EVALUATION OF ANTIULCER ACTIVITY OF WHITE MEMBRANE POMEGRANATE AGAINST ASPIRIN INDUCED GASTRIC ULCERS IN RATS

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ABSTRACT
The objective of this study was to evaluate the antiulcer effects of white membrane pomegranate on aspirin-induced gastric ulcer model rats. Eighteen male albino rats were classified into normal control group. It was carried out on 18 albino Wister rats. Animals were divided into two groups. Gastric ulcer group (T group=9) and control group (n=9). Each group was subdivided into three group (3rd day, 7th day and 15th day after treatment (three animal in each sub group) both groups were fasted for 24 hours, all the rats were sacrificed and the ulcer areas of the gastric walls were determined. Grossly, the ulcer control group exhibited severe mucosal injury, Histological studies of the gastric wall of control group revealed severe damage of gastric mucosa, along with edema and leucocytes infiltration of the submucosal layer compared to rats received pomegranate where there was marked gastric protection along with reduction or absence of edema and leucocytes infiltration of the submucosal layer. It is concluded that, the white membrane of pomegranate possesses antiulcer activity against aspirin-induced ulcers in rats and the antiulcer activity could be due to the presence of flavonoids, tannins, saponins as these compounds have well documented antiulcer activity.

KEYWORDS: Pomegranate, Gastric Ulcer, Rat

INTRODUCTION
Gastric ulcers are a serious problem in many parts of the World. The etiology of gastro duodenal ulcers is influenced by various factors and ulcers are worsened by inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory drugs, stress, hereditary predisposition and infection by Helicobacter pylori (1). Peptic ulcer is thought to result from an imbalance between acid-pepsin secretion and mucosal defense factors. Peptic ulcer is one of the major ailments affecting about 60% human adults and nearly 80% child population in tropical countries (2). The elucidation of the peptic ulcer disease of the stomach and duodenum has accelerated dramatically in the last 20 years due to the identification of several
techniques, which have facilitated the study of the gastric mucosa (4). Inflamed break in the lining of the stomach or the duodenum caused due to either increased acid production or damage to the mucus lining of the stomach leads to formation of peptic ulcer, a term that includes both gastric as well as duodenal ulcer (23). Ulcer healing requires angiogenesis in granulation tissue at the base of ulcer, together with reepithelization starting from ulcer margins and subsequent reestablishment of glandular architecture(27),(31). The pomegranate, *Punica granatum* L., an ancient, mystical, and highly distinctive fruit, is the predominant member of two species comprising the Puniceae family (18). The potential therapeutic properties of pomegranate are wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation. Other potential applications include infant brain ischemia, Alzheimer’s disease, male infertility, arthritis, and obesity (19).

Aim of the study is to investigate and compare the anti-ulcer effect of white membrane of pomegranate against Aspirin gastric ulcer induction.

**MATERIALS AND METHODS**

**Animals:**

This was an experimental study done on 18 male albino rats (weight 180 – 200 gram and 6 – 7 weeks aged). The animals were kept in experimental lab of the animals were maintained under optimal atmospheric and hygienic conditions, with food and water available *ad libitum* before the start of the experiment.

**Preparation of oral suspension of the white membrane of pomegranate:**

The white membranes were obtained from pomegranate fruit after collected washed with tap water and dried, then powdered using electrical blender. Ten grams of the fine powder were soaked in 150 ml of distal water in conical flask for 3 days. After 3 days the mixture was filtered using a fine muslin cloth followed by filter paper and distilled under reduced pressure in an rotary evaporator.

**Chemical analysis for the material :-**

1- Flavonoides test (2).
2- Saponin test (12).
3- Poly saccharides test (9).
4-Tanins test (14).
5-Triterpenoides test (13)

**Induction of gastric ulcer by aspirin:**

The rats fasted for 48 h before the experiment (10), but were allowed free access to drinking water up till 2 h before the experiment to empty the stomach from food. Gastric ulcer was induced by aspirin induction, the dose of aspirin was Aspirin 500mg / kg, suspended in 3ml of 10% ethanol for 3 days according to the method described by (8). After 5 days( the period of gastric ulcer induction), the animals were divided in to two groups 6 animal in each, the treatment group (T group) which administrated the oral suspension of the white membrane of pomegranate. The control group (C group) which administrated Histac drug, all the doses were administered by insulin syringes.

**Macroscopic evaluation:**

In each intervals of the study (3rd day, 7th day, 15th day of treatment), the rats were sacrificed with excess of anesthetic ether. The stomachs were opened along the greater curvature then washed under running water to see ulcers in the glandular portion of the stomach. The observations which recorded around the mucosa of stomach, congestion, redness and erosion as mild, moderate and sever according to the progress of the lesion.

**Histopathological evaluation**

The stomachs were immersed in 10% formalin solution for histopathological examination. These tissues were processed and embedded in paraffin wax. The central part of damaged or ulcerated tissue was cut off along the long diameter, sections of thickness of about 5 μm were cut and stained with haemotoxylin and eosin. These were examined under the microscope for histopathological changes such as congestion, hemorrhage, necrosis, inflammation, infiltration, erosion and ulcers.

