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EVALUATION OF ANTIULCER ACTIVITY OF MENTHA ARVENSIS

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ABSTRACTS

Extract against anti-inflammatory medicine instigated gastric ulcer. Mentha Arvensis extract was prepared by using continuous hor extraction (soxhlet) with ethanol.The extract were subjected to preliminary qualitative phytochemical investigation.The plant extract Mentha arvensis drug delivers a diminishing in the ulcer number in the aspirin instigated alcer model in rat.The remedial proportion in the aspirin initiated gastric model was 42%, 54%,72.9%, and 84.12% utilizing ethanolic extricate (100, 250,500 mg/kg), this shows that the plant has antiulcerogenic, antisecretory, and cytoprotective activities. The present data obtained from ethanolic extract of menthe arvensis showed the presence of a gastro protective effect and improved ulcer healing properties.

Keywords: * Antiulcerogenic, * Antisecretory, * Cytoprotective activities.

INTRODUCTION

A greater part of our populace, especially those living in towns rely generally upon customary medicines. More than 3/4 of the total populace depends principally on the plants and plant removes for medical care. Spices have as of late stood out as wellbeing gainful food sources and as source material for drug improvement. Over 30% of the whole plant species all at once or other, have been utilized for restorative purposes, for example, to treat a wide assortment of clinical conditions including peptic ulcer, liver sickness, ischemia, atherosclerosis, intense hypertension, diabetes mellitus and malignancy.

PEPTIC ULCER

General

Ulcers are cavity like disintegration or sore that happen in the upper gastrointestinal parcel of the body. Stomach ulcers are additionally called peptic ulcers. The word peptic alludes to pepsin, a stomach catalyst that separates protein. A ulcer situated in the stomach is known as a gastric ulcer. A ulcer is the consequence of an unevenness among forceful and protective variables. On one hand, an excess of corrosive and pepsin can harm the stomach coating and cause ulceration. Then again, the harm starts things out from some other reason making the stomach lining powerless to even a normal degree of gastric corrosive. Peptic ulceration is an extremely normal illness and it is assessed that roughly 10%-20% of the grown-up male populace in western nations will encounter a peptic ulcer at some stage in their lives . It produces significant agony and sickness. In 1970 in the USA 3.5 million individuals experienced peptic ulcer and 8600 passing's were ascribed to this infection. At present almost 15 million individuals are experiencing peptic ulcer illnesses and 6000 passings each year are accounted for across the world. As per doctors over 90% of duodenal ulcers are brought about by Helicobacter pylori (H. pylori) disease. Long haul utilization of NSAIDs can likewise cause gastric ulcer. Treatment cost is assessed more than $2 to $4 billion every year. The vast majority of the ulcers mend by utilizing manufactured medications.

A peptic ulcer is an intense, subacute, or persistent mucosal sore that happens in the lower of the throat, in the stomach typically along the lesser curve or in the initial 3 to 4 cm of the duodenum.

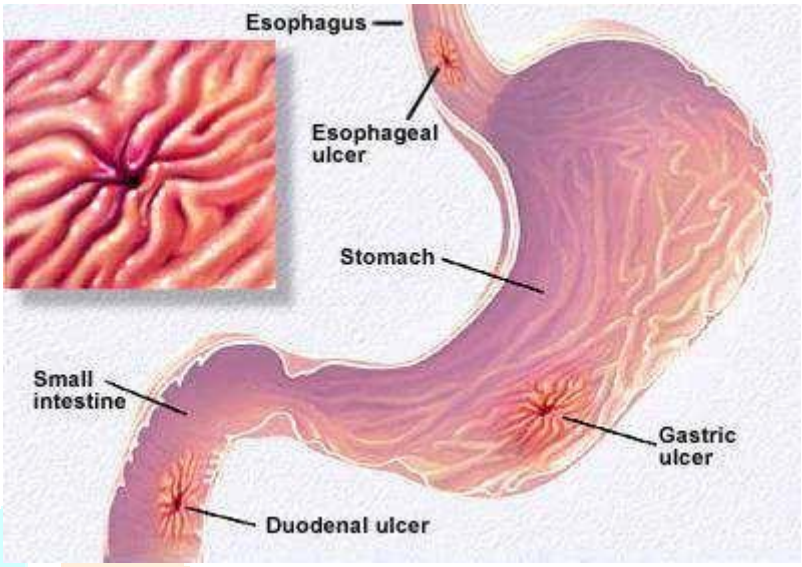


Fig.1 Stomach showing Ulcer

Acute peptic ulcers often are multiple, and range in size from a few milliliters to 3 cm in diameter. The lesions are shallow, extend through the mucosa, and have well-defined margins. Subacute peptic ulcers comprise of sores that are on the move between being intense and constant. Such sores are more profound than the intense ones, and are equipped for entering through the mucosa, submucosa, and periodically the strong layer. Chronic ulcers are quite often single, despite the fact that they might be encircled by scars of recently mended intense or subacute ulcers. The sore is round with a sharp edge. The profundity goes from 10 to 15 mm. The floor of the ulcer is generally clear and covered with stringy tissue. Peptic ulcers heal from the floor upward. In superficial ulcers, healing is completed and gastric glands may regenerate. In chronic ulcers, healing is slow. The mucosa is replaced by smooth, scarred tissue that is devoid of glands.

Classification of peptic ulcer

- Duodenal ulcers
- Gastric ulcers
- Oesophageal ulcer
- Meckel's diverticulum ulcer

EPIDEMIOLOGY

Around 10% of Americans foster constant PUD during their lifetime. In Western nations the predominance of Helicobacter pylori contaminations generally coordinates with age (i.e., 20% at age 20, 30% at age 30, 80% at age 80 and so on). Pervasiveness is higher in underdeveloped nations where it is assessed at about 70% of the populace, though created nations show limit of 40% proportion. In general,

H. pylori contaminations show an overall abatement, all the more so in created

nations. Transmission is by food, polluted groundwater, and through human salivation, (for example, from kissing or sharing food utensils).

