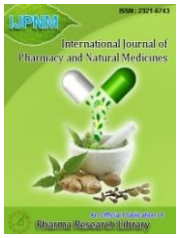

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Evaluation of Antiulcer Activity of *Tagetes Erecta* Flowers in Rats

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A B S T R A C T
Gastric ulcer is the most common gastrointestinal problem affecting many populations worldwide, with high morbidity and mortality rate. The pathogenesis of gastric ulcer has been studied for decades, and it has been found that the disease occurs when there is an imbalance between gastric defensive factor and the aggressive factors (40).Many hearbal medicince and their active ingredients have proved to be effective in treating and even preventing the recurrence of gastric ulcer disease, including one which is the topic of the current investigation. In this study EETE demonstrated significant gastro protective activity against ethanol induced gastric ulcer in the animal model. In the study clinical biochemical parameters, the liver function test, the kidney function test, and the lipid profile were evaluated. The results showed that the rats in the ulcer group exhibited compartable disturbance to the lipid profile and kidney function test. Moreover the results also display an increased serum level of the liver enzymes (aspartate aminotransferase and alanine aminotransferase) as markers of hepatic injury, since an increased level of hepatic enzymes could be ascribed to alcoholic hepatitis due to ethanol administration. This research indicated a significant anti-ulcer activity of ethanolic extract of *Tagetes erecta* flowers by preventing the development of gastric ulcers in ethanol induced rats. It also showed significant protection compared to standard

Keywords: *Tagetes Erecta,* Antiulcer Activity, Rats, hepatitis, EETE

A R T I C L E I N F O

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C o n t e n t s

1. Introduction

Ulcer is defined as disruption of mucosal integrity of the stomach and/or duodenum leading to a local defect or excavation due to active inflammation. Peptic ulcer described a condition in which there is a discontinuity in the entire thickness of the gastric or duodenal mucosa that persists as a result of acid and pepsin in the gastric juice. About 10% of population in developed countries is likely to be affected at some time by peptic ulcer, when the prevalence for active ulcer disease being about 1% at any particular point in time. Peptic ulcer accounts for 10-15% of dyspepsia and oesophagitis for about 20%. However, hospital admission rates for gastro-intestinal bleeding associated with gastric and duodenal ulcers are rising, especially in older patients. Treatment of peptic ulcers is aimed at relieving ulcer pain, healing the ulcer, reducing ulcer associated complications, eradicating H.pylori infection if present and minimizing ulcer recurrence. Most peptic ulcers occur in the presence of acid and pepsin when *H.pylori*, NSAIDS, or other factors disrupt normal mucosal defense and healing mechanisms. Benign gastric ulcers can

occur anywhere in the stomach, although most are located on the lesser curvature, just distal to the junction of the antral and acid secreting mucosa. Most duodenal ulcers occur in the first part of the duodenum.

Tagetes erecta

Plant Description:

Tagetes erecta is a rapid growing annual flowering plant with heights ranging from 6-8 inch to medium and taller. Erect growing plant with height from 10 inch to 3 ft, bearing large pompon-like double flowers up to 5 inch across and have a shorter flowering period from mid-summer to frost. These contain larger flowers, found in two basic shades of yellow and orange. Leaves are toothed and pinnately compounds. The soil must be well drained and moist, plant that brings lot of sunshine. These flowers used in all pujas in india[22].

Plant: Tagetes erecta

Taxonomical classification:

Kingdom : Plantae
Order : Asterales
Family : Asteraceae or Composite
Genus : Tagetes
Spices : Tagetes erect
Scientific : Tagetes erecta.L

Common names:

Marigold, Mexican marigold.

Synonyms: Tagetes corymbosa, T.ernstii, T.excelsa.

