifolia* was used as plant drug and evaluate its antiulcer potential and its efficacy was compared with a known standard antiulcer drug Omeprazole.

MATERIALS AND METHODS

Preparation of plant extract

Mollugo oppositifolia plant was collected from the Sathuragiri Hills Area, Virudhunagar district of Tamil Nadu and the plant was authenticated by the eminent professors in the department of Botany. The voucher specimen (No. 061) was deposited in the department. 1000 gm of *Mollugo oppositifolia* dried plant (areal portion) was taken and extracted with 80% aqueous methanol. To one part of the plant material, six parts of 80% aqueous methanol was added in a suitable round bottom flask fitted with condenser and extracted for 3 h. The 80% methanol extract was filtered and the filtrate was evaporated to dryness in a rotary-evaporator at low temperature. Paste from of the extract obtained was further processed to make in to a dry powder.

Phytochemical study

The extract of *Mollugo oppositifolia* was subjected to analysis for preliminary phytochemicals such as alkaloids, flavonoids, glycosides, phenolic compounds, saponins, terpenenoids, steroids, tannins, protein and carbohydrate according to the standard methods[18][19][20][21].

Experimental animals

Albino Wistar rats of either sex weighing around 150-200 g were taken from inbreed colony animals, which were purchased from reliable source and they were housed in polypropylene cages under standard laboratory conditions (12:12 light and dark period, temperature maintained at 21±2 ºC with a relative humidity of 55%). The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. All procedures involving laboratory animal use were in accordance to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design

Group I : Healthy rats with normal diet.

Group II : Disease control rats, ulcer was induced by alcohol (10 ml /kg-bw of 50% ethanol- single dose) (Pre- treatment).

Group III : Alcohol induced ulcerated rats treated with *Mollugo oppositifolia* extract (400mg/kg- bw) for 21 days.

Group IV : Alcohol induced ulcerated rats treated with standard drug- Omeprazole (20 mg/kg-bw) for 21 days. (Drug control)

Induction of Ulcer in Rats

The rats were given standard rat pellets and tap water *ad libitum*, but they were deprived of food for 24 h before the experiments. Gastric ulcer was induced in group II, III and IV rats. Gastric ulcer in albino Wistar rats was induced by orogastric incubation of 50% ethanol (10 ml/kg-bw). Healthy control group was orally administered with vehicle (carboxymethyl cellulose, CMC, 0.25% w/v, 5ml/kg). The reference group received oral doses of 20 mg/kg Omeprazole in CMC (5 ml/kg) as positive controls. Experimental groups were orally administered with 400 mg/kg of *Mollugo oppositifolia* extract.

On 22nd day (24 h after the treatment period), the animals were sacrificed. The animals were anaesthetized by using ether. The blood samples were collected for hematological study. The abdomen was opened without causing any damage to its blood supply and an incision of 1cm long was made in the abdomen just below the sternum of the stomach and it was exposed. Passed a thread around the pyloric sphincter and applied a tight knot closed the abdomen wall by cervical decapitation and the stomach was removed. The gastric juice was collected in a graduated centrifuge tube and samples of stomach tissues were collected and stored for analysis of total acid, protein and carbohydrate and enzymological assays.

Collection of gastric juice

The gastric content from the stomach was collected carefully in centrifuge tube and centrifuged at 1000 rpm for 10 min. The volume of the juice was recorded and the results were expressed as ml/100 g.

Determination of ulcer index in stomach [22][12].

In the cut opened stomach, the mucosa was washed slowly with saline and the stomach was pinned on frog board and observed under dissection microscope (10×) for ulcer mean score for each animal. The result is expressed as ulcer index by using following formula. % Ulcer index = (USc – USt) x 100 / USc USc = Ulcer surface area in control and USt = Ulcer surface area in treated animals.

Determination of total acidity in gastric fluid [23].

