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ANTI-ULCER POTENTIAL OF VARIOUS SOLVENT EXTRACTS OF *TAGETES ERECTA*

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ABSTRACT

A Peptic ulcer is the most prevailing problem of the gastrointestinal tract correlated with multiple aetiologies occurs due to interferences of aggressive and cytoprotective factors of the gastric mucosa. Medicinal herbs play an essential role in the treatment of various illnesses by maximizing safety, efficacy and minimizing cost. Tagetes erecta Commonly known as Marigold is an ornamental medicinal herb traditionally used to treat various disease including external ulcers. However, there is no proper scientific data to prove its antiulcer activity. Hence the present research was designed to investigate the antiulcer activity of three different extracts (petroleum ether, Dichloromethane and Ethanol) of Tagetes erecta. Antiulcer activity was evaluated using three different models such as pyloric ligation induced gastric ulcer, aspirin-induced ulcer and alcohol-induced ulcer. All the three extracts of Tagetes erecta showed marked anti-ulcer activity by increasing ulcer protection. The present findings revealed the significant antiulcer activity of Tagetes erecta. Hence it can be utilized in a suitable form as an alternative to available synthetic medicines for treating ulcer.

KEYWORDS: ***Peptic ulcer, Aspirin, Alcohol, Pyloric ligation, ulcer index, pantoprazole.***

1. INTRODUCTION

A Peptic ulcer is the most common illness of the gastrointestinal tract associated with multiple aetiologies. It occurs due to the disturbances in aggressive factors (pepsin and hydrochloric secretion, H. Pylori) and cytoprotective factors (mucus secretion, prostaglandin release, bicarbonate secretion and cellular regeneration) of the gastric mucosa [1]. Herbal medicines are the most beneficial over synthetic drugs concerning to cost, safety and long-term usage. Therefore, it is more crucial to identify and investigate the available plants to treat numerous ailments including peptic ulcer. *Tagetes erecta* Commonly known as Marigold is an ornamental medicinal herb belongs to family Asteraceae**.** It is a small herb scattered in Asia, Europe, Africa, America and also in India [2].

Different parts of *tagetes erecta* are used as a folk medicine to treat various ailments. The leaves act as an antiseptic and are effective in muscular pain and kidney troubles. Flowers are having stomachic, carminative and astringent property. It is used to treat epilepsy, hepatic problems and eye disease. Flowers are also utilized in case of fever, cold rheumatism and bronchitis. *Tagetes erecta* is also used in abdominal pain, muscular pain, cough, dysentery and external ulcers. Many more uses were also reported for *Tagetes erecta* [3].

Tagetes erecta is reported to have numerous activities like Antinociceptive, Anti- Inflammatory [4], Antihyperlipidemic [5], Antidepressant [6], Wound healing [7], Hepatoprotective [8], Antibacterial [9] and many more. However, there is no systematic report is available to prove the antiulcer activity of *Tagetes erecta.* Hence the present research was designed to investigate the antiulcer activity of three different extracts (petroleum ether,Dichloromethane and Ethanol) of *Tagetes erecta*.

2. MATERIALS AND METHODS

Preparation of Plant extract

Tagetes erecta Linn plant was acquired locally and had been authenticated. The plant was dried, powdered and subjected to successive soxhlet extraction using three different solvents (petroleum ether, Dichloromethane and Ethanol) based on their polarity. The extract was concentrated and preserved for the experiment.

Preliminary Phytochemical screening

All the extracts of *Tagetes erecta* were analyzed qualitatively to see the presence of Phytoconstituents using standard procedure [10, 11]

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Experimental Animals

Albino mice and rats (either male or female) weighing between 20 to 25 g and 140 to 160 g respectively were procured and maintained at the standard environmental condition. Animals were fed with food and water all the time. Animal experiments were permitted by the Institutional Animal Ethical Committee of Sri Adichunchanagiri College of Pharmacy and ethical clearance no SACCP-IAEC/29/2015 was provided.

Determination of acute toxicity (LD50)

For acute toxicity studies OECD Guideline No. 425 ("up and down" method) of CPCSEA was followed [12].