A few of instances of H. pylori disease will ultimately prompt a ulcer and a bigger extent of individuals will get vague distress, stomach agony or gastritis. Peptic ulcer illness tremendously affected dreariness and mortality until the last many years of the twentieth century, when epidemiological patterns began to highlight an amazing fall in its occurrence. The explanation that the paces of peptic ulcer infection diminished is believed to be the improvement of new compelling medicine and corrosive suppressants and the disclosure of the reason for the condition, H.pylori.

ETIOLOGY

Most peptic ulcers happen within the sight of corrosive and pepsin when H. pylori, NSAIDs, or different variables upset typical mucosal guard and mending mechanisms. Hypersecretion of corrosive is the essential pathogenic system in hypersecretory states like ZES(Zollinger-Ellison syndrome). Ulcer area is identified with various etiologic elements. Generous gastric ulcers can happen anyplace in the stomach, albeit most are situated on the lesser shape, only distal to the intersection of the antral and corrosive emitting mucosa. Most duodenal ulcers happen in the initial segment of the duodenum (duodenal bulb).

Potential causes of peptic ulcer is

- *Helicobacter pylori* infection (90%)
- Drugs - anti-inflammatory (NSAIDs) & Corticostroids
- Hyperacidity eg. Zollinger Ellison syndrome
- Cigarette smoking, Alcohol
- Stress

PATHOPHYSIOLOGY

A physiologic awkwardness between forceful variables (gastric corrosive and pepsin) and defensive components (mucosal protection and fix) stay significant issues in the pathophysiology of gastric and duodenal ulcers. Gastric corrosive is discharged by the parietal cells, which contain receptors for histamine, gastrin, and acetylcholine. Corrosive (just as H. pylori disease and NSAID use) is an autonomous factor that contribute to the disturbance of mucosal uprightness. Expanded corrosive discharge has been seen in patients with duodenal ulcers and might be a result of H. pylori infection. Patients with ZES have significant gastric corrosive hypersecretion coming about because of a gastrin producing tumor.

PLANT PROFILE

Sciencetific classification of pudina

Kingdom : *Plantae* Division : *Magnoliophyta*Class : *Magnoliopsida*

Order : *Lamiales*

Family : *Lamiaceae*

Genus : *Mentha*

Species : *arvensis*

Name of Plant: *Mentha arvensis* Linn.

Common Name: Japanese mint, Corn mint, Field Mint, Pudina.

Botanical Name: *Mentha arvensis* Linn.

Parts Used: Entire plant, Leaves, Flowering tops, Stem

HABITAT:

Mentha arvensis Linn. presented from Japan. It is dispersed all through the Western Himalayas and is developed all through world for utilized as vegetable. It is likewise developed in Uttar Pradesh, Punjab, kumaon, Jammu and Kashmir at an elevation of 270-1500 meter. It is industrially filled in China, Tiwan, Brazil, Thailand, Japan and India. India represents over half of complete world creation of Japanese mint oil. The plant presently fills in North and Central America along the banks of streams and creeks, and in gardens. The plant is found all through the calm districts of Europe, Western and Central Asia (Eastern Siberia and East of the Himalayas) .

DESCRIPTION

Mentha arvensis L. is an erect strongly aromatic, branched perennial herb that grows upto 60 cm in height with suckers.

Stem, Root and Leaves:

The stem is tube shaped with unbending climbing terminal branches and running rootstocks. Stem is dim green to brown hued, have furry surface, solid and sweet-smelling smell and marginally severe taste. Leaves are basic, inverse, to 5 cm long, without further ado petioled or sessile, elliptical praise or lanceolate, coldheartedly or intensely serrate, cuneate at the base, meagerly furry or practically glabrous, strongly toothed, adjusted or obtuse tipped. It has emphatically fragrant and trademark smell and somewhat impactful and marginally severe taste.

Flower and Fruit:

Flowers lilac, arranged in verticillasters, borne on axils of leaves on upper stem, 8 to 12 blossomed false whorls with small linear-lanceolate bracts. The inflorescence is leafy at the apex. The bracts are like the leaves, smaller above. The tepals are 1.5 by 2.5 mm, broadly campanulate and hairy. The corolla is lilac, white to purple or, rarely, pink; 4 to 5 mm long. Fruits are pale brown, nutlets, smooth.

Figure: 2

Mentha arvensis Linn. Herb

CHEMICAL CONSTITUENTS

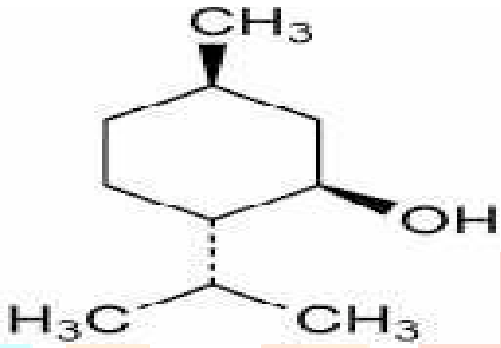
M. arvensis L. contains 70-90% menthol. The main constituents are 4.5-20% menthyl acetate and 15-20% menthone. It also contains isomenthone, menthofuran, limonene, neomenthol, cineole, piperitone, alpha- and beta- pipene, phellandrene, sesquiterpenes (viridiflorol), tannins and flavonoids. Mint plant contains over 40 distinct chemical compounds. It contains the flavonoids acacetin, chrysoeriol, diosmin, eriocitrin, hesperidin, hesperidoside, isorhoifolin, linarin, luteolin, menthoside, methyl rosmarinate, rutin, tilianin, narirutin, quercetin, and nodifloretin. The phenolic acids present are caffeicacid, lithospermic acid, rosmarinic acid, chlorogenic acid, and protocatechuic acid. The anthraquinones aloe-emodin, chrysophenol, emodin; tannins; tocopherols; carotenoids and minerals are the other compounds present.

