The efficiency of aqueous-methanol Pomegranate peel extract against aspirin induced gastric ulcer in rabbits

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Abstract

Ulcer is a common gastrointestinal disorder which is seen among many people. It is basically an inflamed break in the skin or the mucous membranes lining the alimentary tract. Ulcerations occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggregation or diminished mucosal resistance. It may be due to the regular usage of drugs, irregular food habits, stress, and so forth. Peptic ulcers are a broad term that includes ulcers of digestive tract in the stomach or the duodenum. 15, mature male rabbits, of 1-2 years old, weighing 1-1.9 kg were used. After acclimatization for a period of 2 weeks to animal house conditions, left ad libitum to food and water. Basal diet was alfalfa and concentrated food. Rabbits were fasted for 24 h prior to the experiment, but allowed free access to water except for the last hour before the experiment. The animals were randomly classified into 3 groups (5 rabbits each). Rabbits of 1st group not treated with extract, nor exposed to aspirin, as control group. While those of 2nd group, not treated with plant extract but exposed to aspirin at a dose rate of 300 mg / kg b. wt. mixed with 2 ml water orally, meanwhile in 3rd group animals were pretreated with aqueous – methanol (30:70%) extract of pomegranate peel for 9 day at a dose rate of 300 mg / kg. b. wt. orally before aspirin administration at a dose rate of 300 mg / kg in 2 ml water orally. The animals were killed with an ether overdose three hour after aspirin administration. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminate, if any. The surface area of each lesion was counted and scored. The ulcer index for each rabbit was taken as the mean ulcer score. The percentage of ulcer protection (%UP) was calculated.

The results of current study revealed that aqueous- methanol extract of pomegranate peel exhibit high protective effect in protection of stomach from ulcer induced by exposure to aspirin.

Key words: gastric ulcer , aqueous – methanol extract, Pomegranate, Rabbits

كفاءة مستخلص المائي– الميثانول للب الرمان ضد تقرح المعدة المحدث بالتعرض للأسبرين في الارانب

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الخلاصة

التقرح من الاعتلالات المعدية – المعوية الشائعة والتي تلاحظ في العديد من البشر. اساسا انها ، تهتك في الجلد او الغشاء المخاطي المبطن للقناة الهضمية. تحدث التقرحات عندما يكون هناك اضطراب في التوازن الطبيعي المسبب اما بالتكدس المتسارع او ضعف المقاومة للمخاطية. وقد تكون ناتجة من الاستخدام المنتظم للعقاقير، عادة الاكل غير المنتظم ، الاجهاد ، وهكذا. استخدم 15 ارنبا، ذكر، بالغ ، بعمر 1– 2 سنة ،ووزن 1–1.9 كغم في الدراسة . بعد التطبع لمدة 2 اسبوع لظرف مكان التجربة ، حيث تركت حرة للغذاء والماء. الغذاء كان في الاساس من العلف المركز والجت. تم قطع الغذاء ل 24 ساعة قبل البدء بالتجربة. قسمت الحيوانات الى 3 مجموعات (5 ارانب في كل مجموعة) حيوانات المجموعة الاولى (السيطرة) تركت بدون علاج بالمستخلص المائي – الميثانول ، ولم تتعرض للأسبرين. اما حيوانات المجموعة الثانية فقد عرضت للأسبرين بجرعة 300 ملغم \ كغم من وزن الجسم عن طريق الفم مذابة في 2 مل ماء، ولم تعالج بالمستخلص ، في حين عولجت الحيوانات في المجموعة الثالثة لمدة 9 ايام بالمستخلص المائي– الميثانول (30: 70%) من لب ثمار الرمان ، وبجرعة 300 ملغم \ كغم من وزن الجسم ثم عرضت للأسبرين عن طريق الفم بجرعة 300 ملغم \ كغم من وزن الجسم مذابة في 2 مل. تم قتل الحيوانات بالجرعة الفائقة للأثر . بعد مرور 3 ساعات على التعرض للأسبرين. مباشرة معد الحيوانات ازيلت ، وتم فتحها من المنحنى الكبير ، غسلت المخاطية من الدم ان وجد بالمحلول الملحي البارد. ثم عدت الآفات واخذت احجامها وشدتها . مؤشر التقرح لكل ارنب اخذ كمعدل لعدد الآفات. حسبت النسبة المؤية للحماية من التقرح % .