Grouping and dosing of animals

Albino rats weighing between 140-160g were randomly divided into eight groups in each ulcer model containing 6 animals in each group. Group number 1 served as a control, group 2 was treated with pantoprazole 20 mg/kg (standard drug) through oral route. Group 3 and 4 received 200 and 400 mg/kg of petroleum ether extract of *Tagetes erecta*, Group 5 and 6 received 200 and 400 mg/kg of dichloromethane extract of *Tagetes erecta* and Group 7 and 8 received 200 and 400 mg/kg of alcoholic extract of *Tagetes erecta* respectively.

ANTI-ULCER ACTIVITY

Pyloric ligation induced gastric ulcer [13]

In this model, albino rats were fasted for 24 hours before experimentation. Treatment was given to each group as mentioned above.Upon 30 min of drug administration, rats were anaesthetized. Cut opened abdomen by a small incision below the xiphoid process. Identified and lift out pylorus then ligated it without interrupting blood supply. Kept the pyloric part back into the abdomen and closed the abdominal wall by suturing it. Animals were deprived of water and food during the post operative period. After four hours animals were sacrificed. Stomach content was collected and centrifuged for 10 min at 1000 rpm. The supernatant liquid was collected and utilized for further research. The stomach was isolated and its inner part was examined for ulceration and the score was given. Various parameters like pH, gastric volume, total acidity, free acidity, ulcer index and ulcer protection percentage were noted. Ulcer index and percentage of ulcer protection were calculated by a formula:

Ulcer index was calculated by using this formula.An ulcer index UI is calculated:

$UI = UN + US + UP*10^{-1}$

UN= average of a number of ulcers per animal US = average of severity score

UP = percentage of animals with ulcer

$$\text{Percentage of ulcer protection} = \frac{\text{Ulcer index of control} - \text{Ulcer index of test}}{\text{Ulcer index of control}} \times 100$$

Scoring of ulcer were made as follows

Ulcer severity	Ulcer score
Normal stomach	0
Red coloration	0.5
Spot ulcer	1
Hemorrhagic streak	1.5
Ulcers	2
Perforation	3

Measurement of pH

The gastric juice pH was measured by using a pH meter.

Estimation of total and free acidity

Pipette out and added 1 ml gastric juice into a 100 ml conical flask then added Topfer's reagent (2 to 3 drops) into it and titrated against 0.01N NaOH (Sodium Hydroxide) till red color changed to yellowish-orange. Consumption of alkali was noted which correlate to free acidity. Again, added phenolphthalein (2 to 3 drops) and continued the titration till red color reappears. A total volume of added alkali had been noted which correlate to total acidity.

Acidity was calculated by using the formula

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$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1}\text{meq/L/100g}$$

Aspirin-induced ulcer [14, 15]

Albino rats of all groups were fasted for 24 hours before the experimentation with free access to water all the time. Treatment was given accordingly.

After one hour of treatment, aspirin was administered to each animal. Sacrificed the animals 4 hours post aspirin treatment. Stomach was isolated for calculating ulcer index and ulcer protection percentage.

Alcohol-induced ulcer [14, 15]

Rats were kept fasted for 24 hours with free access to water. Treatment was provided to each group as mentioned earlier. One-hour post-treatment 5ml/ Kg of absolute alcohol had been given orally to each rat. Sacrifice all the rats after one hour of alcohol administration. Stomach was detached. The ulcer index and Percentage of ulcer protection were noted.

Statistical analysis

Results were analysed statistically using ANOVA followed by dunnett't' test.

3. RESULTS

Phytochemical Analysis

The qualitative phytochemical evaluation showed to have Carbohydrates, Triterpenoids, Flavonoids, Steroids, Quinones, Glycosides, Alkaloids in the pet ether extract. Carbohydrates, protein, Flavonoids, Alkaloids in Dichloromethane extract and Carbohydrates, protein, Triterpenoids, Flavonoids, Steroids, alkaloids, glycosides in ethanol extract.

Acute toxicity study

No toxicity or mortality was observed in 14 days of study up to the extract dose of 2000 mg/kg bodyweight. Hence 200 and 400mg/kg body weight dose for all three extracts had beenfixed for the study.