Other chemicals include:

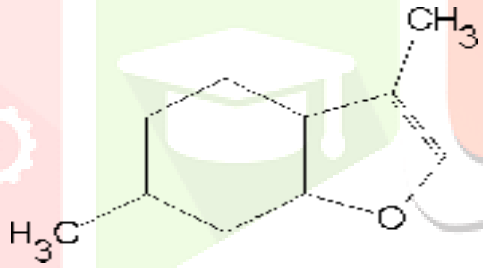
(+)-1,2-Epoxyneomenthyl acetate, (+)-8-acetoxy carvone, (+)-carvone, (+)-isomenthone, (+)-menthofuran, (+)-neomenthol, (+)-octan-3-one, (+)-piperitenone, (+)-piperitenone-oxide, (+)-piperitone, (-)-borneol, (-)-carvone, (-)- linalool, 1,8-cineol, *p*-menthen-3-one, 3',4',5,7-tetrahydoxy-flavone-7-*α*-L- rhamnosyl-*β*-D-glucoside, 3',5,7-trihydroxy-4'-methoxy-flavone-O-*β*-D-glucoside, 3-methylpentanol, acacetin-7-O-*β*-D-glucoside, acetic acid, *α*,*β*-hexenic acid, *α*,g- hexenylphenyl-acetate, anisaldehyde, *β*-car-3-ene, *β*-caryophyllene, Calcium, camphene, caproic acid, carvomenthone, cineol, *cis*-isopulegone, *cis*-ocimene, Copper, D-3-octanol, diosmetin-7-O-*β*-D-glucoside, ethyl-amyl-carbinol, eugenol, formic acid, fulfural, germacrene-D, hesperidine, Iron, isomenthol,

isopulegone, isovaleraldehyde, linalool-acetate, luteolin, Magnesium, Manganese, menthofurolactone, myrcene, neoisomenthol, neoisopulegone, octane-3-ol, *p*- cymene, *p*-cymol, *p*-menthan-*trans*-2,5-diol, Potassium, raffinose, resin, rosmarinic acid, sabinene-hydrate, santene, Sodium, stachyose, tannin, *trans*-isopulegone, Zinc and more recently, linarin (acacetin-7-O-*β*-D-rutinoside) was extracted from the flower of *Mentha arvensis*.

CHEMICAL STRUCTURE



Menthol



Menthofuran

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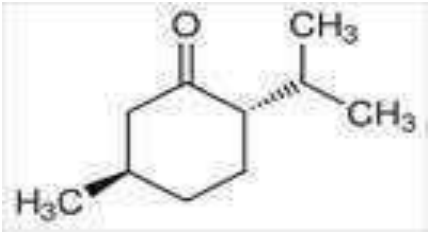
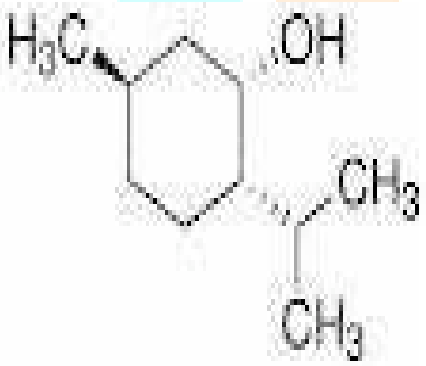
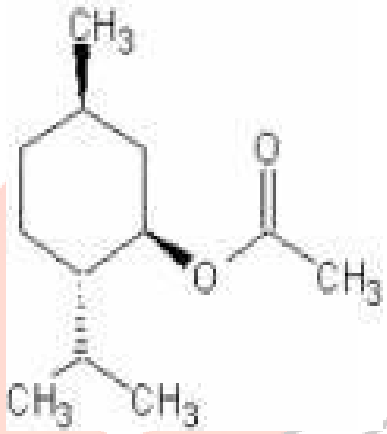


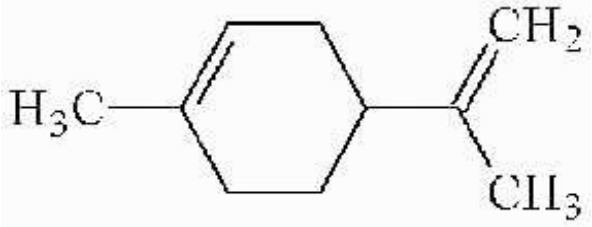
Menthone



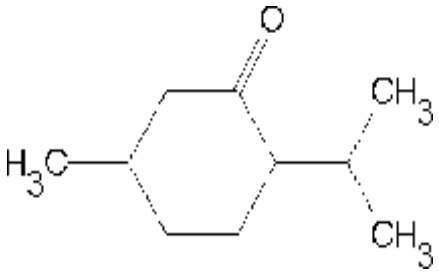
Neomenthol



Menthyl acetate



Limonene



Isomenthone

CULTIVATION

Mentha arvensis developed in India in the semi-calm districts in the lower regions of Himalayas. The mentha in U.P. went up to 40,000 hectares in 1997 from 20,000 hectares in 1996 in light of the fact that a portion of the sugarcane ranchers took up its development taking into account non-installment of unfulfilled obligations by the sugar plants and conclusion of a few factory. Indeed, even in 1998, the region under mentha is accounted for to have gone up. The all-India region under mentha in the nation is assessed at around 70,000 hectares.

Production

India at present delivers around 15,000 tons of mint oil and fares 10,000 tons and acquires unfamiliar trade worth Rs. 4500 crores yearly. In the year 2004-05, complete mentha oil production raise to 15770 MT. From mint oil menthol is likewise delivered in the country. The region under mentha arvensis in the nation is around 70,000 hectares. About 10lakh individuals in the nation are occupied with the development, showcasing and preparing of mentha arvensis, mint oil and menthol.

Exports

At present the significant makers of mint oil on the planet are India, China, Brazil and the US. India sends out various kinds of mint oils to various nations including Argentina, Brazil, France, Germany, Japan, UK, USA, and so on these assortments incorporate the Japanese mint oil (got from Mentha arvensis), peppermint oil (Mentha piperita), dementholised Japanese mint oil, stick mint oil (Mentha spicata), water mint oil (Mentha amphibian), horsemint oil (Mentha sylvestries), Bergamont oil (Mentha citrate) and still others. Mentha Oil send out has consistently expanded from India in the new years. In the year 2004-05 India stunningly sent out 9160 MT. albeit homegrown interest for this item has expanded on the wake of higher action by homegrown restorative and fragrance industry. Present homegrown interest for menthe in homegrown market is assessed to be 4000 MT to 4500 MT.