The gastric fluid was centrifuged at 1000 rpm for 10 min, the volume was noted and pipette out 1ml of supernatant liquid. Diluted it to 10 ml with distilled water and titrated the solution against 0.01N sodium hydroxide (NaOH), using Topfer's reagent -(Dimethyl-amino-azobenzene with phenolphthalein) as indicator. Titrated up to the endpoint when solution turned to yellowish orange colour. Noted the volume of NaOH required for neutralizing the free hydrochloric acid present in the gastric juice. Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. The difference between the two readings indicated the volume of NaOH required for neutralizing the combined acid present in the gastric juice. The sum of the two titrations was the total acid present in the gastric juice.

Acidity was calculated as follow:

Acidity = Vol. of NaOH × Normality of NaOH / Vol. of gastric juice used

The total acidity was expressed as mEq/dl

Estimation of total protein (Modified method of Lowry *et al.* [24][1].

The content of dissolved total protein in the tissue homogenate was estimated in the alcoholic precipitate obtained by adding 90% of alcohol in 9:1 ratio. Then 0.1 ml of alcoholic precipitate was dissolved in 1 ml of 0.1 N NaOH and from this 0.05 ml was taken in another test tube. To this 4 ml of alkaline copper reagent was added and kept for 10 min. Then 0.5 ml of Folin's phenol reagent was added and kept for 10 min and the solution was allowed for colour development. Reading was taken against blank prepared with distilled water at 640 nm. The protein content was calculated from standard curve prepared with bovine albumin and the result has been expressed in terms of µg/mg of tissue. (Standard Bovine serum albumin: 20 mg of bovine serum albumin was dissolved in 100 ml of distilled water. Few drops of NaOH were added to it to aid complete dissolution of bovine serum albumin and to avoid frothing and it was allowed to stand overnight in a refrigerator).

Estimation of carbohydrates (Modified Anthrone method [1].

To 0.1 ml of tissue homogenate, 2 ml of 3N sulphuric acid and 2 ml of sodium tungstate were added and mixed. The content was centrifuged at 3000 rpm for 10 min. From this 0.4 ml of supernatant was taken in a series of test tubes and the blank was prepared without gastric fluid. The volume in all test tubes was made up to 1 ml by adding distilled water. After 10 min of incubation in ice cold water bath, 4 ml of anthrone reagent (freshly prepared by

dissolving 0.2 g of anthrone in 100 ml of concentrated sulphuric acid) was added in to all the test tubes. The test tubes were kept in the boiling water bath for 15 min. After cooling down the solution, read the optical density at 540 nm. The carbohydrate content was calculated from standard curve prepared with different strength standard glucose solution (10-100 µg of glucose dissolved in 1 ml of distilled water) and the results has been expressed in terms of µg/mg of tissue.

Assay of serum alkaline phosphatase [25].

The reaction mixture was prepared by adding 1.5 ml of 0.1M carbonate buffer (pH 10), 1 ml of 0.1M disodium phenyl phosphate and 0.1 ml of 0.1M magnesium chloride and finally 0.1 ml of gastric fluid was added. The reaction mixture was incubated at 37°C for 15 min and the reaction was arrested by the addition of Folin's phenol reagent. Control tubes were treated similarly but serum was added after the reaction was arrested by the Folin's phenol reagent. 1ml of 15% sodium carbonate was added and the colour developed was read after 10 min at 640 nm. The enzyme activity was expressed as IU/ml.

Assay of lipid peroxides [26].

To 0.1ml of tissue homogenate, 4 ml of 0.85N H_2SO_4 and 0.5 ml of 10% phosphotungstic acid were added and stirred well. The content was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the sediment was mixed with 2.0 ml of H_2SO_4 and 0.3 ml of 10% phosphotungstic acid. The mixture was centrifuged for 10 min. The sediment was suspended in 4.0 ml of distilled water and 1 ml of TBA reagent (mixture of equal volumes of 0.67% TBA aqueous solution and glacial acetic acid). The tube was kept in a boiling water bath for 1 hr, after cooling, 5 ml of butanol was added and the colour of the extract in the butanol phase was read at 532 nm. The reaction mixture without tissue homogenate was used as blank. The enzyme activity was expressed as nano moles/mg tissue protein.

Assay of reduced glutathione [27].