Pyloric ligation method

All three *Tagetes erecta* extracts significantly reduced ulcer index and enhanced percentage protection of ulcer. However, free acidity was significantly reduced in standard drug, a higher dose of dichloromethane extract, a higher and a lower dose of alcoholic extract only. On the same time, total acidity was not reduced significantly in any of the extracts as shown in table number 1 and figure number 1 and 2

Table 1. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on parameters ofPylorus ligation model

Treatment	Gastric content(ml)	Free acidity (mEq/L/100g)	Total acidity (mEq/L/100g)	Ulcer index	% Ulcer Protection
Control	4.02±0.11	18.83±0.600	25.16±0.60	10.91±0.071	-
Standard	1.94±0.06	15.16±0.477**	21.33±0.55*	2.15±0.030**	80.29
PETE 200	3.79±0.16	16.83±0.600^{ns}	24.16±1.57^{ns}	6.53±0.030**	40.14
PETE 400	3.28±0.21	17.330±0.666^{ns}	22.16±0.60^{ns}	4.2±0.078**	61.5
DCMTE 200	3.66±0.16	17±0.774^{ns}	22.83±0.60^{ns}	7.53±0.052**	30.98
DCMTE 400	2.40±0.14	15±0.93**	23.5±0.76^{ns}	5.37±0.030**	50.77
AETE 200	3.86±0.14	15.83±0.600*	22.66±0.88^{ns}	4.34±0.023**	60.21
AETE 400	3.68±0.12	15.66±0.881*	22.16±0.70^{ns}	3.2±0.051**	70.66

Values are expressed as mean ± SEM (n = 6). ***P<0.001, **P< 0.01 and *P< 0.05. ns = not significantPETE 400: Petroleum ether extracts of *Tagetes erecta* (400 mg), PETE 200: Petroleum ether extracts of *Tagetes erecta* (200 mg), DCMTE 400: Dichloromethane extracts of *Tagetes erecta* (400 mg), DCMTE 200: Dichloromethane extracts of *Tagetes erecta* (200 mg), AETE 400: Alcoholic extracts of *Tagetes erecta* (400 mg), AETE 200: Alcoholic extracts of *Tagetes erecta* (200 mg).

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Figure 1. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on % Protection ofPylorus ligation model

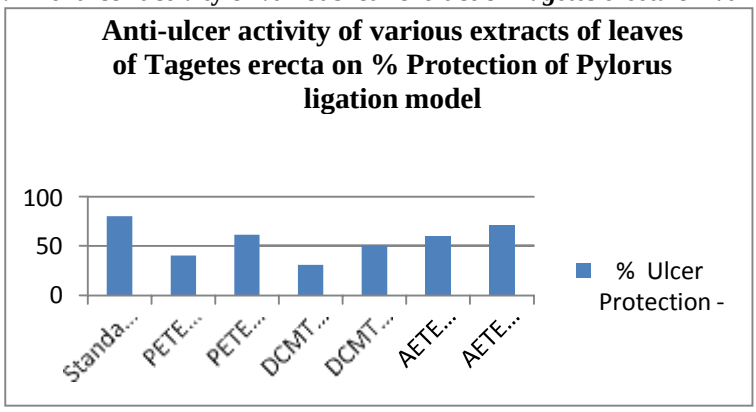
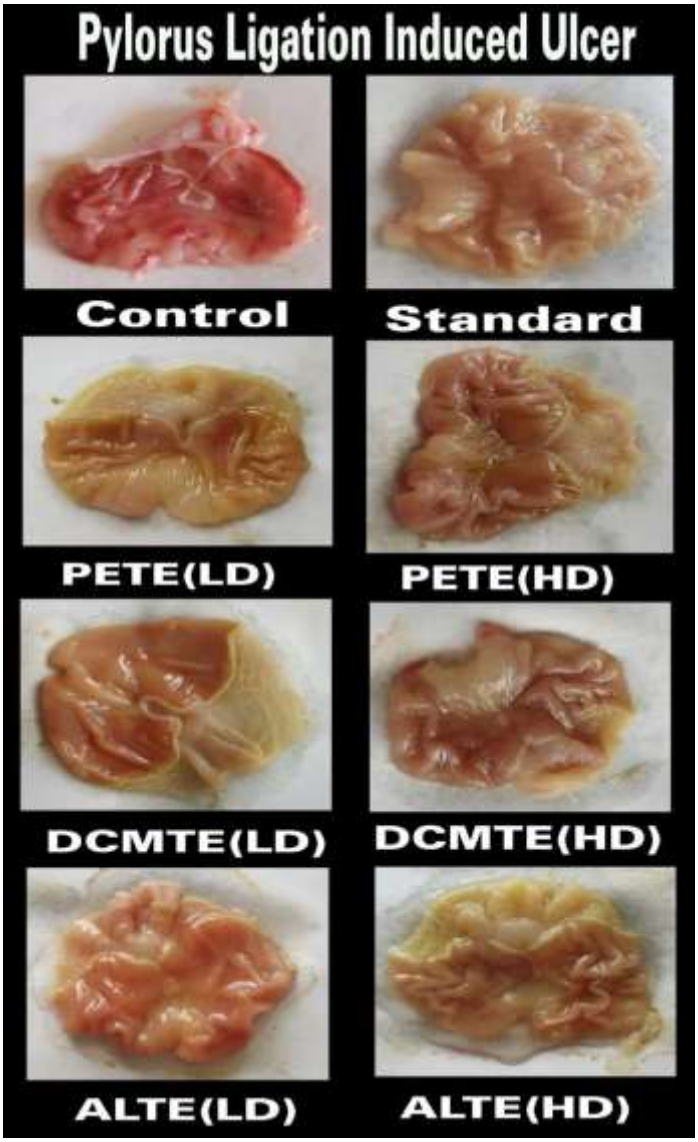


