

RESEARCH ARTICLE

Study the effect of Ethanolic extract of *Anethum graveolens* L. on Aspirin induced Gastric Ulcer in Male Guinea Pigs.

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ABSTRACT:

The objective of the present study is to investigate the effect of ethanolic extract of *Anethum graveolens* L. on Aspirin – induced gastric ulcers in guinea pigs. thirty guinea pigs divided in to five groups (6 each group), first group received oral administration 2ml of normal saline served as negative control. 500mg/kg B.W of Aspirin was used to induced ulcer in guinea pigs for 3 days. after checking for presence of ulcers, the ulcerogenic guinea pigs were divided in to four groups, 2 groups were treated with ethanolic extract of *Anethum graveolens* L. at doses of (150mg/kg, 300mg/kg) respectively, while a group received cimetidine at dose 20mg/kg for 10days and the last group was the ulcer untreated control (control positive). At the end of the study the animals were sacrificed, the stomach was prepared for estimating, gastric PH , gastric ulcer index, total gastric area, gastric fluid volume, serum MDA and tissue MDA, in addition a gross and histological examination of the stomach. There were significant decrease in PH in control positive , while it showed significant increase($P \leq 0.05$) of gastric ulcer index, total gastric area, gastric fluid volume, serum MDA and tissue MDA compared with the negative control group. The study also revealed significant decrease ($P \leq 0.05$) in gastric ulcer index, gastric volume, total gastric area, serum MDA and tissue MDA with a significant increase($P \leq 0.05$) in gastric PH in groups treated with ethanolic extract and in the group treated with cimetidine compared with the positive control group. Histological studies showed many pathological changes in stomach in control positive group but in the treated with ethanolic extract of *Anethum graveolens* L at dose 300mg/kg group the histopathological changes near to the normal status, whereas, higher dosage of extract (300mg/kg) was more superior to cimetidine drug in its protection of the stomach

KEYWORDS: *Anethum graveolens* L, gastric PH , gastric ulcer index, total gastric area, MDA.

INTRODUCTION:

Gastric ulcers are common disease, which have been major cause of morbidity and mortality for more than a century (1). Some of the causes of gastric ulcer is stress, smoking, nutritional deficiency and ingestion of non-steroidal anti-inflammatory drug (2).

The pathogenesis of gastric ulcer is based on an imbalance between aggressive and protective factors in the stomach (3). Pepsin and gastric acid make up the offensive factors, whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding cell proliferation and prostaglandins (4). The NSAIDS like aspirin and indomethacin are useful agents for a variety of diseases like rheumatic musculoskeletal and cardiovascular diseases. However they cause gastrointestinal complications such as ulcers and erosions. NSAIDS also generates oxygen free radicals that are known to play a role in the pathogenesis of mucosal injury (5). The systemic gastro toxic effect of

aspirin is related to the inhibition of cyclooxygenase1 (cox-1) and the subsequent disruption of prostaglandin synthesis and to the antiplatelet function that promotes bleeding complication (6 , 7).

In recent years, more interest has been growing in alternative therapies as using natural products especially that derived from plants (8,). Dill (*Anethum graveolens* L.) from *Umbelliferae* family. Is an annual herb growing in the Mediterranean region, Europe, central, southern Asia and south eastern region of Iran. Now it is cultivated widely throughout the world (9,10). It is used traditionally as a popular aromatic herb and spice that has a very long history of use going back to more than 5,000 years. It was used as a remedy for indigestion, and flatulence and as milk secretion stimulation. Moreover, it is used as an anti-convulsion, anti-emetic, anti-inflammatory, analgesic, anti-cramp (in children), as a wound healer and to increase the appetite and strengthen the stomach (11,12), and used to treat cough, dropsy, jaundice, sclerosis, hyperlipidaemic and tumors (13,14,15). *Anethum graveolens* contained essential oils comprising of major compounds like carvone, limonene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, furanocoumarin and falcariindiol (16,17). it also contains phenols and flavonoids, tannins and alkaloids (18,19, 20). The objective of this investigation is to study the effect of ethanolic extract of *Anethum graveolens* on aspirin – induced gastric ulcer in guinea pigs.