اظهرت الدراسة ان المستخلص المائي– الميثانول للب الرمان ابدى فعل عالي لحماية المعدة من التقرح المحدث من التعرض للأسبرين.

كلمات المفتاح : المعدة والامعاء، مستخلص المائي – الميثانول ، لب الرمان ، الارانب

Introduction

Gastric ulcers are illness that affect a considerable number of people in the world, some of the causes of this disorders are stress, smoking, nutritional deficiencies and bacterial infection, and ingestion of non-steroidal anti- inflammatory drugs (1).

The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs (2).

Herbal remedies can act either as gonists or antagonists that potentiate some drug therapies (3).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (4).

The anti-oxidative effect is mainly due to phenolic components, such as flavonoids (5), phenolic acids, and phenolic diterpenes (6). Typical phenols that possess antioxidant activity have been characterized as phenolic acids and flavonoids (7).

Pomegranate

Several disorders such as cardiovascular diseases, diabetes, metabolic syndrome, cancer. have become more prevalent. Free radicals such as reactive oxygen species (ROS) have been implicated in their etiology. However, the defense mechanism of the body against ROS could be overwhelmed in many circumstances by an over- production of these species. Therefore, synthetic antioxidants and food rich in antioxidants have been shown to boost our defense against ROS. The pomegranate molasses, are believed to have antioxidant effects but without much scientific evidence. Pomegranate is considered one of the oldest fruits and one of the earliest to appear in human diet. The juice contains a considerable amount of carbohydrates, ascorbic acid, Vit. B, pectin, cellulose, tannins, and ash. Moreover, the pericarp is a good source of polyphenols such as anthocyanin, leucoanthocyanins, catechins, and flavonoids and contains about 30% of tannins. This could explain the benefit of pomegranate compounds when used as additives or anti- bacterial due to phenols, pigments and citric acid. Because of their rich content in tannins, the pericarp, the roots, and the peel are used in medicine against dysentery as they are included in the pharmaceutical preparations containing biological active substances (8 and 9).

(10) Negi et al., (2003) reported that pomegranate peel extracts exert anti-oxidant and anti- mutant activities in vitro due to their content of polyphenols (tannins, ellagic and gallic acids). On the other hand, (11), (12 and 13) have reported an anti-oxidant and anti- sclerotic effects of pomegranate syrup on animal models in vitro. Pomegranate juice has a remarkable ability to decrease oxidative stress by 40-80% and to increase the antioxidant enzymes- paraoxonases (serum PON1 and macrophage PON2) by 50-100% (14 and 15).

Flower juice is recommended as a gargle fore sore throat, in leucorrhea, hemorrhages and ulcers of the uterus and rectum (16).

The biological activities, viz. antibacterial, antifungal, anthelmintic and antifertility, of the various extracts of different parts of this plant Pomegranate have also been reported. Different parts of the plant have anti-hepatotoxicity, anti-tumor and anti-inflammatory activities (17).

Chemical composition of pomegranates

About 50% of the total fruit corresponds to the peel, which is an important source of bioactive compounds such as phenols, flavonoids, ellagitannins and proanthocyanidin compounds (18), minerals (19), and complex polysaccharides (20).

Pomegranate fruit extracts constituents possesses immense biological activities such as anticarcinogenic (21; 22), antibacterial (23); Antidiarrheal (24), antifungal (25), antioxidant activity and free radical scavenging capability (9, 12), strengthening of the immune systems (26), Prevention of heart disease and liver fibrosis (27), and inhibition of lipid peroxidation even at lower concentrations than vitamin E (28). All these therapeutically activities are related to the presence of diverse 'phenolic compounds', Including gallic acid, protocatechinunic acid, chlorogenic acid, caffeic acid, ferulic acid, coumaric acid, and catechin and hydrolysable tannins (such as punicalin, pedunculagin, punicalagin, coreliagin, casuarinin, punicacortein, granatin and ellagic acid), and anthocyanins (delphinidin, cyanidin, and pelargonidin 3- glucosides and 3,5-diglucosides) (29-33).