Figure 2. Anti-ulcer activity of the various leaf extract of *Tagetes erecta* on experimentalanimals by pylorus ligation method



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Aspirin-induced ulcer

All the three extracts (Petroleum ether, Dichloromethane and Ethanol) of *Tagetes erecta* were showed a marked reduction in ulcer index and increased ulcer protection. The percentage protection of ethanolic extract at a higher dose showed more significant antiulcer activity as compared to all other extracts as shown in table number 2, figure number 3 and 4.

Table 2. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on parameters ofAspirin induced gastric ulcer model.

Treatment	Ulcer index	% Ulcer Protection
Control	3.90±0.047	-
standard	3.15±.0.042**	19.23
PETE 200	3.46±0.051**	11.28
PETE 400	3.36±0.042**	13.84
DCMTE 200	3.36±0.039**	13.84
DCMTE 400	3.53±0.055**	9.48
AETE 200	3.44±0.041**	11.79
AETE 400	3.33±0.033**	14.61

Values are expressed as mean ± SEM (n = 6). ***P<0.001, **P< 0.01 and *P< 0.05. ns = not significantPETE 400: Petroleum ether extracts of *Tagetes erecta* (400 mg), PETE 200: Petroleum ether extracts of *Tagetes erecta* (200 mg), DCMTE 400: Dichloromethane extracts of *Tagetes erecta* (400 mg), DCMTE 200: Dichloromethane extracts of *Tagetes erecta* (200 mg), AETE 400: Alcoholic extracts of *Tagetes erecta* (400 mg), AETE 200: Alcoholic extracts of *Tagetes erecta* (200 mg).

Figure 3. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on % Protection byAspirin induced gastric ulcer method.