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Manufacture of Volatile Oil & Menthol:

Refining of dried leaves is less expensive than that of new leaves. By steam refining and filtration, a brilliant yellow unstable oil is acquired. Leaves and blooming tops give the best return. About half of menthol can be isolated out in glasslike structure on cooling the oil. The leftover (dementholised) oil is utilized as peppermint oil. Assembling of menthol from dementholised oil has been taken up on business scale by three or four firms in Bombay, West Bengal and Gujarat. There are a few little refineries in U.P likewise for the refining of oil.

Storage of Oil:

The peppermint oil is put away in shaded jugs, sealed shut aluminum or stirred holders in cool dry spot. Presence of dampness in the oil may rancidify the oil. Since it is acidic in nature, it ought not be put away in tin compartments.

Uses of Mentha arvensis L.

Traditional uses:

The leaves are carminative and are utilized to treat like dyspepsia, bacillary loose bowels, tooting, gastritis, and enteritis. It is likewise utilized as cholagogue, emmenagogue, vermifuge, to upgrade lactation, energizer, dentifrice, expectorant, cardiotonic, febrifuge, preventative, diuretic and antiperspirant.

- It has antifungal, antibacterial, germ-free, anthelmintic, antiemetic and antispasmodic action.
- The leaves are valuable in the treatment of bronchitis, asthma, hack, fever, amenorrhoea, dysmenorrhoea, skin illnesses, the runs, queasiness, torment, colic, peptic ulcer, jaundice, irritation of liver, hepatopathy, splenopathy, general shortcoming, respiratory and urinary parcel contaminations.
- Dried plant is sweet-smelling; given as "sharbat" or syrup for its cooling and diuretic impact.
- Juice of the leaves is given in the runs and diarrhea.
- Infusion of leaves in utilized in stiffness and heartburn.

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• Indian Medicine: The spice is utilized for joint torments, dyspeptic protests, the runs and regurgitating, hacks and asthma, migraine and toothaches, just as broad weakness.

• Chinese Medicine: The spice is utilized for cerebral pains, dyspeptic grievances, loose bowels and regurgitating, toothaches and skin rashes.

Medicinal uses:

- Carminative, energizer, stomachic, sweet-smelling, germ-free, antispasmodic, sudorific, Emmanogogue, antibacterial, antifibrile and antifungal.
- Pudina, generally acclaimed as carminative, stomach related and fragrant is esteemed as energizer, expectorant, antispasmodic enemy of intestinal worms and as pain relieving spice.
- It is successful in migraine, rhinitis, hack sore throat, colic and regurgitating. Menthol acquired from the plant is utilized in analgesics.
- It is likewise utilized as seasoning specialist in culinary arrangements, cardio tonic in drug arrangements. It is a decent blood cleaning agent.
- Due to its germicide and antibacterial property, it very well may be utilized in swollen gums, mouthwash or mouth ulcers and toothache.
- The Commission E endorsed interior utilization of mint oil for fart, useful gastrointestinal and gallbladder problems, catarrhs of the upper respiratory lot, and outer use for myalgia and neuralgic diseases.

MATERIALS AND METHODS

METHODOLOGY

Animal selection- Albino rats of either sex (Wistar strain) weighing between 100-200 gm were chosen for present examination. They were kept up under uniform lab conditions in standard steel confines and gave food and water not indispensable. Every one of the creatures were saved for 15 days under standard lab conditions in a 12h: 12h light and dim cycles and kept up under controlled temperature 27± 20C for acclimatization.

Drugs and chemicals-

Standard drug- Ranitidine (Dose : 50mg/kg)

Inducing agent- Aspirin (Dose : 200mg/kg)

Apparatus/ Instruments

Table:1

Sr. No.	Apparatus/Instrument
1.	Burette
2.	Digital pH Meter
3.	weighing balance
4.	Heating mantle size (1000 ml)
5.	Laboratory oven
6.	Microscope

PHARMACOGNOSTIC INVESTIGATION:

Collection and Authentication of *Mentha arvensis* Linn.

The *Mentha arvensis* Linn. plant were collected from the Local garden Sitapur Uttar Pradesh. The plant was authenticated by Dr. D.C.Saini,Scientist 'E', Birbal Sahni Institute of Palaeobotany, 53, University road, Lucknow-226007. The registration no. is 13400.

Preliminary Pharmacognostic Characterstics

In the present study, the plant of *Mentha arvensis* Linn. and the powder of *Mentha arvensis* Linn. were investigated for its macroscopic characteristics. The results were given in (Table:4,Table: 5).

EXTRACTION OF THE PLANT *MENTHA ARVENSIS* LINN.

In the current investigation, the shade dried plant of Mentha arvensis L. were diminished to coarse powder and powdered were exposed to hot ceaseless extraction (soxhlet) with Ethanol. 60 gm of plant material were taken in a soxhlet device and removed with 250 ml of ethanol for 6 hours. The concentrate was separated. The filtrate was taken and dissolvable was taken out under decreased tension on a water shower until it turns out to be totally dried. The concentrate of the medication was put away at 40C for the examination.