Non- steroidal anti- inflammatory drugs (NSAIDS) are known as one of the most common pathogenic factors associated with gastric mucosal damage. Acetyl salicylic acid (Aspirin, ASP) Acetyl salicylic acid powder was suspended in 5 ml distilled water and given orally through stomach tube in a dose of 500 mg /kg

The aim of this study was to investigate the efficiency of aqueous-methanol Pomegranate peel extract against aspirin induced gastric ulcer in rabbits.

Materials and methods

Plant materials

Ripe Pomegranate peel were collected from the farms in Diyala province, Iraq.

Preparation of extract:

The Pomegranate peel were cleaned carefully and washed several times with running tap water. The aqueous- methanolic (30:70%) extract was prepared by soaking 500g of Pomegranate peel in 1 liter of a solvent composed of 700 ml methanol 95% and 300 ml distilled water, with frequent shaking for 2 days and kept in a refrigerator. The infusion was filtered by a piece of double layer gauze. The filtrate was evaporated using a rotary evaporated apparatus (34).

Experimental animals

15 mature, male rabbits of 1-2 years old, weighing 1-1.9 kg were used after acclimatization for a period of 2 weeks to animal house, left ad libitum to food and water. Basal diet was alfalfa and concentrated food. All the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. The experiment was conducted at department of Medicine, College of Veterinary Medicine, University of Diyala, Iraq.

Experimental design:

Rabbits were fasted for 24 h prior to the experiment but allowed free access to water except for the last hour before the experiment. The animals were randomly classified into 3 groups (5 rabbits each). Rabbits in 1st group not treated with the extract, nor exposed to aspirin, while those in 2nd group not treated with plant extract but exposed to aspirin at a dose rate of 300 mg / kg b. wt. mixed with 2 ml water orally, meanwhile animals in 3rd group were pretreated with plant extract for 9 day at a dose rate of 300 mg / kg. b. wt. orally before aspirin administration at a dose rate of 300 mg / kg in 2 ml water orally.

Assessment of gastric mucosal lesions:

The animals were killed with an ether overdose three hour after aspirin administration. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminate, if any. The surface area of each lesion was counted and scored as described by (35). The severity factor was defined according to the length of lesion.

Severity factor:

0= no lesion.

1= lesion < 1 mm.

2= lesion 2-4 mm length.

3 =lesions > 4 mm length.

Lesion scores for each rabbit was calculated as the number of lesions in the rabbits x their respective severity factor

UI for each group= mean lesion score of all the rabbits in that group

%=

CUI = ulcer index of control group

TUI= ulcer index of treated group

Prevent index(PI)=

Biochemical estimation

Determination of gastric juice volume and pH:

The volume and pH of centrifuged gastric juice were measured by pipette and digital pH meter. The volume was expressed as ml (36).

Determination of total and free acidity:

The total and free acidity were determined by titrating with 0.01 N NaOH using phenolphthalein and Methyl orange (37).

Reagents

- 0.01N NaOH

- Phenolphthalein.
- Methyl orange.

Procedure

Pipette 1 ml of filtered gastric contents into a small beaker, add 2 to 3 drops of methyl orange and titrate with 0.01 N NaOH until all trace of the red color disappears and the color is yellowish orange. Note the volume of alkali added that indicate free acidity. Then add 2 or 3 drops of phenolphthalein and continue titrating until a definite red tinge reappears. Note the total volume of alkali added that indicate total acidity. The results expressed as Meq/ L.

Acidity=

Histopathological studies

The stomach from all groups were removed rapidly, opened along the greater curvature, and thoroughly rinsed with saline. After recording the ulcers produced in the stomach a longitudinal section of the gastric tissue was taken from the anterior part of the stomach and fixed with a 10% formalin solution, for hematoxylin- eosin staining for histological assessment of the gastric mucosa according to (38).