MATERIALS AND METHODS:

Collection of plant materials and extraction:

The plant of dill was purchased from the local market at Basra-Iraq. The shade dried plant was finely powdered in chemical mixer, 50gm of powder were put in the round bottle flask, 200ml of ethanol (70%) were added to flask and extracted for 12 hours at 70 °C. the extract was filtered by using whatmann filter paper, then the extract were put in the petridish and left at room temperature under the shade, the collected extracts were kept in tight closed container and stored until using (21).

Experimental animals:

Thirty healthy adult guinea pig weighting 900-1000 gm. were used in this study, the guinea pigs kept in suitable cages in the animal house of veterinary medicine college / Basra university, animals were acclimatized to holding facilities for two weeks prior to the experiment and were feed standard diet and water *ad libitum*.

The animals were fasted and allowed access to water *ad libitum* to empty the stomach of food for 24 hours prior to their use in the experiment and then given Aspirin at dose (500 mg/ kg B.W) orally. The animals were divided into five groups, each group containing 6

animals as follow;

Group 1: normal guinea pigs received 2ml distilled water (control negative).

Group 2: guinea pigs served as positive control group in which ulcers induced by giving aspirin at dose of 500 mg/kg orally for 3 days.

Group 3: served as a treated group, in which ulcers induced by giving aspirin at dose 500 mg/kg orally for 3 days, then treated with ethanolic extract of *Anethum graveolens* at dose 150 mg/kg orally for 10 days.

Group 4: served as a treated group, in which ulcers induced by giving aspirin at dose 500 mg/kg orally for 3 days, then treated with ethanolic extract *Anethum graveolens* at dose 300mg/kg orally for 10 days.

Group 5: served as a treated group, in which ulcers induced by giving aspirin at dose 500 mg/kg orally for 3 days, then treated with cimetidine at 20 mg/kg orally for 10 days.

At the last day of each experimental period, blood samples were taken by cardiac punctures then the animals were scarified. then the blood samples were put in a screw tube without anticoagulant, then centrifuged at 5000 rpm for 15 minutes to get serum for Biochemical parameter such as serum MDA. a mid-line abdominal incision was performed, stomachs ligated from esophageal and pyloric opening, and rapidly removed, opened along their greater curvature and spread on a paraffin plate and gently washed with saline. total gastric area, gastric lesions induced by aspirin and ulcerated areas as ulcer index were measured by digital caliper. The gastric juice was collected in test tube. Then the stomach was examined for macroscopical mucosal lesions. Parts of gastric mucosa was removed for other laboratory tests. Finally the stomach was immersed in 10% formalin solution for histopathological examination.

Gastric Ulcer Index:

The mucosal layer of stomach was observed under magnifying lens and was checked for ulcers, The gastric mucosal lesions were expressed in terms of ulcer index (mm).The sum of length for all lesions of each animal was measured and served as the Determination of the curative ratio (22).

Determination of the curative ratio:

The curative ratio was calculated to each group by using equation of (23),

Curative ratio = [(Control ulcer index) – (Test ulcer index) / (Control ulcer index)] x100.

Determination of Gastric Juice Volume:

Gastric juice collected from each stomach, was centrifuged at 3000 rpm for 10 minutes to remove any solid debris, the volume of the supernatant was

measured by graduated cylinder (24).

Determination of Gastric Juice Acidity:

acidity degree (pH) of gastric juice from animal stomach was determined by using pH meter apparatus (HI 9021).

Lipid Peroxidation Test:

1- Blood MDA Assay:

serum lipid peroxide levels were determined by measuring thiobarbituric acid (TBA) reactivity as described by (25).

Gastric Tissue MDA Assay-2:

Gastric tissue MDA (as an important indicator of lipid peroxidation) levels were measured according to the method (26).

Histological examination:

The animals were sacrificed at the end of the experiment and the stomachs were taken. these organs were fixed in 10% buffered formalin, dehydrated progressively in increased ethanol concentrations, treated with xylene and embedded in paraffin. five microns thickness sections of paraffin-embedded tissue were mounted on glass slides and stained with Haematoxyline and Eosin (H & E stain) (27).

Statistical analysis:

Data obtained from experiments was expressed as mean \pm SD , the results were analyzed statistically using (ANOVA) by SPSS programming difference were considered significant at $P < 0.05$ (28).