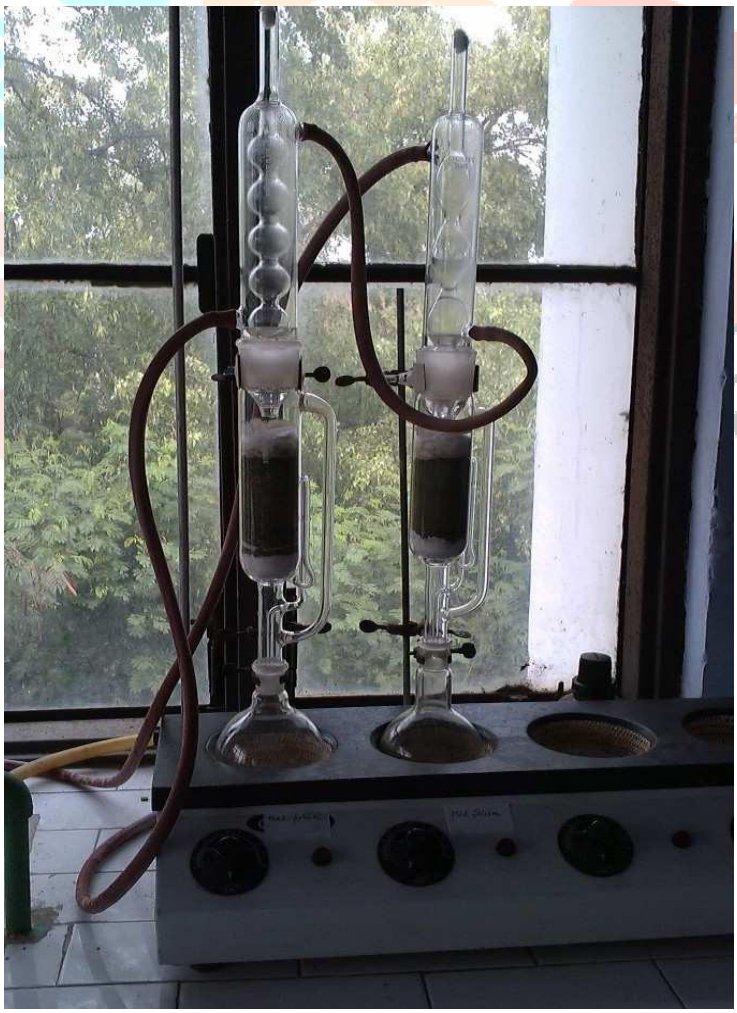


Fig.3- Soxhlet Apparatus Assembly

PRELIMINARY PHYTOCHEMICAL INVESTIGATION:

The concentrates were exposed to starter subjective phytochemical examination. The accompanying strategies were received to test for the presence of different compound constituents in the concentrates and perceptions were recorded in (Table 6)

TESTS FOR ALKALOIDS:

Planning of test arrangement: The test arrangement was set up by dissolving extricates in the weaken hydrochloric corrosive arrangement.

Mayer's test: The acidic test arrangement with Mayer's reagent (Potassium mercuric iodide) gave cream hued accelerate

Hager's test: The acidic test arrangement with Hager's reagent (Saturated picric corrosive arrangement) gave yellow encourage.

Dragendorff's test: The acidic arrangement with Dragendorff's reagent (Potassium bismuth iodide) showed ruddy earthy colored hasten.

Wagner's test: The acidic test arrangement treated with Wagner's reagent (Iodine in Potassium iodide) gave earthy colored hasten.TEST FOR FLAVONOIDS:

Shinoda test: To the test solution add few fragments of magnesium ribbon and conc. HCl dropwise , pink to magenta red colour or occasionally green to blue colour appeared after few minutes.

Lead acetate solution test: To a little amount of test arrangement when lead acetic acid derivation arrangement was added, it shaped yellow shaded encourage.

Alkaline reagent test: Test arrangement when treated with sodium hydroxide arrangement showed expansion in the force of yellow tone, which gets boring on expansion of not many drops of weaken corrosive.

Zinc hydrochloride test: Test solution when treated with a mixture of zinc dust and conc. HCl showed red colour after few minutes.

TESTS FOR GLYCOSIDES:

Tests for Cardiac Glycosides:

Baljet's test: The test solution treated with picric acid or sodium picrate gave yellow to orange colour.

Raymond's test: The test solution treated with dinitrobenzene in hot methanolic alkali gave violet colour.

Keller-Killiani test for digitoxose: To 2 ml extract, add glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 observed for reddish brown colour at junction of the two liquid and upper layer bluish green.

Legal's test: The test solution when treated with pyridine and alkaline sodium nitroprusside solution gave pink to red colour.

TESTS FOR SAPONIN GLYCOSIDES:

Foam test: The drug extract was shaken vigorously with water. Persistent foam was observed.

Haemolytic test: Added drug extract to one drop of blood placed on glass slide. Haemolytic zone appeared.

TESTS FOR ANTHRAQUINONE GLYCOSIDES:

Borntrager's test: To 3 ml extract, added dil.H_2SO_4. Boiled and filtered. To cold filtrate, added equal volume benzene or chloroform. Shaken well. Separated the organic solvent. Added ammonia. Ammonical layer turned pink or red.

Modified Borntrager's test: To 5 ml extract, added 5 ml. 5% $FeCl_3$ and 5 ml dil. HCl. Heated for 5 min. in boiling water bath. Cooled and added benzene. Shaken well and separated organic layer, added equal volume dil. ammonia. Ammonical layer showed pinkish red colour.

TESTS FOR CYANOGENETIC GLYCOSIDES:

Guignard's test: Doused a channel paper strip first in 10% picric corrosive, then, at that point in 10% sodium carbonate; dried. Dampened powdered medication was put in a conelike cup. Stopper it; put the above channel paper strip in the cut in the plug. The channel paper turned block red or maroon.

TESTS FOR TANNINS AND PHENOLIC COMPOUNDS:

To 2-3 ml of test solution, add few drops of following reagents: 5% $FeCl_3$ solution : Deep blue- black colour

Lead acetate solution: White precipitate.

Gelatin solution: White precipitate.

Bromine water: Decoloration of bromine water.

Acetic acid solution: Red color solution.

Dilute Iodine solution: Transient red colour.

Dilute HNO_3 : Brownish to yellow colour

TESTS FOR STEROID

Salkowski Reaction: In two ml of concentrate, mix 2 ml chloroform and 2 ml concentrated H2SO4. Flustered properly. Chloroform flake seemed red and corrosive flake exhibit greenish yellow fluorescence.

Liebermann-Burchard Reaction:

Blended 2 ml extricate with chloroform. Added 1-2 ml acidic anhydride and 2 drops conc.H2SO4 from the side of test tube. First red, then, at that point blue lastly green tone showed up.

Liebermann's Reaction: Blended 3 ml extricate with 3 ml acidic anhydride. Warmed and cooled. Added not many drops conc. H2SO4. Blue hued showed up.