Vernacular names:

Language	**: Name**
Telugu	: Banti
Hindi	: Genda
English	: Marigold
Sanskrit	: Sandu
Malayalam	: Chendumalli
Urdu	: Genda
Tamil	: Turkamalli

Chemical constituents

Flavonoids

Tagetes erecta stem, leaves and flowers is very rich in flavonoids. Various flavonoids reported to be present are querectin, myricetin, quercetagetrin, kampeferol, 6-hydroxykaempferol-7-O-glucoside, kampeferol, apigenin, patuletin, rhamnetin kampeferol and luteolin[22,23,24]

Phenolic Compounds:

The polyphenols isolated from the synergic acid, ethyl gallate, methyl-3,5-dihydroxy-4-methoxy benzoate, ferulic acid, ortho- coumaric acid.

Carotenoids: Tagetes erecta whole plant shows presence of different carotinoids like lutein, lutein esters, carotene, violaxanthin and 9'-(Z)-neoxanhin, and xanthophylls.

Vitamins: Tagetes erecta plant contains high concentration of vitamin A, E, C, and K and also folic acid.

Uses:

Traditional uses: In piles, kidney troubles, muscular pains, painful mouth, stomach ulcers, wounds, burns, astringent, carminative, stomachic, scabies, liver complaints, disease of eyes, rheumatism, cold, bronchitis.

Essential oils:

The essential oil of the Tagetes erecta plant was reported to contain d-limonene, ocimene, α-pinene,β-pinene, dipentene, linalool, gereniol, tagetone, β-myrecene, β-phellandrene. Other chemical constituents such as Alkaloids, Saponins, Tannins, Carbohydrates, Proteins, Glycosides, Quinones, Coumarins, and Vit C,E.

Folkoric uses: Pimples, eczema, bruising, other skin allerges.

Medicinal uses:

Anti-inflammatory, Analgesic, Antipyretic, Antioxidant, Anti-epileptic, Hepatoprotective, Wound healing, Antidiabetic, Hypolipedemic activity.

Other uses:

Larvicidal, Insecticidal, Mosquitocidal, Antibacterial, antimicrobial activity, Food colorants and dyes.

2. Materials & Methods

1. Animals:

Healthy wistar rats weighing 150-200gms were purchased from Mahaveer Enterprizes, Mwdipally and Hyderabad. The animals were kept in a well- ventilated room and the animals had exposed to 12hrs day and night cycle with a temperature between 22±3°C.The animals were housed in large spacious,hygoenic polypropylene cages during the course of the experimental period.The animals were feed and water ad libitium,supplied by this institution.All experiments were performed after obtaining prior approval from Institutional Animal Ethics Committee.

2. Drugs and Chemicals: Ethanol, Lansoprazole, CMC, Phenolphthalein, Methyl orange reagent, Sodium hydroxide, Normal saline, SGOT, SGPT kit, Cholesterol, HDL, Triglyceride kit and Creatinine kits.(25,26)

3. Procurement of plant material:

The flowers of Tagetes erecta were collected from local markets in Hanamkonda; collected flowers were shade dried and made into coarse powder by using a mixer grinder, and the powder is allowed for extraction of chemical constituents by Maceration technique.

4. Preparation of extraction:

The flowers of Tagetes erecta were selected for the present study. Ethanolic plant extract were prepared by soaking coarse powder of plant seeds in ethanol and allowed to stand for 7days with occasional shaking and stirring. when the solvent become concentrated, the liquid alcohol content was filtered through cotton and then through whatmann filter paper #1.then the solvent were allowed to evaporate using rotary evaporator at temperature 40-45˚C.Thus the highly concentrated crude extracts were obtainted extract was preserved for experimental use.[28,30,31]

5. Preliminary Phytochemical screening of extraction:

Qualitative chemical tests were conducted for ethanolic extract identify the various phyto constituents by employing standard screening tests.

Tests for carbohydrates:

Mollish's test: To 2-3ml of extract, few drops of α-napthol solution in alcohol were added, shake and con. sulphuric acid added from the side of the test tube. It was observed for violet ring at the junction of two liquids.

Test for glycosides:

Liebermann-buchard's reaction: Mixed 2ml of extract with chloroform added 1-2ml of acetic anhydride and 2 drops of concentrated sulphuric acid from the side of the test tube. Observed the first red then blue and finally green colour.