RESULTS:

Effect of ethanolic extract of *Anethum graveolens* and cimetidine drug on Gastric ulcer index, Total gastric ulcer Gastric fluid volume and Curative ratio in guinea pigs : The results of gastric ulcer index are shown in table (1) there are significant increase ($P \leq 0.05$) in positive control compared with another treated groups, while the results showed a non- significant change ($P \leq 0.05$) in gastric ulcer of guinea pig treated with ethanolic extract (300mg/kg) compared with negative control group, but the results showed a significant decrease ($P \leq 0.05$) in gastric ulcer index in guinea pig treated with ethanolic extract at dose 150mg/kg and cimetidine group compared with positive control group. The results of Total Gastric Area reveled significant increase ($P \leq 0.05$) in control positive compared with control negative group, while the results showed a significant decrease ($P \leq 0.05$) in Total gastric area of guinea pig treated with ethanolic extract (150mg/kg, 300 mg/kg) and treated with cimetidine group compared with positive control group (table 1).

Table 1. Effect of ethanolic extract of *Anethum graveolens* on Gastric ulcer index, Total gastric area and Gastric fluid volume guinea pig with Gastric Ulceration..

Groups	Gastric ulcer index(mm)	Total gastric area	Gastric fluid volume (cm3)	Curative ratio %
Control negative	0.000 \pm 0.000 D	13.756 \pm 0.181 E	6.033 \pm 0.103 B	-
Control positive	1.933 \pm 0.121 A	29.833 \pm 0.500 A	8.200 \pm 0.451 A	-
Aspirin+ extract 150mg/kg	1.066 \pm 0.121 B	23.676 \pm 0.509 B	6.066 \pm 0.816 B	48.1
Aspirin+ extract 300mg/kg	0.108 \pm 0.020 D	20.686 \pm 0.583 D	4.033 \pm 0.103 C	94.8
Aspirin+ cimetidine 20mg/kg	0.616 \pm 0.147 C	22.533 \pm 0.233 C	6.066 \pm 0.136 B	68.3

Values are expressed as mean \pm SD of 6 guinea pig in each group. Capital letters denote differences between groups, $P \leq 0.05$ vs. control.

The obtained results in table (1) revealed a significant increase ($P \leq 0.05$) in Gastric fluid volume of guinea pig in control positive compared with control negative and another treated group but the results showed a non-significant change ($P \leq 0.05$) in gastric fluid volume in group treated with ethanolic extract (150mg/kg) and cimetidine group compared with control negative, while the results showed a significant decrease ($P \leq 0.05$) in group treated with ethanolic extract (300mg/kg) compared with control negative and another treated groups. The results showed that there was a significant increase ($P < 0.05$) in the mean values of curative ratio of treated group with ethanolic extract at dose 300mg/kg as compared with groups treated with cimetidine or ethanolic extract at dose 150mg/kg.

Effect of ethanolic extract of *Anethum graveolens* and cimetidine drug on PH, Serum MDA and Gastric tissue MDA in guinea pigs : The obtained results in table (2)

revealed a significant decrease ($P \leq 0.05$) in gastric PH of positive control compared with another groups, but the results showed non-significant in gastric PH of treated with ethanolic extract at dose 150 mg/kg and cimetidine group with control negative group, while in treated group with ethanolic extract at dose 300mg/kg showed a significant increase ($P \leq 0.05$) compared with another groups. A significant increase ($P \leq 0.05$) in serum MDA of guinea pigs of positive control group compared with another groups, while the results showed significant ($P \leq 0.05$) decrease of MDA in guinea pigs serum with gastric ulceration treated with ethanolic extract (300mg/kg) compared with another treated groups but show non-significant change ($P \leq 0.05$) in guinea pig treated with ethanolic extract at a dose of 150mg/kg compared with cimetidine group. The results of gastric tissue MDA showed a significant decrease ($P \leq 0.05$) of guinea pigs treated with ethanolic extract at a dose of (150mg/kg, 300mg/kg) and cimetidine group compared

with control positive group, but the results revealed non-significant change in gastric tissue MDA of guinea pigs treated with ethanolic extract at dose 300mg/kg and cimetidine compared with control negative group (Table2)..

Table (2) : Effect of ethanolic extract of *Anethum graveolens* on serum MDA and Gastric Tissue MDA guinea pig with gastric ulceration .