Statistical analysis:

Data were expressed as Mean ± S.E.M., with a value of $P < 0.05$ considered statistically significant. Statistical evaluation was performed by ANOVA followed by the Student's t- test. (39).

Results

The results revealed that body weight, body temperature, respiratory rates, heart rated did not showed significant changes (Table-1-).

Table-1- Showing body weight, body temperature, respiratory rates, heart rates of rabbits in experiment.

Day			group	Parameter
18	9	0		
1.35± 0.15	1.33± 0.22	1.49 ± 0.23	treated	Body weight Kg
1.53± 0.30	1.52 ± 0.20	1.50 ± 0.10	control	
38.6 ± 0.35	38.5± 0.20	38,.02 ± 0.47	treated	Body temp. ^{o}C
38.8± 0.20	38.9 ± 0.12	39.0 ± 0.15	control	
135 ± 5.5	140 ±8.17	131.5 ± 11.79	treated	Resp. rate / min.
138.0± 10.0	135.0 ± 4.0	135.0± 5.50	control	
188.0 ±6.8	190 ± 10.0	185± 9.57	treated	Heart rate/ min.
180.0± 6.50	185.5 ± 10.0	187.5± 10.0	control	

The results revealed that bleeding and clotting times time decreased in treated group in comparison with values of control group(Table-2-).

Table- 2- showing bleeding and clotting times (seconds) of rabbits suffered from hepatitis

Day				
18	9	0	Group	Parameter
30.5± 8.09	28.75 ± 7.18	37.5± 10.31	Treated	Bleeding / sec.
42.5 ± 0.25	40 ± 0.0	40.0 ± 0.5	Control	
40.20 ± 3.80	37.5 ± 4.33	41.25± 4.27	Treated	Clotting / sec.
48.0± 4.0	50± 30	45.20 ± 3.0	Control	

The revealed that total erythrocytes count, Hb concentration, PCV, MCV, and MCH were increased in treated group in comparison with control one (Table-3-).

Table- 3- showing total erythrocytes count , Hb, PCV and erythrocyte indices (MCV, MCH, MCHC) of rabbits in experiment.

Day			Group	Parameter
18	9	0		
5.8 ± 0.35	5.55 ± 0.40	5.23 ± 0.43	Treated	Erythrocytes $X10^6$/cmm
5.3 ± 0.10	5.4 ± 0.08	5.70± 0.35	control	
12.0± 0.25	12.33 ± 0.34	11.4 ± 0.32	Treated	Hb g%
9.90 ± 0.28	10.85± 3.15	10.20 ± 0.25	control	
35.6 ± 0.90	36.25± 1.18	33.5 ± 0.87	Treated	PCV %
34.2± 0.80	34.0 ± 9.0	33.7± 0.65	control	
60.12 ± 2.55	67.11 ± 5.37	54.67± 3.55	Treated	MCV ft
50.25± 1.85	49.6 ± 14.91	55.40 ± 3.15	control	
20.25 ± 1.50	22.58 ± 1.84	19.52 ± 2.14	Treated	MCH pg
19.0± 0.85	18.19± 5.23	19.5 ± 0.90	control	
33.0 ± 0.50	34.0 ± 0.18	33.86 ± 0.20	Treated	MCHC%
33.9 ± 0.18	33.9 ± 0.38	34.9 ± 0.38	control	

The results revealed that total leucocytes count and Heterophil% were decreased , while Lymphocyte%, Esinophil%, Monocyte% were increased in treated group in comparison with that of control group (Table-4-).