To 0.5 ml of tissue homogenate, 20% TCA was added and precipitated. The contents were mixed well for complete precipitation of protein and centrifuged. To aliquots of clear supernatant, 2.0 ml of DTNB reagent (0.6mM DTNB in 0.2 M Phosphate buffer, pH 8.0) was added and 0.2 M phosphate buffer was added to make a final volume of 4.0 ml. The absorbance was read at 412 nm against a blank containing TCA instead of sample. A series of standard solutions (prepared from 10 mg of reduced glutathione dissolved in 100 ml of water)

were treated in a similar way to determine glutathione content. The amount of glutathione was expressed as nano moles of GSH oxidized/mg protein.

Activity of superoxide dismutase [28].

To 0.1ml of tissue homogenate, 0.75 ml of ethanol and 0.15 ml of ice cold chloroform were added and centrifuged. The supernatant was taken and 0.5 ml of 0.6nM EDTA solution and 1 ml of buffer (0.1 M pH 10.2) were added and mixed well. The reaction was initiated by the addition of 0.5 ml of fresh epinephrine (1.8nM) and the increase in absorbance was measured at 480 nm. The reaction mixture without tissue homogenate was used as blank. The enzyme activity was expressed as U/ml.

Assay of catalase [29].

20 µl of tissue homogenate added 1.5 ml of the 0.01N phosphate buffer (pH 7.0). To this 0.5 ml of hydrogen peroxide was added to start the enzyme reaction. Then add 0.25 ml of potassium dichromate and the tubes were kept in boiling water bath for 10 min. green colour was developed and the decrease in absorbance was measured at 240 nm at 60 sec intervals for 3 min. the enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. Activity of catalase was expressed as µ moles of hydrogen peroxide utilized/min/mg protein.

Assay of glutathione peroxidase [30].

The reaction mixture (consisted of 0.2ml each of 0.8M EDTA, 0.2M reduced glutathione, 10mM sodium azide, 2.5mM H_2O_2, 0.4 ml 0.2M phosphate buffer (pH 7.0) and 0.1ml tissue homogenate) was incubated at 37^{o}C at different time interval. The reaction was arrested by adding of 0.5ml of 10% TCA and the tubes were centrifuged at 3000 rpm for 10 min. To 0.5ml of supernatant, 4ml of 3mM disodium hydrogen phosphate and 0.5ml of DNTB solution were added and the colour developed was read at 420 nm immediately. The level of glutathione peroxidase was expressed as µmoles of glutathione oxidize/min/mg protein.

Determination of haemoglobin [31].

A series of (6 Nos.) test tubes were taken and 0.02 ml of blood sample was added in to each test tube. Then 5ml of Drabkin's reagent was added and the test tubes were allowed to stand for 4 min. The blank was prepared similarly without adding blood sample and the read reaction solution at 540 nm in a calorimeter.

Enumeration of RBC count [32].

The anticoagulated blood was sucked into pipette up to the marking of 0.5. It was diluted with RBC diluting fluid (Hayem's diluting fluid) by sucking up to 10 marking. The pipette was shaken for a min and wiped away the excess of blood by using blotting paper. Placed a drop of blood at the edge of haemocytometer and placed a cover slip on it, which covered the counting chamber. The cells were counted under microscope on the 16 squares in 5 different parts of the fluid.

Enumeration of WBC count [32].

The uncoagulated blood was sucked into pipette up to the marking of 0.5. It was diluted with WBC diluting fluid (Turkey's fluid) by sucking up to 10 marking. The pipette was shaken for a min and wiped away the excess of blood by using blotting paper. Placed a drop of blood at the edge of the hemocytometer and placed a cover slip on it, which covered the counting chamber. The cells were then counted under microscope on the 4 corner squares.