**RESULTS**

**Chemical composition of the white membrane of Pomegranate:**

Table (1) explain the Chemical composition of the white membrane of Pomegranate

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Flavonoids</th>
<th>Saponin</th>
<th>Poly saccharides</th>
<th>Tanins</th>
<th>Triterpenoides</th>
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**Macroscopic Examination:**

The effect of the administration of oral suspension of white membrane of pomegranate on the gastric ulcer induced by aspirin, showed along the period of treatment mild ulcerregency
lesion in the epithelial lining of stomach such as hemorrhage and congestion, gastric mucosal layer also show mild degree of lesion in erosion and redness, while in the control group which administered Histac drug showed different degree of ulcer in the epithelial lining and gastric mucosal layer along the period of treatment averaged from sever to moderate and mild for the above categories. Fig(1,2)

**Microscopic Examination:**

In the present study, the oral suspension of white membrane of pomegranate for the treated group was given for the treatment of gastric ulcer after ingestion of Aspirin for three days. After 3 days of treatment, treated group animal show mild erosion sites with mild degenerative changes and congestion in mucosal surface and presence of few inflammatory cells, while control group which was given Histac drug for the treatment the gastric ulcer, showed sever erosion sites which infiltrated with sever inflammatory cells, and presence of hemorrhage and necrotic lesions. Fig.(3,4). After a week (seven days) on treatment with oral suspension of pomegranate, the site of erosion on mucosal layer, because superficial erosion with infiltration of lymphocytes and appearance of granulation tissue and new blood capillaries formation near the site sites of the erosion. In control group, the erosion still appear with moderate granulation tissue and moderate inflammation with infiltration of inflammatory cells (neutrophils, macrophage). Fig.(5, 6). The results showed throughout the period of study that treated group undergo reparative changes in mucosal surface layer with appearance of granulation tissue and complete healing of gastric ulcer with the progress of treatment (after 15th days on treatment). In control group animal showed ulcerative region.
which become still superficial with presence of granulation tissue, non complete healing of gastric ulcer till the end of study 15 days on treatment Fig. (7, 8 ), and presence of edema with infiltration of leukocytes in submucosal layer Fig.(9,10 ).

Fig.(3)T1 showed the ulcerative region in mucosal layer (arrows).10X.H&E stains.

Fig.(4)C1 showed the ulcerative region in mucosal layer (arrows).10X.H&E stains.

Fig (5 )T2 showed the ulcerative sites still appear superficial and presence of new epithelial layer(arrow).10x H&E stains

Fig.(6) C2,showed the ulcerative sites still appear deep (arrows)10x.H&E stains
DISCUSSION

Nowadays gastric ulcer is one of the most important concerns as a result of many factors. Because of poorly understanding the pathophysiology of this disease (20), it is necessary to develop more effective agents that are also less toxic, with medicinal plants being an attractive source for the development of new drugs because of their wide array of active ingredients (5). In accordance with the obtained results, aspirin has been reported to induce the gastric ulcer in rat, the present results that oral suspension of white membrane of pomegranate possess gastro protective property Which inhibit the formation of gastric ulcer lesion. Our findings have revealed protection of gastric mucosa and inhibition of leucocytes.
infiltration of gastric wall in rats pretreated with white membrane of pomegranate. (16) reported that teprenone exerts a protective effect against mucosal lesions through inhibition of neutrophil infiltration in the ulcerated gastric tissue while (21) demonstrated that the reduction of neutrophil infiltration into ulcerated gastric tissue promotes the healing of gastric ulcers in rats induced by Aspirin drug. According to table (1) which explained the chemical composition of white membrane of pomegranate which possesses chemical compounds such as saponins, tannin, flavonoids which considered and known as ulcer protective agents (1). Flavonoids strengthening the mucosal defense system through stimulation of gastric mucus secretion, (22) explained in their study that the leaf extract of *Avicenna officinal* in albino rat induced gastric ulcer by Aspirin has anti ulcer activity and this effect belonged to presence of Flavonoids in this plant extract which has anti ulcer properties. Tannins also play as anti ulcer compound which has astringent action and help precipitating micro protein on the ulcer site and then protect the underlying mucosa from irritation (1). The researcher (20) observed the antiulcer effects of pomegranate tannins in animal models. Pomegranate tannins play a protective role against gastric ulcer. It’s antiulcer effect is related to the increasing secretion of adherent mucus and free mucus from the stomach wall. (29) and (3) showed throughout their study, that flavonoid and tannin may promote mechanical barrier that protect stomach from ulcer formation and Facilities ulcer healing. All the histopathological examination of the stomach of the ulcerated animals which showed severe erosion of gastric mucosa, sub-mucosal edema and neutrophil infiltration were found to be normal in treated groups. Form above explanation, the results indicate that oral suspension of white membrane of pomegranate has beneficial effect on Aspirin gastric ulcer rats by reduction in ulcerative lesions, promote healing of gastric ulcer and anti ulcer properties.

**CONCLUSION**

It is worthy to note that the antiulcer capacity of pomegranate white membrane is three times higher than the histac drug. The results can provide an extra income and may contribute to have good nutritional values of this product.

**REFERENCES**