Table -4- showing total and differential leucocyte counts of rabbits suffered from induced hepatitis

Day		Group	Parameter

18	9	0		
11.0 ± 2.0	10.86± 1.05	14.09± 3.66	Treated	Total leucocytes $X10^3$/cmm
13.8 ± 0.5	12.8± 0.6	13.5 ± 1.50	Control	
35.,5 ± 3.80	34.5± 4.94	50.75± 4.40	Treated	Heterophil%
49.0± 2.5	50.5± 12.6	48.0 ± 2.5	control	
52.8 ± 3.09	50.75 ± 6.14	41.25± 4.84	Treated	Lymphocyte%
48.0± 2.80	48.0 ± 4.0	46.8 ± 3.5	control	
6.0 ± 1.0	6.5 ± 1.26	4.0± 0.0	Treated	Eosinophil%
3.5 ± 0.5	3.5 ± 0.5	3.9± 0.7	control	
3.0 ± 0.60	3.0 ± 0.71	2.75 ± 0.75	Treated	Basophil%
1.8 ± 0.15	1.5± 0.5	2.8± 0.25	control	
5.0 ± 0.10	5.5 ± 0.5	1.5± 0.14	Treated	Monocyte%
1.8 ± 0.5	1.5 ±1.5	1.5 ± 0.5	control	

The results revealed that values of RBS decreased while that of ALT increased in those treated with extract and exposed to aspirin, and those exposed to aspirin in comparison with normal group (Table-5-)

Table- 5- showing values of RBS, ALT, AST, TSB, TSP of rabbits in experiment

Group			parameter
Normal	Aspirin	Melia + Aspirin	
90± 2.7	25 ± 0.5	35.0± 0.45	RBS mg/dl
66.05± 1.85	85.3 ±0.30	75.33± 6.06	ALT U/L
70±3.0	74.55± 5.65	68.43 ± 4.54	AST U/L
0.5±0.06	0.5 ±0.01	0.45 ± 0.0	TSB mg /dl
8.0±0.9	8.2 ±0.20	8.17± 0.09	TSP g/dl

Antiulcer activity

The effect of orally administered Pomegranate peel on gastric damage induced by aspirin is shown in table -6-

Pomegranate no of lesion 4;

2=lesion 2-4mm; and 2 lesion > 4

lesion scores = 10

UI = 10 x 5

Aspirin

8 lesions 4 lesions = lesion 2-4 mmm; 4 lesions > 4 mm.

Lesions scores 20

UI = 20 x5

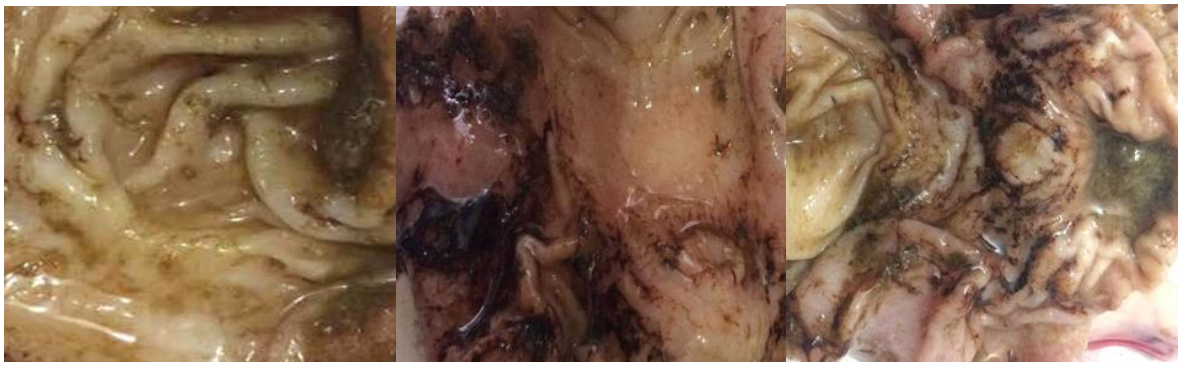
Prevent index(PI)=

== = 75%

It was observed that increase in lesions of stomach mucosa in treated and control groups. Significant reduction in lesion was observed in treatment with pomegranate. It is significant to note that increased in volume, total acidity and free acidity and decreased pH of gastric juice. Administration of pomegranate decreased the volume, total acidity and free acidity and increased pH of gastric juice were observed as compared to control rabbits the percentage of gastric protection was 75% . Animal groups treated with the Pomegranate 300mg / kg b. wt. exhibit a reduction of gastric damage against aspirin induced gastric damage, bleeding and congestion pic(1,2,3).