Test for flavonoids:

- To small quantity of residue, added lead acetate solution observed for yellow colored precipitate.
- To the test solution added few drops of ferric chloride solution observed for intense green.

Test for Alkaloids:

Mayer's test: 2-3ml of filtrate with few drops of mayer's reagent was observed for precipitate.

Hager's test: 2-3ml of filtrate with few drops hager's reagent was observed for yellow precipitate.

Test for proteins:

Millon's test:

Mixed 3ml of test solution with 5ml of millon's reagent, white precipitate obtained. Precipitate warmed turns brick red or precipitate dissolves given red solution.

Test for tannins and phenolic compounds: To 2-3ml of test solution added few drops of following solutions was looked for respective coloration or precipitate.

- 5% ferric chloride solution –Deep blue black coloured.
- Lead acetate solution-white precipitate.
- Gelatin solution-white precipitate
- Acetic acid solution-red colour solution.

Test for amino acids:

Ninhydrin test:

3ml of test solution and 3 drops of ninhydrin were heated in boiling water bath for 10mins.Observed for purple or bluish colour.

Saponins test: Shake the drug extract or dry powder vigorously with water. persistant foam observed.

Test for Terpenoids:

To 0.5ml of extract, 2ml of chloroform was added and conc. sulphuric acid was added carefully. Formation of red brown color at the interface indicated the presence of terpenoids.

Test for Glycosides:

Libermann-buchared'reagent:

Mixed 2ml of extract with chloroform added 1-2ml of acetic anhydride and 2 drops of concentrated sulphuric acid from the test tube. observed the first red then blue and finally green.

Acute toxicity studies:

The acute toxicity was determined on albino rats by fixed dose method of OECD guide line no 420 given by CPCSEA. Group of 6 rats were administered test drug by oral route at a dose of 200,400mg/kg(6 animals in each dose)and mortality was observed after 24hrs.The safe dose was found to be mg/kg body weight for this study two doses were selected. [28]

Screening methods:

Ethanol induced gastric ulcer
Pylorus-ligation induced gastric ulcer
Aspirin induced gastric ulcer
Indomethacin induced gastric ulcer.

Ethanol: Ethanol is a a clear liquid alcohol that is made by the fermentation of different biological materials, this alcohol is know to have many uses, but one is particular is becoming more popular. Ethanol, the most widely used biofuel, is made in a process similar to brewing beer the ethanol is blended with gasoline to improve vehicle performance and reduce air pollution. Ethanol is a liquid alcohol that is manufactured by the fermentation by the of a wide variety of biological materials. These materials include grains such as wheat, barey, corn, wood and sugarcane. Ethanol is miscible in all proportions with water and most organic solvents .It is useful as a solvent for many substances are called tinctures; if the solute is volatile, the solution is called spirit.[32]

Properties:

Molecular Formula: CH_3CH_2OH
Molar mass: 40.07 g mol^{-1}
Appearance: Colorless liquid
Density: 0.789 g/cm^3
Melting point: 114ºC, 159K-173ºF
Boiling point: 78.37ºC,352K, 173ºF
Log P: 0.18
Acidity: 15.9
Basicity: 1.9
Refractive index: 1.36
Dipole moment: 1.69D
Vapour pressure: 5.95KPa (at 20ºC) [32].

Ethanol has been made since ancient times by the fermentation of sugars. All beverage ethanol and more than half of industrial ethanol is still made by this process. Simple sugars are the raw materials. Zymase, an enzyme from yeast, changes the simple sugars into ethanol and carbondioxide and fermentation and reaction represented by the simple equation.