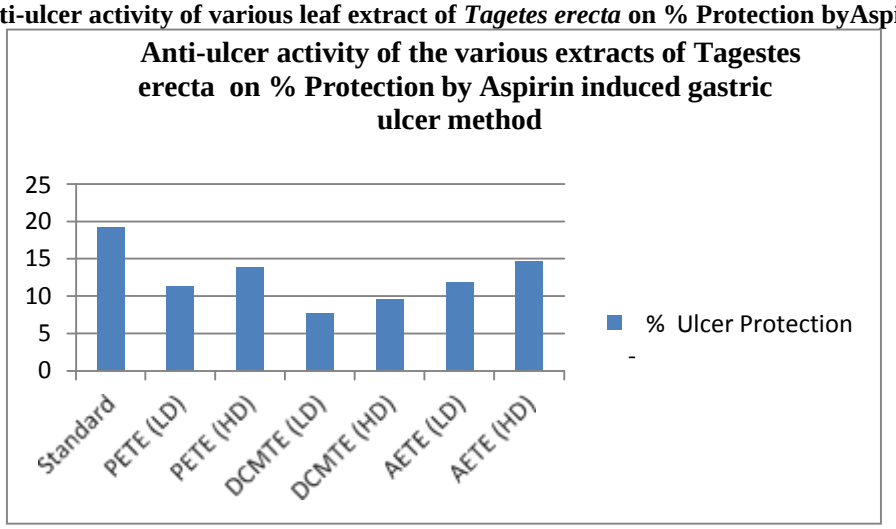

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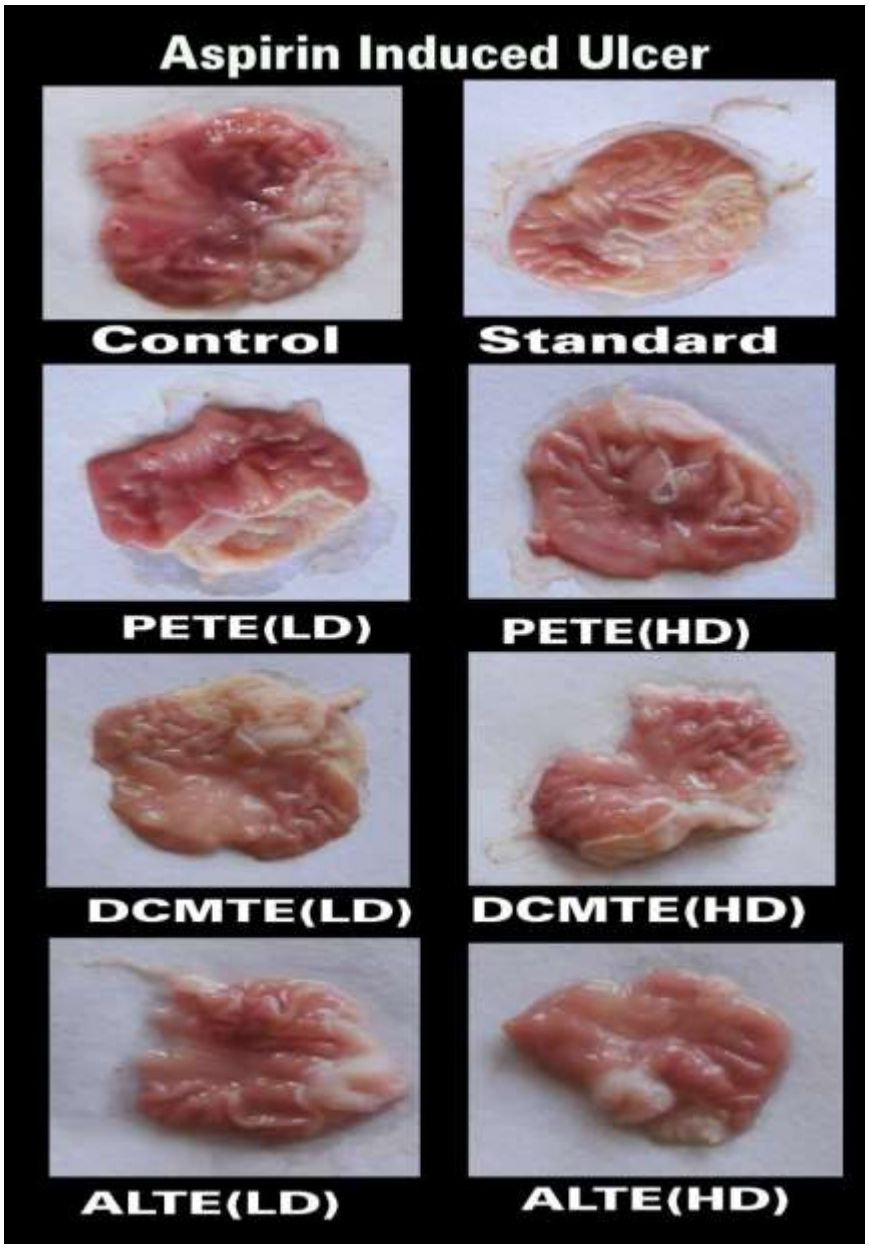
Figure 4. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on parameters ofAspirin induced gastric ulcer model.