TEST FOR TRITERPENOIDS:

Salkowaski Test: At the point when a couple of blob of conc. H2SO4 were added to the testing arrangement, shaken well and permitted to stand, lower layer became yellow.

Liebermann-Burchard Reaction: The test arrangement treated with acidic anhydride, blended well and conc. H2SO4 was added from the sides of the test tube. Dark red tone shaped.

TESTS FOR CARBOHYDRATES:

Preparation of test solution: The testing solution was make ready by liquefy the test remove with water. Then, it was hydrolyzed with 1 volume of 2N HCl and exposed to following substance tests.

Molisch's test (General test): To 2-3 ml watery concentrate, added not many drops of α-naphthol arrangement in liquor, shaken and added concentrated H2SO4 from sides of the test tube. Violet ring was shaped at the intersection of two fluids.

Test for Reducing Sugars:

Fehling's test: 1 ml Fehling's A and 1 ml Fehling's B arrangements were blended and bubbled briefly. Added equivalent volume of test arrangement. Warmed in bubbling water shower for 5-10 min. noticed for a yellow, then, at that point block red accelerate.

Benedict's test: Same quantity of Benedict's reagent and test arrangement in test tube were assorted. Warmed in bubbling water bath for 5 min. Arrangement may seem green, yellow or red according to measure of reducing sugar present in testing arrangement.

TEST FOR MONOSACCHARIDES:

Barfoed's test: Equivalent volume of Barfoed's reagent and test arrangement were added. Heated for 1-2 min in bubbling water shower and cooled. Red accelerate was noticed

Test for Non-reducing sugar:

Test arrangement didn't offer reaction to Fehling's and Benedict's tests. Hydrolysed test arrangement Fehling's and Benedict's tests were positive.

Test for Non-reducing Polysaccharides (Starch):

Iodine test: Blended 3 ml. test arrangement and few drops of weaken Iodine arrangement. Blue tone showed up; it vanished on bubbling and returned on cooling.

Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

TESTS FOR PROTEINS:

Biuret test (General test): To 3 ml test solution, added 4% NaOH and few drops of 1% $CuSO_4$ solution observed for violet or pink colour.

Millon's test (for proteins): Mixed 3 ml test solution with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour.

Xanthoprotein test (For protein containing tyrosine or tryptophan): Mixed 3ml test solution with 1 ml concentrated H_2SO_4 observed for white precipitate.

Test for protein containing sulphur: Blended 5 ml test arrangement with 2 ml 40% NaOH and 2 drops 10% lead acetic acid derivation arrangement. Arrangement was bubbled it became dark or earthy shading.

TESTS FOR AMINO ACIDS:

Ninhydrin test (General test): Warmed 3 ml test arrangement and 3 drops 5% Ninhydrin arrangement in bubbling water shower for 10 min. Purple or pale blue tone showed up.
Test for Tyrosine: Heated 3 ml test solution and 3 drops Million's reagent. Solution showed dark red colour.
Test for Cysteine: To 5 ml. test arrangement added not many drops of 40% sodium hydroxide and 10% lead acetic acid derivation arrangement. Dark ppt. of lead sulfate was framed.

PHARMACOLOGICAL METHODS FOR EVALUATION OF ANTIULCER ACTIVITY

Aspirin –induced (ASP) gastric ulcer model in rats.

Creatures were haphazardly isolated into five gatherings: control, standard, test bunch, each containing 6 rodents. The benchmark group got just vehicle (refined water, 1ml/100g p.o.), and standard gathering got Ranitidine at a portion 50 mg/kg , orally. The excess experimental groups got portion (100, 250, 500mg/kg) of Mentha arvensis plant removes orally as indicated by body weight of creatures. Following 7 days of dosing, creatures were abstained for 24 hours and later headache medicine at a portion of 200mg/kg was given orally. The animals were then sacrificed by euthanasia five hour after administration of Aspirin, the animal were sacrified by cervical dislocation, and the stomach was then excised and cut along the greater curvature , washed carefully with 5.0 ml of 0.9% Nacl. The stomach was examined by a 5x magnifying lens to assess the formation of ulcers. Then ulcer score, ulcer index and % ulcer inhibition were measured.

Animal study protocol

Dose selection. Dose selection was done on the basis of previous literature study.

Standard drug. Ranitidine 50 mg/kg, b.wt.

Group	Treatment	Dose and route
1.	Control	10 ml/kg, p.o.
2.	Standard	50 mg/kg, p.o. + 200 mg/kg Aspirin.
3.	Test drug (Treated)	100 mg/kg, p.o. + 200 mg/kg Aspirin.
4.	Test drug (Treated)	250 mg/kg, p.o. + 200 mg/kg Aspirin.
5.	Test drug (Treated)	500 mg/kg, p.o. + 200 mg/kg Aspirin.

Table:2 Animal study protocol for Aspirin induced ulcer model

MACROSCOPIC EXAMINATION OF STOMACH

The stomach was opened alongside more prominent arch, washed with saline to eliminate gastric substance and blood clusters. The stomach was analyzed by a 5x amplifying focal point to survey the development of ulcers. The quantity of ulcer was checked Ulcer scoring was under taken as following manner: 0 = no ulcer

0.5 = red colouration

1 = superficial (spot) ulcer 2 = deep ulcer

3 = perforation

An ulcer index *U*I was measured by using following formula

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

- *U*N= average of number of ulcers per animal
- *U*S = average of severity score
- *U*P = percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

% inhibition of ulceration = (ulcer index control – ulcer index test) × 100/ ulcer index control

Sr. No.	Stomach colour	Ulcer scores
1.	Normal colour	0
2.	Red colour	0.5
3.	Red spot	1
4.	Hemorrhagic streaks	1.5
5.	Ulcers > 3 but < 5	2
6.	Perforation (>5)	3

Table:3 Ulcer scores

STATISTICAL ANALYSIS

All results were expressed as mean ± SEM and analyzed using one-way ANOVA following by Dunnett's multiple comparision test using GraphPad Prism software (GraphPad software Inc., Version 4.0.0.255). $P<0.05$ was considered to be statistically significant data.