$$C_6H_{12}O \longrightarrow 2CH_3CH_2OH+2CO_2$$

Ethanol passes through the stomach into the small intestine where the ethanol is rapidly absorbed and distributed throughout the body.The ethanol enters body tissue in proportion to their water content. Therefore, more ethanol is found in the blood and the brain than in muscle or fat tissue. The ethanol is greatly diluted by body fluids. Ethanol is toxic and the body begins to dispose of it immediately upon its consumption. Over 90% of its processed by the liver. In the liver,the alcohol dehydrogenase enzyme converts ethanol in to acetaldehyde, which is itself toxic. [33]

$$CH_3CH_2OH \longrightarrow CH_2\text{-}CO_2\text{-}H+2H$$

Ethanol acts as a drug affecting the central nervous system. Its behavioral effects stem from its effects on the brain and not on the muscles or senses themselves. It is a depressant, and depending on dose, can be a mild tranquilizers or a general anesthetic. It suppresses certain brain functions.

However, as concentration increases, further suppression of brain functions produce the classic symptoms of intoxication: slurred speech, unsteady walk, disturbed sensory perception, and inability to react quickly. At very high concentrations, ethanol produces general anesthesia; a highly intoxicated person will be a sleep and very difficult to walk, and if awakened, unable to move voluntarily.[33]

Methodology:

The wistar rats were randomly divided into five groups of six rats in each group were fasted for 24hr before experiment but with free access to water.

Table.1 Experimental design of Ethanol induced gastric ulcer model

Groups	Treatment
1Normal control	Vehicle
2 Disease control	Ethanol
3 Standard	Lansoprazole(10mg/kg p.o)
4 Test drug	Tagetes erecta 200mg/kg
5 Test drug	Tagetes erecta 400mg/kg

Experimental procedure:

Ethanol induced ulcer model*:* Wistar rats of either sex weighing between 150-200 g were divided into six groups, each consisting of six rats. The animals of group I served as normal control were pre-treated with vehicle. Group II served as disease control received vehicle for 7 days. Group III received lansoprazole (10mg/kg p.o.) for 7 days as reference during for ulcer protective study. Group IV received ethanolic extract of Tagetes erecta (EETE) Flowers extract (200mg/kg) and Group V received ethonolic extract of Tagetes erecta (EETE) Flower extract (400mg/kg) in 1% CMC for 7 days. The gastric ulcers were induced on 7th day in all treated rats expect group 1by administrating ethanol (95%)(1ml/200 g.p.o), after 1 hour of respective treatment. The animals were then sacrificed by cervical dislocation after six hours. The stomach was taken out and cut open along the greater curvature. Ulcer index was determined.[29,35,36]

Ulcer index:

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

UI=Ulcer index

UN=Average of number of ulcer per animal

US=Average of severity score

UP=Percentage of animal with ulcer[34,37,38]

Ulcer scores:

0=Normal coloured stomach

1.0=Isolated haemorrhagic spot

2.0=Dense haemorrhagic spot

3.0=Small ulcer

4.0=Large ulcers

5.0=Perforations.[39,40]

Percentage protection was calculated by using the formula:

Percentage protection

$$= \frac{(ulcerindex)control-(ulcerindex)test}{(ulcerindex)control} \times 100$$

Estimation of gastric pH:

A volume of 1ml of the gastric juice was diluted with 1ml of distilled water and the pH of the solution was measured using a pH meter.[41]

Estimation of free acidity and total acidity:

1ml of gastric juice was pipette into a 100 ml of conical flask,2 0r 3 drops of methyl orange reagent was added and titrated with 0.01N sodium hydroxide untill the colour of the solution become yellowish. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was titrated until a definite pink colour appears. The total volume of NaOH added was noted and this corresponds to total acidity.

Acidity=volume of NaOH X Normality of NaOH X 100/0.1 m.eq/lit

Serum biochemical analysis:

The collected blood samples were centrifuged at 2500 rpm for 10min, which was stored at 80ºC before use for biochemical analysis. Serum samples were analyzed to evaluate possible changes in serum biochemical parameter: liver function test, kidney function test, and lipid profile. [47]

Statistical Analysis:

All the data were expressed as mean ± S.D (n=6). Statistical comparison was performed by using one-way ANOVA coupled with Dunnet's multiple comparison tests. Results were considered as significant when P<0.05.