Table -6- showing Volume and PH, free acidity and total acidity of gastric juice, No of gastric lesions and % protection in rabbits of experiment

% of ulcer protection	Gastric ulcer lesion (No.)	Total acidity	Free acidity	Volume	pH	Groups
-	0.0±0.00	280± 12.5	250 ±18.9	1.5 ± 0.06	2.6 ±0.08	control
-	8±0.35a	450 ± 5.0a	325 ± 7.5a	2.67± 0.03a	1.33 ± 0.12a	aspirin
75	4±0.10b	245 ± 11.67b	223.3 4.91b	1.8 ± 0.61b	1.62 ± 0.03b	treated

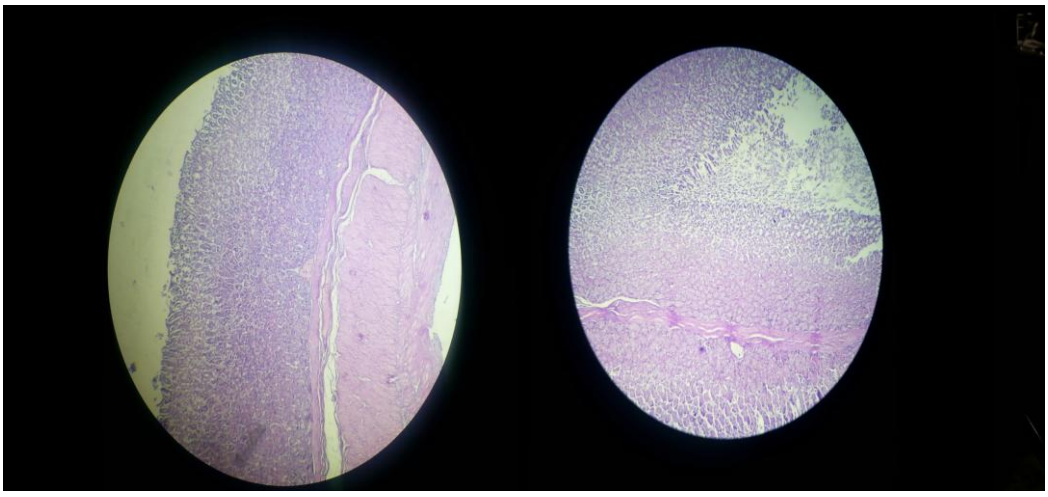


Picture (1) showed normally grossly finding apparent of the stomach in control group

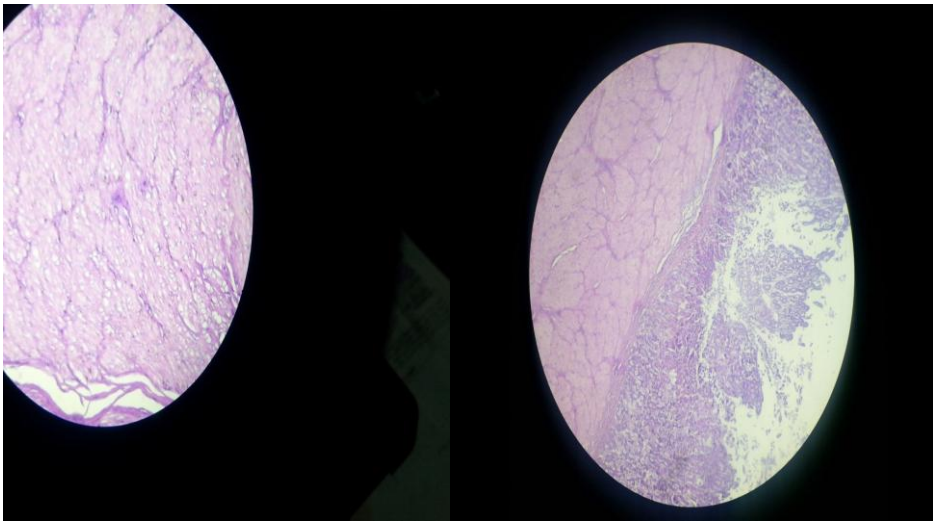
Picture (2)grossly apparent of stomach showed bleeding ,congestion in the (group 2)