Groups	PH	Serum MDA (nmol/mg of pt)	Gastric Tissue MDA(nmol/mg)
Control negative	2.608±0.142 B	0.325±0.041 D	0.101±0.004 B
Control positive	1.166±0.163 C	1.393±0.021 A	1.100±0.126 A
Aspirin+ extract 150mg/kg	2.633±0.133 B	0.978±0.007 B	0.323±0.005 C
Aspirin+ extract 300mg/kg	3.100±0.322 A	0.520±0.016 C	0.148±0.007 B
Aspirin+ cimetidine 20mg/kg	2.716±0.194 B	1.006±0.049 B	0.196±0.013 B

Values are expressed as mean± SD of 6 guinea pig in each group. Capital letters denote differences between groups, P≤0.05 vs. Control.

Grossly examination of stomach:



Fig.(1):stomach of guinea pig (negative control). showed normal gastric mucosa ,



Fig.(2): stomach of guinea pig (positive control). showed gastric damage including gross mucosal lesion and hemorrhagic lesion.

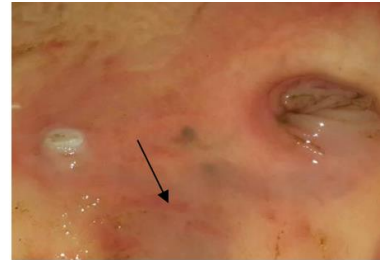


fig.(3): Stomach of guinea pig treated with extract at dose(150mg/kg) showed erosion was limited to the superficial mucous layer in glandular region



fig.(4): Stomach of guinea pig treated with extract at dose (300mg/kg) showed no lesion at all tissues of stomach.

Histopathological Examination:

The stomach of control negative group in guinea pig appeared normal gastric mucosa, submucosa and muscular layers shown in figure(6). while the stomach of control positive group in guinea pig revealed histopathological change include sloughing of mucosal layer, vacuolated epithelial cells, haemorrhagic area and show excessive edematous fluid in muscular layer shown in figure (7). In the stomach of guinea pig treated with ethanolic extract of *Anethum graveolens* at a dose of 150mg/kg appeared mild erosion of mucosal layer, vacuolated cells and edematous fluid in submucosal layer with congestion of blood vessels figure(8) while the stomach of guinea pig treated with ethanolic extract of *Anethum graveolens* at a dose of 300mg/kg revealed mild degenerative epithelial cells in mucosal layer with normal muscular layer, no congestion of blood vessels shown in figure(9). the stomach of guinea pig treated with cimetidine at a dose of 20 mg/kg appeared mild erosion of mucosal layer, edematous fluid in submucosal layer and congestion of blood vessels figure(10).

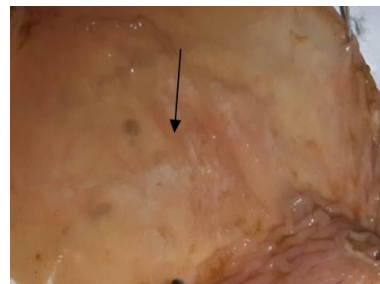
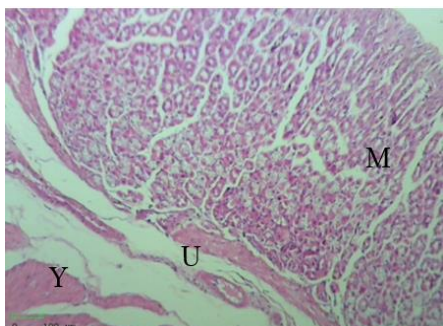


fig.(5): stomach of guinea pig treated with cimetidine at dose (20mg/kg) showed marked necrotic area in the glandular area of mucosal layer.



Fig(6): section of stomach of guinea pig of control negative group Showed normal gastric mucosa (M), submucosa(U), muscular layers(Y) (H&E) stain 10X.

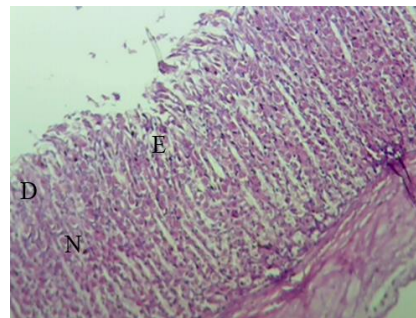
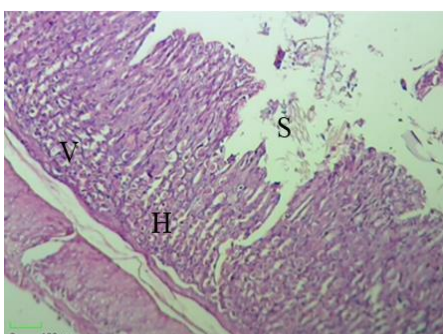


Fig.(10):Section of stomach treated with cimetidine at dose20 mg/kg B.W. mild erosion of mucosal layer(E), some cells are degenerated (D) and others are necrotized(N) (H&E) stain 10X.