RESULTS

In the present study the *Mentha arvensis* Linn. plant were collected from the Local garden Sitapur Uttar Pradesh. The plant was authenticated by Dr. D.C.Saini,Scientist 'E', Birbal Sahni Institute of Palaeobotany, 53, University road, Lucknow-226007. The registration no. is 13400.

Macroscopic characteristics of leaves:

Sr. No.	Parameters	Observation of leaves
1.	Color	Green
2.	Odour	Strongly aromatic and characteristic
3.	Taste	Pungent and bitter
4.	Size	2.5-5 cm long and 1-2 cm wide
5.	Shape	Ovate or lanceolate

Table no.4

Macroscopic powder characteristics:

Sr. No.	Parameters	Observation of leaves
1.	Nature	Coarse powder
2.	Color	Light brownish-Green
3.	Odour	Aromatic and characteristic
4.	Taste	Pungent and bitter

Table no.

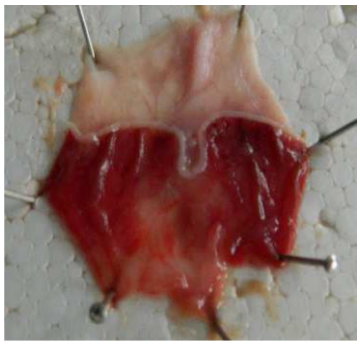
Preliminary phytochemical investigation:

Sr. No.	Chemical Tests	Ethanolic Extract
1.	Test for Alkaloids	+
2.	Tests for Flavonoids	+
3.	Tests for Glycosides	+
4.	Tests for Carbohydrates	+
5.	Tests for Tannins and Phenolic Compounds	+
6.	Tests for Steroids	-
7.	Tests for Triterpenoids	+
8.	Tests for proteins	-
9.	Tests for Amino acids	-
10.	Tests for Fat and oils	-

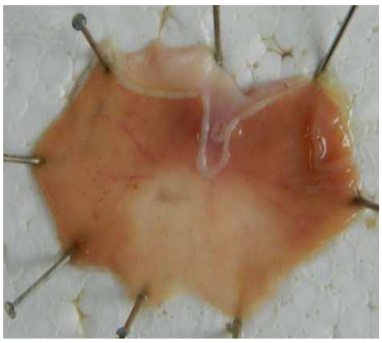
Table : 6

PHARMACOLOGICAL SCREENING OF ANTIULCER ACTIVITY:

To assess the Antiulcer activity of *Mentha arvensis* extract by using Aspirin induced model and effect of ethanolic extract of *Mentha arvensis* once a day, for seven days pretreatment prevented acute gastric ulcers in dose related manner. As shown in the fig.5,the extract at 100, 250 and 500mg/kg treated rat shows significant decreases in acute gastric ulcer in comparison to control non treated for 7 days and the result are statistically significant ($p<0.05$) which is also comparable to the rat treated with Ranitidine, was taken as positive standard.



(A)



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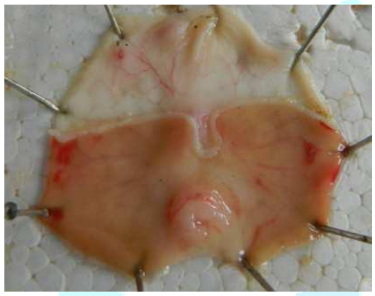

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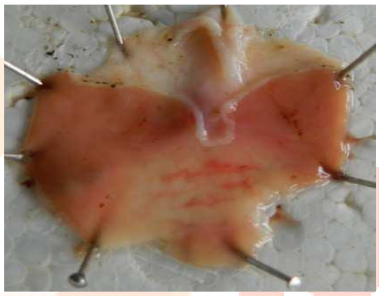


(D)

(E)

Figure: 4 A)- Aspirin treated rat shows mucosal damage B)Ranitidine treated rat

C)-MAE 100mg/kg treated rat D)-MAE 250mg/kg treated rat E)-MAE

500mg/kg treated rat

MAE- *Mentha arvensis* extract.

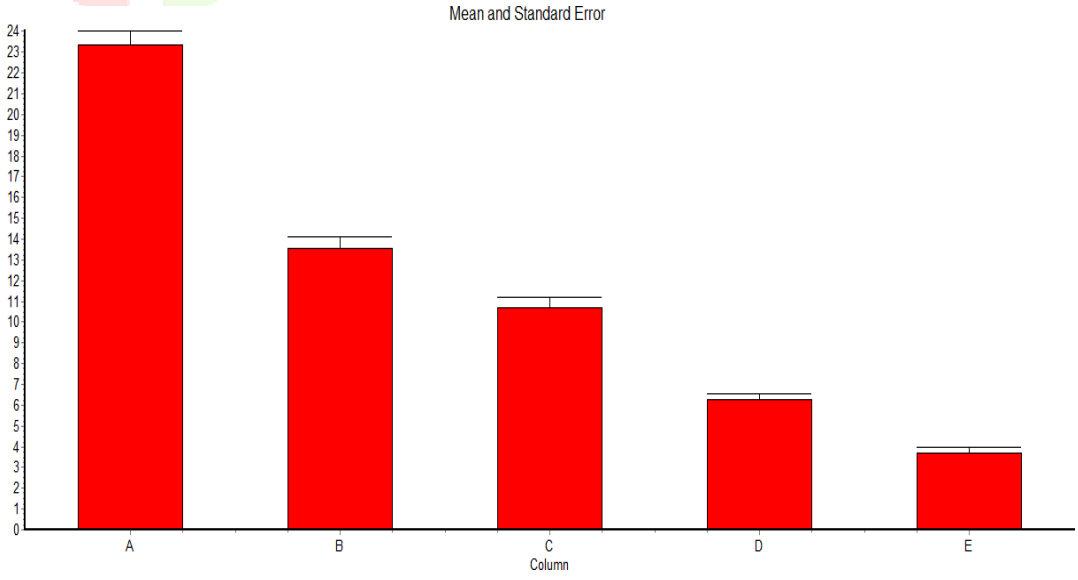
Effect of Mentha arvensis L. extract on Aspirin induced ulcer model

Sr. No.	Group	Ulcer Index(mean±S.E.M)	Percentage Inhibition
1.	Control (Aspirin 200mg/kg)	23.3±0.69	
2.	Treated 100mg/kg(MAE)	13.5±0.53	42%
3.	Treated 250mg/kg(MAE)	10.7±0.46	54%
4.	Treated 500mg/kg(MAE)	6.3±0.25	72.9%
5.	Standard(Ranitidine 50mg/kg)	3.7±0.27	84.12%

Table 7

Table 7: showing ulcer index and percentage inhibition of acute gastric ulceration in Aspirin induced gastric ulcer rat model. Data are expressed as means±S.E.M.(n=6), $p<0.01$ MAE(100,250,500 mg/kg) and Ranitidine(50mg/kg) for versus control group using ANOVA followed by Dunnett's test.