Statistical analysis

The data of results obtained were subjected to statistical analysis and expressed as mean ± SD. The data were statically analyzed by one way analysis of various (ANOVA) and to compare the means of the studied groups with *post hoc* Duncan multiple range tests at 5% and 1% for those results where significant difference was indicated. Values are expressed as mean±S.E.M (n=6) *p<0.05, **p<0.01, ***p<0.001 Compared with control

RESULTS AND DISCUSSION

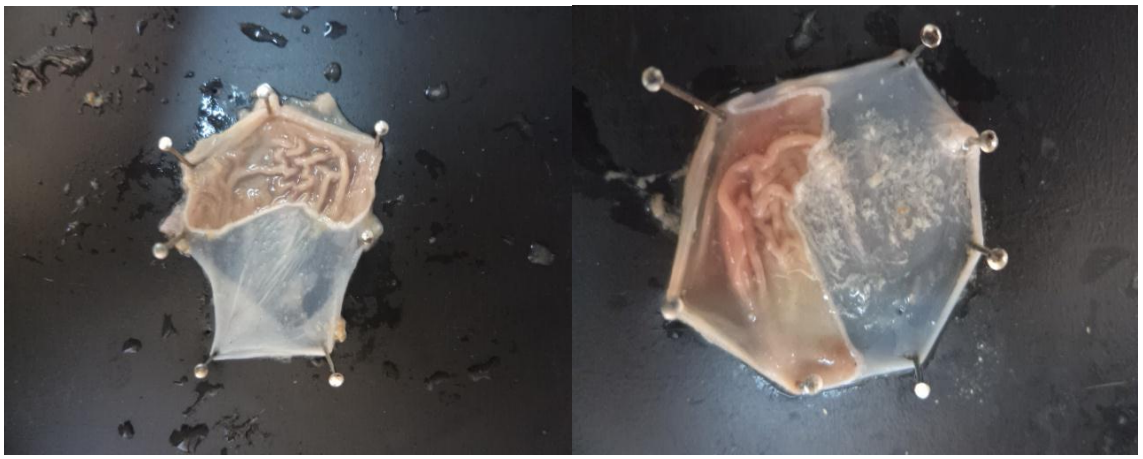
The phytochemical studies revealed the presence of following phytochemicals, alkaloids, flavonoids, glycosides, phenolic compounds, saponins, terpenenoids, steroids, tannins, protein and carbohydrates (Table 1)

Table 1: Screening of phytochemicals in *Mollugo oppositifolia* extract.

Pytoconstituents*	Qualitative determination
Alkaloids	++
Flavonoids	+++
Glycosides	++
Phenolic compounds	+++
Phytosterols	+
Saponins	+
Tannins	++
Triterpenoids	+

Carbohydrates	++
Proteins	+

'+'Present, '-' Absent. *Values are means of triplicate determination.



Group I (Healthy control) **Group II (Disease control)**

Group III (Plant extract treated)
Group IV (Omeprazole treated)

Fig. 1: Photograph showing the ulceration in the mucosa of experimental animals

Table. 2. Effect of *Mollugo oppositifolia* leaf extract on ulcer index, total acidity, protein, and carbohydrate levels in alcohol induced ulcerated in rats.

Parameters	Group I	Group II	Group III	Group IV
Ulcer index (No. of lesions)	00.00	3.23±2.1**	0.66±0.13***	0.31±0.22**
Total Acidity (Meq)	38.25±4.2	86.35±3.1**	43.98±4.6*	35.75±4.2**
Volume of gastric secretion (ml/100 g)	3.2±0.11	6.8 ±0.13***	3.0±0.11	3.5±0.13*
Protein (µg/mg tissue)	9.65±1.6*	6.13±1.2**	17.56±0.44***	14.6±0.71***
Carbohydrate (µg/mg tissue)	165.00±8.2	135.66±6.5**	186.66±6.1	175.66±8.6*

Values are expressed as mean± S.E.M (n=6) *p<0.05, **p<0.01, ***p<0.001 Compared with control group

Table .3. Effect of *Mollugo oppositifolia* leaf extract on Alkaline phosphatase, lipid peroxidase, reduced glutathione and superoxide dismutase activity in alcohol induced ulcerated in rats.