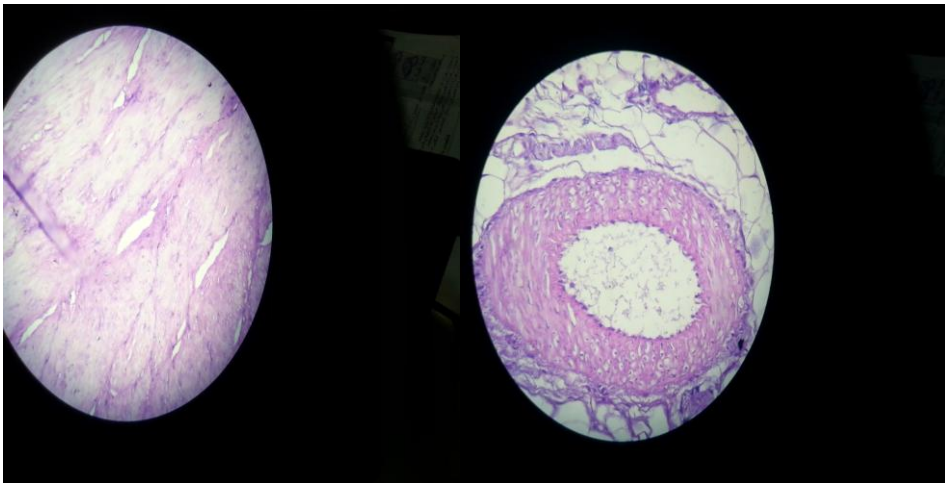
Picture (3)grossly lesion in the stomach showed congestion in the (group 3)

The histopathological results revealed to desquamation in the epithelium surface of the stomach(in the pitts),cellular debris ,degeneration in the pyloric glands in the mucosa of stomach ,there is granulation tissue in the lamina muscularis layer of the stomach pic(4,5) . The histopathological results of group two revealed to desquamation in the mucosal epithelium surface of the stomach(in the pitts)and extended to pyloric gland more than group one , degeneration in the pyloric glands in the mucosa of stomach ,some granulation tissue in the lamina muscularis layer of the stomach , Presence of granulation tissue in the wall of artery that enriched the stomach, presence of fibrinoid necrosis in the lamina muscularis pic(6,7,8,9). The histopathological results of group three revealed to degeneration in the surface epithelium , desquamation in the pyloric glands but some of them are still normal ,slight granulation tissue in the lamina muscularis, slight presence of fibrin in the muscular lamina pic(10,11).

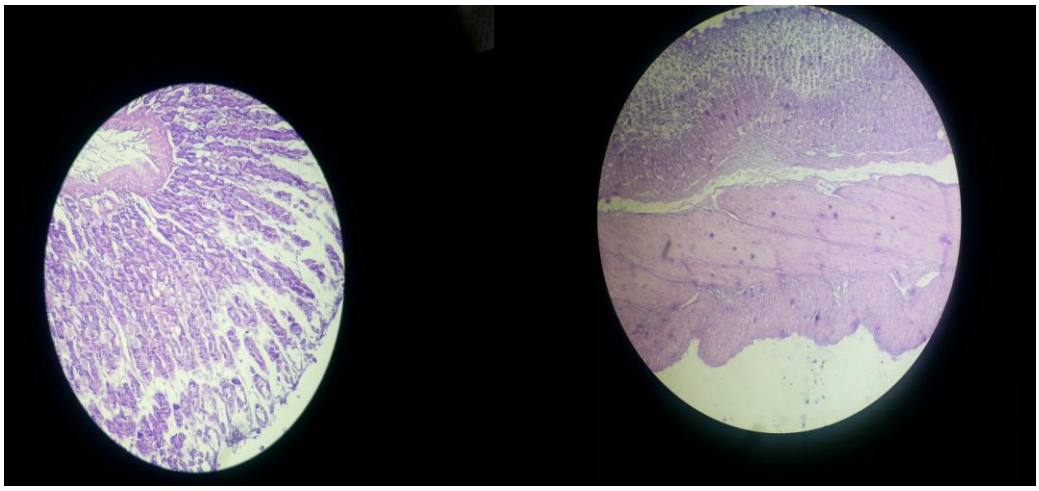


Picture (4,5)of group one showed desquamation in the epithelium surface of the stomach(in the pitts),cellular debris ,degeneration in the pyloric glands in the mucosa of stomach ,there is granulation tissue in the lamina muscularis layer of the stomach.