GRAPHICAL PRESENTATION OF ULCER INDEX



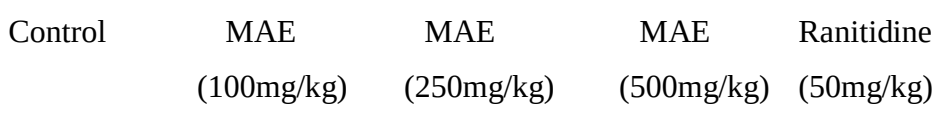


Figure 5: Ulcer index graph

Fig. 5 Effect of administration of control MAE(100,250,500mg/kg) and Ranitidine (50 mg/kg) for 7 days in Aspirin induced gastric ulcer model. Data are expressed as means±S.E.M. (n=6), $p<0.05$ MAE (100,250,500mg/kg) and Ranitidine (50 mg/kg) for versus control group ANOVA followed by Dunnett's test.

Following Fig. 5 shows the Ulcer index of the control, MAE (100, 250 and 500mg/kg), and standard group (Ranitidine 50mg/kg) and their corresponding percentage inhibition in acute gastric ulceration induced by Aspirin after treatment course of 7 days. Data are expressed as means±S.E.M (n=6), $p<0.05$, MAE(100,250,500mg/kg) and Ranitidine (50mg/kg) for versus control group. It shows decrease in the ulcer score after treating with the ethanolic extract (100,250,500mg/kg) and ranitidine.

The current assessment displayed the sufficiency of Mentha Arvensis plant remove against gastric ulceration incited by Aspirin initiated gastric ulcer model.The plant separate Mentha arvensis and standard medications creates a lessening in the ulcer number in the Aspirin actuated ulcer model in rodents. The remedial proportion in the Aspirin actuated gastric model was 42%, 54%, 72.9% and 84.12% utilizing ethanolic separate (100,250,500mg/kg) and standard medication ranitidine, individually. This demonstrates that the plant has antiulcerogenic, antisecretory and cytoprotective activities. A few specialists have detailed similar outcomes after plant remove treatment. Gastric bodily fluid is known to ensure the gastric mucosa against tissue harm by HCl delivered by parietal cells. It comprises of thick, versatile, follower and straightforward gel framed by 95% water and 5% glycoproteins that covers the whole gastrointestinal mucosa. Besides, bodily fluid is fit for going about as a cell reinforcement subsequently can decrease mucosal harm interceded by oxygen free extremists. The defensive properties of the bodily fluid hindrance depend on gel structures as well as on the thickness of the layer covering the mucosal layer . This demonstrates cytoprotective activities in the plant removes. Plant compound substances like flavonoids, tannins, terpenoids and so on have been displayed to scrounger free revolutionaries and in this way are seen as promising restorative medications with the expectation of complimentary extremist pathologies. Phytochemical tests uncovered the presence of flavonoids and terpenoids in the concentrates of Mentha arvensis. A portion of the triterpenes are known as an antiulcer specialists and their activity has been referenced to be because of actuation of cell proteins, decrease of mucosal prostaglandin digestion, cytoprotective activities and decrease of gastric vascular permeability. Be that as it may, the system by which this concentrate creates an antiulcer result isn't totally clear. The outcomes in present investigation appears to offer help for the utilization of Mentha arvensis as an antiulcer drug in people medication. Consequently, additionally considering its enormous use in India more itemized phytochemical and pharmacological examinations on the antiulcer impacts and harmfulness contemplates are required. In Aspirin prompted gastric ulcer exploratory model the ethanol separate shows the best antiulcerogenic activity, due topresence of tannins and flavonoids, as in writing references.

The present data obtained from ethanolic extracts of *Mentha arvensis* L. showed the presence of a gastroprotective effect and improved ulcer healing properties.

CONCLUSION

Phytochemical examinations had revealed the presence of Carbohydrates, Phenolic compounds, Glycosides, Alkaloids, Triterpenoids and Flavonoids in the concentrates of Mentha arvensis Linn.

Plant synthetic substances like flavonoids, tannins, terpenoids and so on have been displayed to scrounger free revolutionaries and accordingly are seen as promising helpful medications with the expectation of complimentary revolutionary pathologies. Phytochemical tests uncovered the presence of flavonoids and terpenoids in the concentrates of Mentha arvensis. A portion of the triterpenes are known as an antiulcer specialists and their activity has been referenced to be because of enactment of cell proteins, decrease of mucosal prostaglandin digestion, cytoprotective activities and decrease of gastric vascular porousness. In any case, the mechanism by which this concentrate creates an antiulcer impact isn't altogether clear. The outcomes in present examination appears to offer help for the utilization of Mentha arvensis as an antiulcer drug in society medication. Thusly, likewise taking into account its huge use in India more definite phytochemical and pharmacological examinations on the antiulcer impacts and poisonousness considers are required. In Aspirin prompted gastric ulcer trial model the ethanol extricate shows the best antiulcerogenic activity, because of the presence of tannins and flavonoids, as in writing references.

Ethanolic concentrate of Mentha arvensis 100, 250, and 500 mg/kg body weight unmistakably shows a defensive impact against corrosive emission and gastric ulcers in Aspirin initiated ulcer model.

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