Parameters	Group I	Group II	Group III	Group IV
Alkaline phosphatase (IU/L)	86.53±4.2	128.13±5.4**	87.45±4.4*	91.13±5.4*
Lipid peroxides (nmoles/mg)	34.13±2.7	78.55±2.9***	31.53±2.2**	33.15±5.5 *
Reduced glutathione (nmoles/mg)	85.46±5.2	41.65±3.4**	80.65±4.5**	86.80±8.7*
Superoxide (U/mg)dismutase	56.43±4.3	25.25±3.1***	52.70±5.5*	55.30±7.9*
Catalase (mmoles/ min/mg protein	6.68±0.9	3.75±0.6***	7.68±0.7**	6.31±0.9
Glutathione peroxidase nmoles/min/mg protein	32.55±1.9	15.48±2.3***	36.60±1.6*	30.68±1.5

Values are expressed as mean± S.E.M (n=6) *p<0.05, **p<0.01, ***p<0.001 Compared with control

Table. 4. Effect of *Mollugo oppositifolia* leaf extract on hemoglobin, Red Blood Cells (RBC) and White Blood Cells (WBC) content in alcohol induced ulcerated in rats.

Parameters	Group I	Group II	Group III	Group IV
Haemoglobin (g%)	16.83±3.4*	8.25±4.2**	19.66±8.2*	20.21±4.4*
Red Blood Cells (RBC) (million cells/mm^3)	8.60±0.8*	7.10±0.4*	8.40±0.8*	8.50±0.5*
White Blood Cells (WBC) (thousand cells/ mm^3)	6760±20.0	9560±17.2***	7445±12.5*	7710±18.5**

Values are expressed as mean± S.E.M (n=6) *p<0.05, **p<0.01, ***p<0.001 Compared with control

The experimental animals were divided in to 4 groups and each group consisting of 6 animals. Animals in group I were maintained as healthy control where as animals in the group II, III and IV were administered with 50% ethanol and ulcer was induced. An animal in the group II was sacrificed after 24 hours of ulcer induction and checked its stomach for ulcer. Ulcer was noticed and the observation conformed that ethanol treated animals were ulcerative. The plant extract (*Mollugo oppositifolia*) and Omeprazole (standard drug) were started administered after 24 hour in group III and group IV animals respectively. On 22nd day, 24 h after treatment, the animals were sacrificed and analyzed for the antiulcer activity of the test drugs. Administration of alcohol has induced the peptic ulcer in the experimental animals (Fig.1) and increased the levels of offensive factors such as ulcer index, gastric secretion and total acidity. Administration of plant drug and Omeprazole significantly decreased the levels of offensive factors.

Oral administration of ethanol in rats caused severe gastric mucosal damage by disruption its barrier and provokes rapid, strong microvascular events in mucosal capillaries [33][34]. The *Mollugo oppositifolia* leaf extract showed significant decrease in rat mucosal injury induced by ethanol. The deleterious effect of ethanol on gastric mucosa is consequences of enhanced lipid peroxidation, decreased glutathione (GSH) level and excessive generation of free radicals such as hydroxyl ethyl radical, superoxide radical, hydroxyl radical and hydrogen peroxide[35].

Mollugo oppositifolia leaf extract (400mg/kg- bw) treatment significantly healed the ulcer wounds, which was shown in Fig-1. A significant ($P<0.01$) increase in the gastric volume level was observed in the ulcer induced rats when compared to healthy control rats. Plant extract and standard drug treatment significantly ($P<0.01$) reduced the gastric volume when compared diseased rats.

Ulcer index was significantly increased ($P<0.01$) in disease control rats when compared to healthy control animals. Administration of both plant extract and Omeprazole significantly ($P<0.001$) decreased the ulcer index. The maximum inhibition of ulcer index was noticed in animals treated with plant extract than the rats treated with standard drug (Omeprazole). Total acidity was significantly increased ($P<0.01$) in disease control rats when compared to healthy control rats. After the treatment it was significantly ($P<0.001$) decreased in the animals treated with plant extract and Omeprazole. The decrease was more pronounced in group III rats than group IV rats (Table-2). This may be due to the antacid effect and/or cytoprotective effect of *Mollugo oppositifolia* extract. Apart from antiulcer activity *Mollugo oppositifolia* extract demonstrated the other properties such as antisecretory activity. It reduced the ulcer index and total acidity and thereby improving the cytoprotective effect.