Alcohol-induced ulcer.

All the three *Tagetes erecta* extracts proved a significant fall in ulcer index and increase in ulcer protection. Among all the extract, a higher dose of the alcoholic extract showed better ulcer protection but less potent than standard drug as shown in table number 3, figure number5 and 6.

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Table 3. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on parameters ofAlcohol induced gastric ulcer model.

Treatment	Ulcer index	% Ulcer Protection
Control	10.97±0.044	-
Standard	2.25±.0.054**	79.48
PETE 200	8.45±0.036**	22.96
PETE 400	4.36±0.054**	60.25
DCMTE 200	7.52±0.047**	31.38
DCMTE 400	5.34±0.043**	51.36
AETE 200	4.41±0.051**	59.84
AETE 400	3.31±0.044**	69.77

Values are expressed as mean ± SEM (n = 6). ***P<0.001, **P< 0.01 and *P< 0.05. ns = not significantPETE 400: Petroleum ether extracts of *Tagetes erecta* (400 mg), PETE 200: Petroleum ether extracts of *Tagetes erecta* (200 mg), DCMTE 400: Dichloromethane extracts of *Tagetes erecta* (400 mg), DCMTE 200: Dichloromethane extracts of *Tagetes erecta* (200 mg), AETE 400: Alcoholic extracts of *Tagetes erecta* (400 mg), AETE 200: Alcoholic extracts of *Tagetes erecta* (200 mg).

Figure 5. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on % Protection byAlcohol induced gastric ulcer method.