3. Results and Discussion

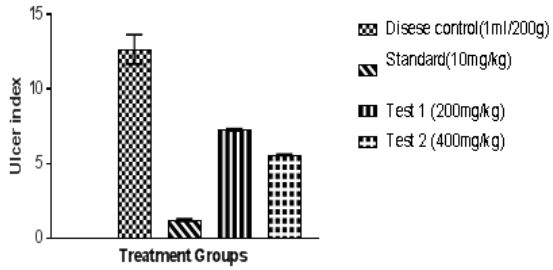

Figure.1. Figure: Showing Effect of extract on Ulcer index of ethanol induced rats

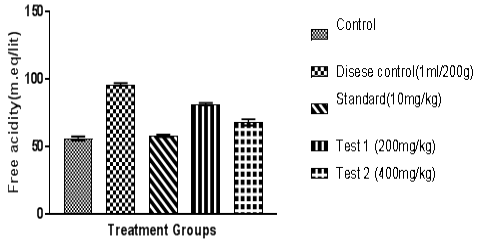

Figure.2: Showing Effect of extract on free acidity of ethanol induced rats

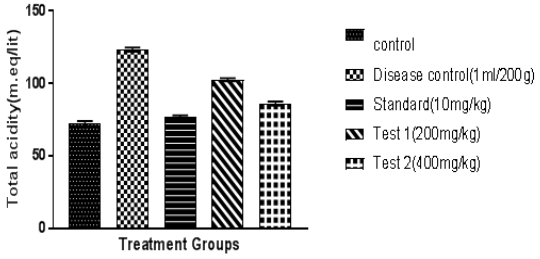

Figure.3: Showing Effect of extract on pH of ethanol induced rats

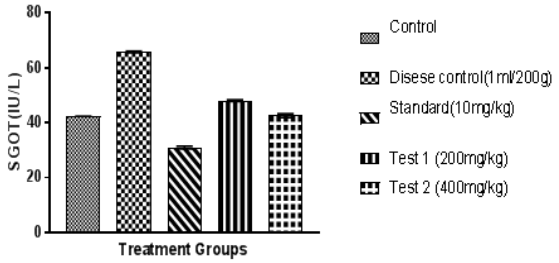

Figure.4: Showing Effect of extract on SGOT levels of ethanol induced rats

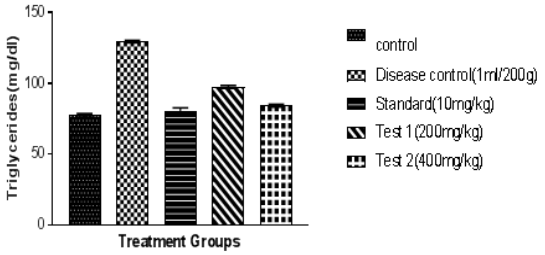

Figure.7: Showing Effect of extract on Triglyceride levels of ethanol induced rats