The content of total proteins and carbohydrates significantly decreased ($P<0.01$) in the mucosal tissue of diseased animals, when compared to healthy animals. But after treatment with drugs (in group III and group IV animals) the content of proteins and carbohydrates significantly ($P<0.01$) increased. Administration of plant extract influenced higher increase in the protein and carbohydrate contents than the animals administered with Omeprazole (Table-2). The decrease in the content of proteins and carbohydrates in ulcerogenic rats may be due to the tissue damage in ulcerated condition and subsequent leakage of these constituents in to the gastric juice. Administration of the plant extract has increased the content of proteins and carbohydrates in the plant drug treated animals. Treatments with

drugs healed the ulcer wounds and prevent the leakage of protein and carbohydrates so the contents were increased and the increment was significantly (P<0.001) higher in plant drug treated animals than that of healthy ones. This may be due to biosynthesis triggered by phytochemicals present in the plant drug. The phytoconstituents such as alkaloids, flavonoids, terpenenoids, steroids, phenolic compounds, tannins, proteins and carbohydrates present in the plant extract were responsible for healing the ulcer wounds in the stomach and prevent the disintegration of protein and carbohydrate. These phytoconstituents have been reported as gastroprotective agents in several animal model anti-ulcer studies[36]**.** Flavonoids, tannins and triterpenes are among the cytoprotective active materials and their antiulcer efficacy has been extensively confirmed[37][38][39][40]. These biologically active compounds will be able to stimulate mucus, bicarbonate and secret prostaglandins and counteract with the deteriorating effects of reactive free radicals in gastrointestinal lumen**.**

Alcohol ingestion caused damages to gastric mucosa and results in the significant (P<0.001) enhanced activity of marker enzyme, alkaline phosphatase (ALP) in disease control animals when compared to healthy control animals. Oral administration of plant extract significantly (P<0.05) reduced the level of ALP, which indicates that the gastric damage was cured in group III animals. Omeprazole treated animals also showed decreased the level of ALP. But the reduction in alkaline phosphatase level was significantly (P<0.001) higher in plant drug treated animals than in the Omeprazole treated animals. The results of the present study shows that the *Mollugo oppositifolia* extract performed better in protecting mucosal damage than Omeprazole.

The administration of ethanol induced the ulcer and results in excessive generation of free radicals such as hydroxyl ethyl radical, superoxide radical (O2-), hydroxyl radical (OH-), peroxyl radical and hydrogen peroxide [41]**.** All these radicals formed from the ethanol-mediated process have a great potential to react rapidly with lipids and turns into lipid peroxides (LPO) [42]**.** So in the alcohol induced ulcerated condition the level of LPO will be increased. Due to the same reason, in the present study also a significant (P<0.001) level of increase in lipid peroxides was observed in disease control animals than the healthy control animals. After the treatment with plant extract and Omeprazole the level of LPO was reduced. The reduced level of LPO was more pronounced in plant extract treated animals than Omeprazole treated animals. The result authenticated that the *Mollugo oppositifolia* was having more efficacy in scavenging free radicals. Both plant extract and Omeprazole brought

back the normalcy. Under the normal healthy condition, low level of lipid peroxides occurs in body tissue [14].

Reduced glutathione is one of the most abundant non-enzymatic antioxidant bio-molecules present in the tissues[43]. Its functions are removal of free oxygen species such as H_2O_2, superoxide anions and alkoxy radicals, maintenance of membrane protein thiols and to act as a substrate for GPx and glutathione *S*- transferase (GST) [44]. In the present study the level of reduced glutathione (GSH) was significantly ($P<0.01$) decreased in disease control animals when compared to healthy control animals. After treatment with plant drug and Omeprazole the reduced glutathione level was significantly ($P<0.01$) increased and the level was comparable to the healthy animals. It may be understood that the effect of *Mollugo oppositifolia* may be due to increased synthesis of glutathione.

Free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are known to be the first line cellular defense factors against oxidative damage, disposing O_2 and H_2O_2 before their interaction to form the more harmful hydroxyl ($OH^{\cdot}$) radical[45]. Superoxide dismutase level was significantly ($P<0.01$) decreased in disease control animals than healthy control animals. Animals treated with plant extract and Omeprazole showed a significant ($P<0.05$) increase in the activity of superoxide dismutase. The enhanced level of superoxide dismutase was higher in the plant extract treated animals than in the Omeprazole treated animals. In the present study SOD activity decreased significantly in the stress induced group of animals, which may be due to an excessive formation of superoxide anions. SOD is an important defense enzyme that catalyzes the dismutation of superoxide anions into O_2 and H_2O_2 [46] [6].