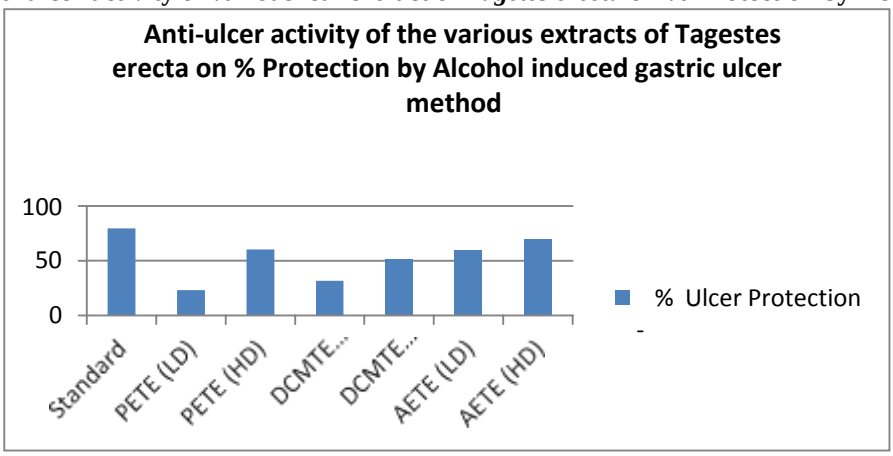

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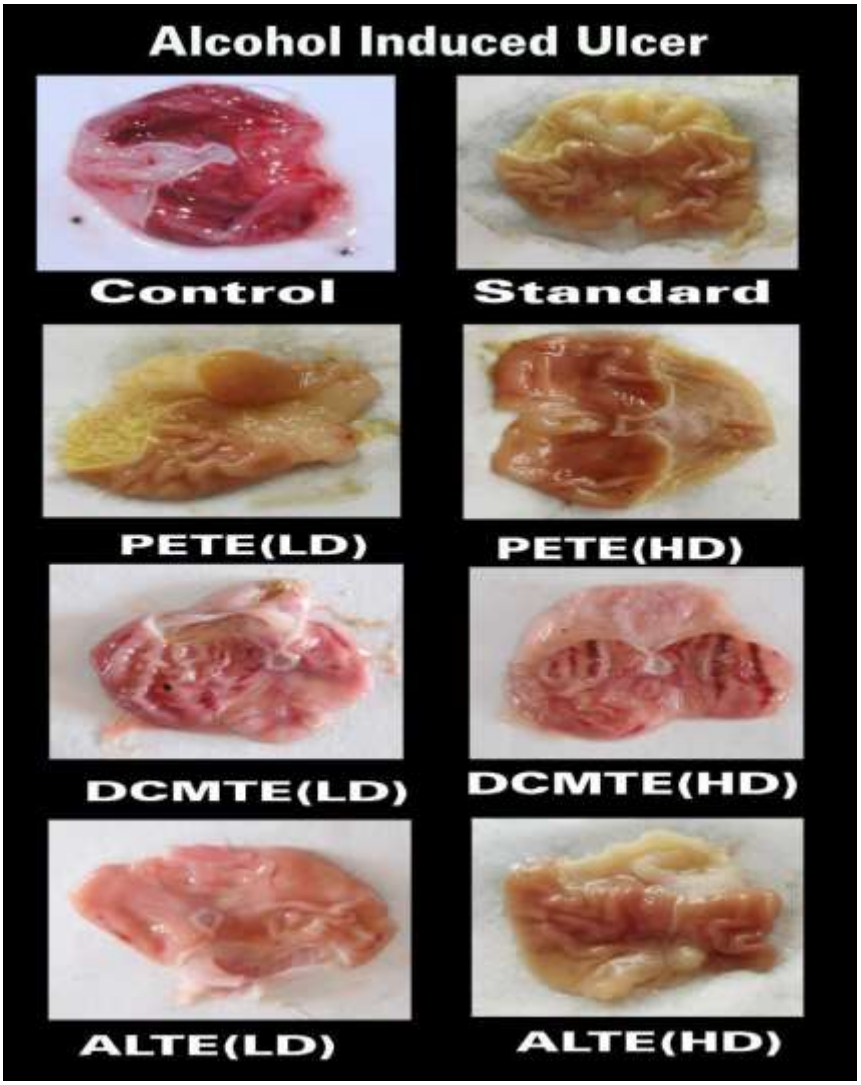
Figure 6. Anti-ulcer activity of various leaf extract of *Tagetes erecta* on parameters ofAspirin induced gastric ulcer model



4. DISCUSSION

Gastric acid is a major component of the stomach. It has been involved in varied gastrointestinal functions like protein digestion, iron and calcium absorption and even protects against bacterial infection. However, an abnormal quantity of gastric acid brings abnormal pathological conditions including gastric ulcer. A peptic ulcer is an outcome of Imbalance between aggressive and cytoprotective factors of the gastric mucous membrane. So it can be treated by balancing these factors [16].

The anti-ulcer activity of *Tagetes erecta* plant was evaluated by three different models (Pyloric ligation ulcer model, Aspirin and Alcohol induced ulcer model) which represent ulceration in human beings.

In Aspirin-induced ulcer model, aspirin administration leads to the reduction of prostaglandins synthesis by inhibiting the enzymes cyclooxygenases [17, 18]. Prostaglandin protects mucus layer of the gastrointestinal tract by secreting mucus and bicarbonate. Thus, causes vasodilatation and inhibit acid secretion. In this model all the extract significantly reducing ulceration probably due to prostaglandin involvement, mucus secretion or by showing its anti- inflammatory effect [19]

In the case of Alcohol-induced ulceration, administration of alcohol causes the generation of oxygen free radicals that lead to the enhancement of lipid peroxidation. Thus, damages cell promotes tissue injury and ulceration [20, 21]. Hence *Tagetes erecta* have a cytoprotective effect against alcohol-induced ulcer.

In pyloric ligation, gastric juice accumulation and hindrance of gastric blood circulation are answerable for ulceration induction [22]. Here there is a rise in various parameters like gastric volume, free acidity, total acidity and ulcer index. The antiulcer potential of all the three extracts of *Tagetes erecta* is evidenced by decreasing all these parameters in this model.

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Flavonoid is the major Phytoconstituents which protect gastric mucosa and responsible to show anti-ulcerogenic activity is a flavonoid [23, 24]. A Phytochemical investigation had beenproved the presence of flavonoid in *Tagetes erecta*. Hence the antiulcer potential of this plant might be due to the existence of flavonoid.

5. CONCLUSION

In conclusion, the plant *Tagetes erecta* Linn could be a drug of demand in thetreatment for ulcer. However further study is required to elucidate the exact mechanism of action and constituent responsible for its antiulcer activity. Also, suitable formulation should be decided for superior delivery and efficacy of a drug.

6. ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

Declared None

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