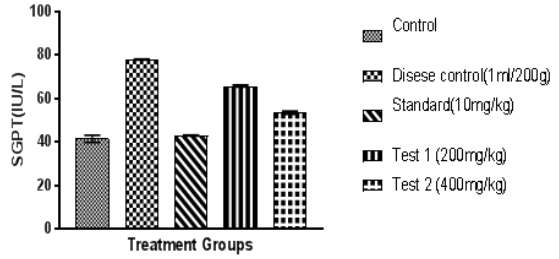

Figure.5: Showing Effect of extract on SGPT levels of ethanol induced rats

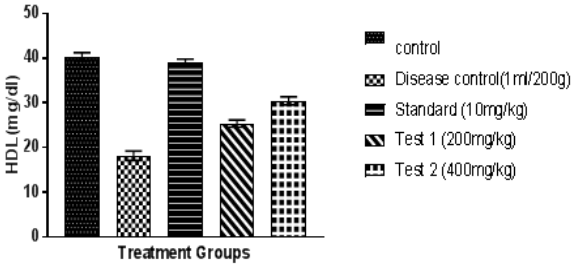

Figure.8: Showing Effect of extract on HDL levels of ethanol induced rats

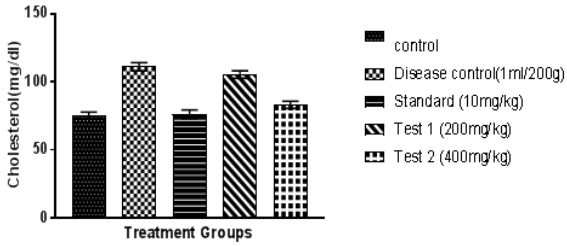

Figure.6: Showing Effect of extract on Cholesterol levels of ethanol induced rats

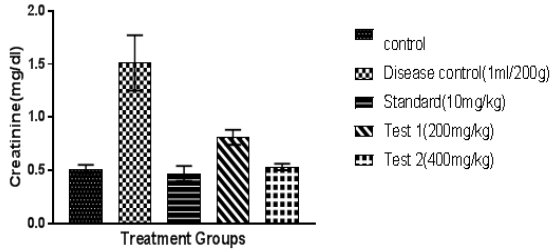

Figure.9: Showing Effect of extract on Creatinine levels of ethanol induced rats

Table 1: Phytochemical analysis

Phytoconstituents	**EETE flowers Present/absent**
Carbohydrates	Present
Glycosides	Present
Saponins	Present
Flavonoids	Present
Alkaloids	Present
Tannins and phenolic compounds	Present
Proteins	Present
Terpenoids	Present
Quinones	Absent

Table 2: Effect of EETE on Ulcer index and percentage protection

Group	Dose(mg/kg)	Ulcer index	%protection
Control	-	-	-
Disease control	-	12.68±0.19	-
Std	10	1.25±0.08***	93.1
Test 1	200	7.32±0.07***	42.2
Test 2	400	5.63±0.08***	55.8

Mean±SD (N=6). *P<0.05, **P<0.01,***P<0.001 compared to disease control, analyzed by one-way ANOVA followed by Dunnet's multiple comparison test.

Table 3: Effect of EETE on free acidity and total acidity

Group	Dose(mg/kg)	Free acidity(m.eq/lit)	Total acidity (m.eq/lit)
Control	-	56.23 ±1.41	72.13± 0.75
Disease control	-	96.32± 0.89***	123.23± 1.37***
Std	10	58.35 ±0.88***	76.56± 1.36***
Test 1	200	81.02 ±0.89**	102.42± 0.98***
Test 2	400	68.32± 0.68***	86.03± 1.36***

Mean±SD(N=6). P<0.05, P<0.01, P<0.001 compared to disease control, analyzed by one-way ANOVA followed by Dunnet's multiple comparison test.

Table 4: Effect of EETE on Gastric pH

Group	Dose(mg/kg)	pH
Control	-	4.61 ±0.051
Disease control	-	1.92± 0.16
Std	10	4.52± 0.04***
Test 1	200	3.03± 0.04**
Test 2	400	3.71± 0.06***

Mean±SD (N=6).P <0.05, P<0.01, P<0.001 compared to disease control, analyzed by one-way ANOVA followed by dunnet's multiple comparison test

Table 5: Effect of EETE on liver enzymes

Group	Dose(mg/kg)	SGOT(IU/L)	SGPT(IU/L)
Control	-	42.41± 1.02	41.60 ±1.63
Disease control	-	65.81 ±0.32***	78.08 ±0.20***
Std	10	30.94 ±0.46***	43.08± 0.21***
Test 1	200	48.11± 0.28***	65.82 ±0.41***
Test 2	400	42.62 ±0.66***	53.63 ±0.69***

Mean±SD(N=6).P<0.05,P<0.01, P<0.001 compared to disease control, analyzed by one way ANOVA followed by Dunnet's multiple comparison test.