Picture (6,7,8,9)of group two showed desquamation in the mucosal epithelium surface of the stomach(in the pitts)and extended to pyloric gland more than group one , degeneration in the pyloric glands in the mucosa of stomach ,some granulation tissue in the lamina muscularis layer of the stomach , Presence of granulation tissue in the wall of artery that enriched the stomach, presence of fibrinoid necrosis in the lamina muscularis.



Picture (10,11)of group three showed degeneration in the surface epithelium , desquamation in the pyloric glands but some of them are still normal ,slight granulation tissue in the lamina muscularis, slight presence of fibrin in the muscular lamina .

Discussion

The etiology of gastro- duodenal ulcers is influenced by various aggressive and defensive factors such as acid- pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermic growth factors (40). According to (41), some other factors, such as inadequate dietary habits, cigarette- smoking, excessive ingestion of non- steroidal anti- inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori may be responsible for the development of peptic ulcer.

The volume of acid present in gastric secretion which encompasses HCL, pepsinogen, mucus, Bicarbonates, intrinsic factor and protein reflect acid volume. Exposure of unprotected lumen of the stomach to accumulative acid could facilitate ulceration (42).

(43) recorded that Indomethacin injection, a representative of NSAIDs family caused a remarkably significant increase in ulcer index, gastric juice, free acidity and total acidity. Oral administration of RAN significantly reduced ulcer index, gastric juice, free and total acidity. Treatment with CME protected the gastric mucosa from damage by increasing the mucin content significantly.

Peptic ulcer is defined as disruption if the mucosal integrity of the stomach and or duodenum leading to a local or excavation due to active inflammation.

Several experimental models were used to assess the anti- ulcerogenic activity of test drugs in rabbits. They include, cold restraint stress, pyloric ligation, aspirin,

indomethacin, cysteamine, ethanol, etc (44). Mucus, which continuously coats over the gastric mucosa, is well known as a mucous barrier to prevent the injury of luminal acid, bacteria and noxious agent's injuries. Mucus might implicate in scavenging oxygen – derived free radicals. Mucus glycoproteins and lipids bound to mucin might involve in the antiradical process. (44).

In the present investigation, it has been demonstrated that Pomegranate can significantly enhance gastric mucus secretion while reduce in the acidity of the gastric juice in rabbis. Gastric mucus is an important protective factor for the gastric mucosa and it is capable of acting as an antioxidant agent and reducing mucosal damage mediated by oxygen free radicals. However, the protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface. Pomegranate possess marked gastro- protective properties as evident by its significant inhibition of the formation of gastric lesion (in terms of length and number) induced by aspirin. in the present study, the decrease in volume of the gastric juice and concomitant decrease in the acidity and increase in pH providing the antiulcer activity of Pomegranate and this result complements the earlier findings.

Administration of Pomegranate significantly increased the amount of mucus produced by the rabbit gastro mucosa compared to their respective controls. Therefore, the enhanced mucous secretion after administration of Pomegranate may help to protect against the aspirin induced damage by preventing the action of acid on the stomach mucous epithelium.

Gastroprotective effect of flavonoids has been reported (45). Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulceration and erosion lesion (46).

Conclusion

Gastroprotective role for Pomegranate against gastric mucosal damage induced by aspirin were investigated in the present study. aspirin induced gastric ulcer in rabbits shows that increased gastric volume, acidity and depleted pH. The observed gastroprotection is possibly mediated to a major extent by a gastric mucosal secretion mechanism as the Pomegranate were able to restore the increased volume, acidity and depleted pH by aspirin almost toward s normal levels seen in control.

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