Fig(7): section of stomach of guinea pig of control positive group Showed sloughing of mucosal layer (S), vacuolated epithelial cells (V), with haemorrhagic area(H). (H&E) stain. 10X.

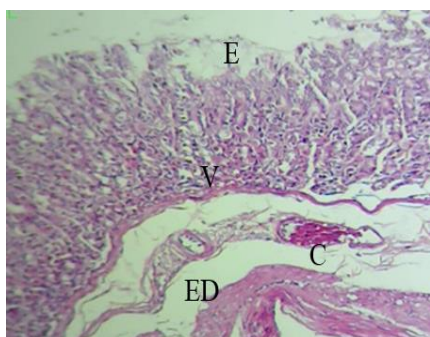


Fig.(8): Section of stomach treated with extract at dose 150 mg/kg, show mild erosion of mucosal layer (E), vacuolated cells(V) and edematous fluid in submucosal layer(ED) with congestion of blood vessels(C). (H&E) stain 10X.

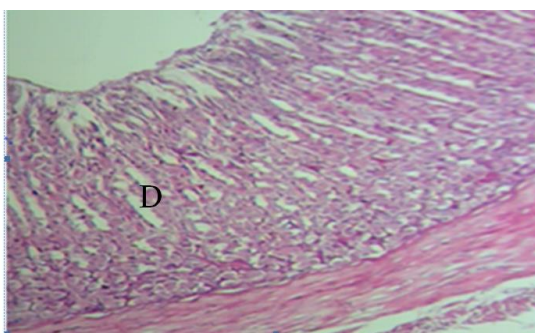


Fig.(9): Section of stomach treated with extract at dose 300mg/kg. Showed mild degenerative epithelial cells in mucosal layer (D), no congestion of blood vessels, with normal muscular layer. (H&E) stain 10X.

DISCUSSION:

Gastric ulcer is gastrointestinal disorder, characterized by disruption of the mucosal integrity attributed to various aggressive factors (acid, pepsin, stress, *Helicobacter pylori* and NSAIDS) and defensive factors (mucus, bicarbonate, blood flow and prostaglandins) (29). In our study, the significant decrease in gastric PH, increased gastric index, total gastric area and gastric volume of the untreated Aspirin group, Gastric lesions with aspirin administration consisted of multiple erosions and frank bleeding ulcerations in the mucosa. there was almost complete inhibition, however, of the mucosal generation of prostaglandins. loss of normal protection by endogenous prostaglandins is considered to be the major factor in the pathogenesis of acidified aspirin-induced gastric ulcerations (30). The significant increase in gastric volume following oral administration of Aspirin is due to increased production of hydrochloric acid as it evident from the total acidity of the gastric juice (31). Low PH value is manifestation of increase H^+ ion concentration in gastric juice (6). In treated group with cimetidine significantly increase gastric PH, decrease in gastric ulcer index, total gastric area and gastric volume due to its ability to inhibits the action of histamine at the H_2 - receptor site located primarily in gastric parietal cells, resulting in inhibition of gastric acid secretion (32). Administration of ethanolic extract of *Anethum graveolens* significantly increasing in gastric PH, decreasing in gastric volume and gastric index proving antisecretory activity because it contains flavonoids and tannins, flavonoids are not only free radical scavengers, but are important increasing mucosal prostaglandin content and can decrease histamine secretion from mast cells by the inhibition of histidine decarboxylase (33). flavonoids also have anti-inflammatory properties without any ulcerogenic action as a side effect and thus show a great advantage in the treatment of peptic ulcers (34). Tannins prevent ulcer development by improving vasoconstricting effects (35). Our study showed that administration of Aspirin caused significant increase in serum and gastric tissue MDA. Oxidative stress plays an important role in the

pathogenesis of gastric ulcer that lead to increase the MDA activity. While in the ethanolic extract of *Anethum graveolens* treated group cause significant reduction in serum and gastric tissue MDA because the extract contains many compounds like alkaloid and flavonoid which are powerful antioxidant and scavenge of free radicals (36).

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