Table 6: Effect of EETE on lipid profile

Group	Dose(mg/kg)	Cholesterol (mg/dl)	Triglycerides (mg/dI)	HDL(mg/dI)
Control	-	75.15±2.58	77.30 ±1.21	40.32 ±0.89
Disease control	-	111.16 ±2.92	129.21 ±0.89	18.15± 1.04
Std	10	76.16±3.10***	80.12± 2.40***	39.03 ±0.75***
Test 1	200	105.32±2.82***	97.30 ±1.04***	25.36± 0.81***
Test 2	200	83.13±2.73***	84.13 ±0.89***	30.46 ±0.89***

Mean±SD (N=6). P<0.05,P<0.01, P<0.001 compared to disease control, analysed by one-way ANOVA followed by Dunnet's multiple comparison test

Table 7: Effect of EETE on Creatinine

Group	Dose(mg/kg)	Creatinine
Control	-	0.51± 0.04
Disease control	-	1.51 ± 0.26
Std	10	0.47 ± 0.07***
Test 1	200	0.81 ± 0.07***
Test 2	400	0.53 ±0.03***

Discussion

Gastric ulcer is the most common gastrointestinal problem affecting many populations worldwide, with high morbidity and mortality rate. The pathogenesis of gastric ulcer has been studied for decades, and it has been found that the disease occurs when there is an imbalance between gastric defensive factor and the aggressive factors[40].Many hearbal medicince and their active ingredients have proved to be effective in treating and even preventing the recurrence of gastric ulcer disease, including one which is the topic of the current investigation. In this study EETE demonstrated significant gastro protective activity against ethanol induced gastric ulcer in the animal model. Ethanol is the most common gastric ulcer model that is frequently used due to its characteristics; the easy and rapid penetration properties into the gastric mucosa can cause numerous pathologic events, resulting in mucosal injuries.[47] Ethanol stimulates gastric juice production, even when food is not present and as a result, its consumption will stimulate acidic secretion normally intended to digest protein molecules.

Consequently, the excess acidity may harm the inner lining of the stomach. Ethanol provoked gastric mucosal lesions

are caused by the direct toxic effect of ethanol through the reduction in mucus production, gastric mucosal blood flow and bicarbonate secretion. Moreover by intragastric administration of ethanol a rapid and time dependent release of endothelin-1 into the systemic circulation preceded the development of hemorrhagic mucosal erosions by vasoconstriction. Endogenous glutathione and prostaglandin (PG) levels are also lowered by ethanol while the release of histamine, influx of calcium ions and generation of free radicals and production of leukotrienes are all increased.[11,50]

Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the stomach. There are various mechanisms involved in the ulcer production in different experimental models. Many experimental evidences have shown that antioxidant significantly strengthen the gastric walls and protect tissue from oxidative damage. Furthermore, gastric acid secretion now accepted to play an important role in the formation of gastric ulcer.[50]

In the present study we observed the dose of EETE (200mg/kg) significant decreases the total acidity, free acidity, ulcer index as well as increase gastric pH due to the restoration of the normal gastric acid condition. As per result we observed more significant improvement in EETE (400mg/kg) treated rats when compared to control rats. In the study clinical biochemical parameters, the liver function test, the kidney function test, and the lipid profile were evaluated. The results showed that the rats in the ulcer group exhibited compartable disturbance to the lipid profile and kidney function test. Moreover the results also display an increased serum level of the liver enzymes (aspartate aminotransferase and alanine aminotransferase) as markers of hepatic injury, since an increased level of hepatic enzymes could be ascribed to alcoholic hepatitis due to ethanol administration. Pretreatment with EETE showed a significant decrease in such parameters near to that of the normal control level when compared to control rats. The results could presumably be due to the high efficacy of this natural compound against ethanol-induced tissue damage.

4. Conclusion

This research indicated a significant anti-ulcer activity of ethanolic extract of *Tagetes erecta* flowers by preventing the development of gastric ulcers in ethanol induced rats. It also showed significant protection compared to standard. Extract of *Tagetes erecta* are very effective herbal alternative for the treatment of ulcers.

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