The catalase (CAT) activity was significantly ($P<0.01$) decreased in diseased control animals than healthy control animals. After the administration of plant extract and Omeprazole the activity was significantly ($P<0.01$) increased in experimental animals. The increase in catalase level was higher in plant extract treated animals when compared to Omeprazole treated animals (Table-3). The phytoconstituents especially flavonoids, tannins, polyphenolic compounds available in *Mollugo oppositifolia* extract (Table-1) may be responsible for the enhanced catalase activity. Catalase acts as a protective antioxidant against the deleterious effects of LPO. And it is a highly reactive enzyme that reacts with H_2O_2 to form water and molecular oxygen [6]. The enzyme was act cooperatively at different sites in the metabolic

pathway of free radicals. The activity of SOD and CAT is associated with the elimination of highly reactive free radicals, leading to integrity and function of cell membrane [14].

Glutathione peroxidase (GPx) activity was significantly (P<0.01) decreased in the disease control animals than the healthy control animals. After the administration of plant drug and Omeprazole the enzyme activity was significantly (P<0.01) increased. The level of glutathione peroxidase significantly (P<0.01) increased more in plant extract treated animals than Omeprazole treated animals (Table-3). Glutathione peroxidase activity is important for the elimination of hydrogen peroxide and lipid hydroperoxides in the gastric mucosal cell [47]. Thus, inhibition of this enzyme activity in the gastric mucosa by ethanol may result in the accumulation of hydrogen peroxide with subsequent oxidation of lipids. The reversal of GPx activity in ethanol treated animals by *Mollugo oppositifolia* may therefore be due to the replenishment of glutathione level.

The Hemoglobin (Hb) content and Red Blood Cells (RBC) count were significantly (P<0.01) decreased when compared to healthy control animals. In ulcerated condition there is a profound hemorrhage due to the lesions in the gastric mucosa. This was evidenced from the decreased level of Hb and RBC count found in diseased animals. After the administration of plant extract there was a significant (P<0.01) raise in the level of Hemoglobin and RBC count. Hemoglobin and RBC count also increased in Omeprazole treated animals (Table-4).

In disease control animals the WBC count was significantly (P<0.001) higher than the healthy control animals. After the administration of plant extract and Omeprazole the WBC count was significantly (P<0.01) reduced (Table-3). Ulcerogenesis drastically increased the production of White Blood Cells (WBC) in the rats due to the over production of WBC during disease condition to combat the pathogens which were infected in the ulcerated tissues[3].

Ethanol produces severe gastric haemorrhagic lesions. The pathogenesis of ethanol induced gastric damage in rats is complicated and involves superficial aggressive cellular necrosis as well as the release of tissue derived mediators such as histamine and leukotriene C4. These mediators act on gastric microvasculature, triggering a series of events that lead to mucosal and sub mucosal damage [48]. So the cytoprotective mechanism of the *Mollugo oppositifolia* extract may therefore include mechanisms other than simple acid neutralization.

CONCLUSION

Prevalence of peptic ulcer disease is reported as one of the major diseases. Alcohol consumption is one of the reasons for the disease. From the present animal model study it is proved that *Mollugo oppositifolia* extract is potent antiulcer and gastroprotective agent. Treatment with this plant extract in ulcer induced rats reduced the offensive factors such as ulcer index, volume of gastric juice and total acidity of gastric juice and improved the defensive factors such as enhanced level of total protein and carbohydrates; decreased the activity of ALP and LPO; improved the level of GSH and increased activity of SOD, CAT and GPx. It was proved that the performance of *Mollugo oppositifolia* extract was better than the standard drug, Omeprazole. In continuation to the present investigation, it is planned to isolate and elucidate the structure of the active molecules from the plant which are responsible for antiulcer activity and further clinical studies with these molecules will provide important clue to produce and/or develop a highly potent antiulcer drug.

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