Comparative study of the therapeutic effect of pomegranate alone or in combination with bone marrow mesenchymal stem cells on experimentally induced gastric ulcer in adult male rats. Histological and immunohistochemical study.

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Corresponding Author: Nahla El-Erakfy E-Azaz, Lecturer
Benha University Faculty of Medicine
Benha, EGYPT

Corresponding Author's Institution: Benha University Faculty of Medicine

Corresponding Author's Secondary Information:

First Author: Nahla El-Erakfy E-Azaz, Lecturer

First Author Secondary Information:

Order of Authors: Nahla El-Erakfy E-Azaz, Lecturer
Aisha E Mansf, MD
Abeer M El-Mahalawef, MD

Order of Authors Secondary Information:

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Abstract: Gastric ulcers are erosion of the gastric mucosa, which are the most prevalent gastrointestinal disorders. Pomegranate fruits have many beneficial therapeutic effects. Bone marrow mesenchymal stem cells (BMSCs) are a novel approach with promising therapeutic potential for curing human diseases. Objective: to evaluate the effect of pomegranate and BMSCs on experimentally induced gastric ulcer in adult male rats.

Materials and methods: Fifteen four adult male rats were divided into four groups. Group I (control). Group II (Gastric ulcer): Rats received 100% ethanol orifice of gastric tube. Group III (subgroup Ilia: gastric ulcer treated with pomegranate and BMSCs) & subgroup IIb: gastric ulcer treated with BMSCs only.

Results: Group IIb & IV showed various changes as deep erosion, marked damage of most of the cells. The presence of the inflammatory cells in gastric mucosa were supported by a significant increase of tumor necrosis factor α (TNF-α) expression and collagen fibers deposition (P<0.01). A significant decrease of Periodic acid Schiff (PAS) positive reaction and vascular endothelial growth factor (VEGF) expression (P<0.01). Subgroup IIb showed attenuation of some histological changes, while subgroup IIb & IIc showed more improvement of the histological and immunohistochemical changes described before.

Conclusion: BMSCs can heal experimentally induced gastric injury and protect the gastric tissue, but addition of pomegranate with BMSCs can field special efficiency
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<td>Amany Elshawarif, MD</td>
<td>head of Histology and Cell Biology, Ain Shams Faculty of Medicine, Egypt <a href="mailto:ElshawarifAmany@yahoo.com">ElshawarifAmany@yahoo.com</a></td>
</tr>
<tr>
<td>Soheir Kamal Ahmed, MD</td>
<td>professor, Ain Shams Faculty of Medicine, Egypt <a href="mailto:dr.soheirkamal@yahoo.com">dr.soheirkamal@yahoo.com</a></td>
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Comparative study of the therapeutic effect of pomegranate alone or in combination with bone marrow mesenchymal stem cells on experimentally induced gastric ulcer in adult male rats. Histological and immunohistochemical study.

Nahla El-Eraky El-Azab1* & Aisha E. Mansy2** & Abeer M. El-Mahalawy3*** & Dina Sabry4****

1,2,3Departments of Histology and Cell Biology, Benha Faculty of Medicine, Benha University, Egypt.
4Department of Medical Biochemistry and molecular biology, Faculty of Medicine, Cairo University, Cairo, Egypt.
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Nahla El-Eraky El-Azab1,2 & Aisha E. Mansy2* & Abeer M. El-Mahalaway3*** & Dina Sabry4****

1,2,3Departments of Histology and Cell Biology, Benha Faculty of Medicine, Benha University, Egypt.
2Department of Medical Biochemistry and molecular biology, Faculty of Medicine, Cairo University, Cairo, Egypt.

The corresponding author information:
Nahla El-Eraky El-Azab, MD, Department of Histology and Cell Biology, Faculty of Medicine, Benha University, Benha, Egypt.
Tel.: +20 102 763 6337, fax.: +013-3227518.
E-mail: eleraky_nahla@yahoo.com

Background: Gastric ulcers are erosion of the gastric mucosa, which are the most prevalent gastrointestinal disorders. Pomegranate fruits have many beneficial therapeutic effects. Bone marrow mesenchymal stem cells (BMSCs) are a novel approach with promising therapeutic potential for curing human diseases.

Objective: to evaluate the effect of pomegranate and BMSCs on experimentally induced gastric ulcer in adult male rats.

Materials and methods: Fifty four adult male rats were divided into four groups. Group I (Control). Group II (Gastric ulcer): Rats received 100% ethanol orally by gastric tube. Group (III) subdivided into 3 subgroups: subgroup IIIa: gastric ulcer treated with pomegranate & subgroup IIIb: gastric ulcer treated with BMSCs & subgroup IIIc: gastric ulcer treated with
pomegranate and BMSCs. Group IV (Untreated gastric ulcer). Fundus of stomach specimens were prepared and examined using histological and immunohistochemical techniques.

Results: Group II & IV showed various changes as deep erosion, marked damage of most of the cells. The presence of the inflammatory cells in gastric mucosa were supported by a significant increase of tumor necrosis factor α (TNF-α) expression and collagen fibers depositions (P<0.01). A significant decrease of Periodic acid Schiff (PAS) positive reaction and vascular endothelial growth factor (VEGF) expression (P<0.01). Subgroup IIIa showed attenuation of some histological changes, while subgroups IIIb & IIIc showed more improvement of the histological and immunohistochemical changes described before.

Conclusion: BMSCs can heal experimentally-induced gastric injury and protect the gastric tissue, but addition of pomegranate with BMSCs can yield special efficiency over the BMSCs therapy alone.

Key words: BMSCs, gastric ulcer, pomegranate, tumor necrosis factor -α, vascular endothelial growth factor.

Short title: pomegranate and stem cell on gastric ulcer.

Introduction

Peptic ulcers are the most prevalent gastrointestinal and global disorders and are included gastric and also duodenal ulcer. Gastric ulcer is known as an interruption of the continuity of the gastric or duodenal mucosa that extend towards the muscularis mucosa and may reach deeper into submucosa [1,2,3]. It can cause major complications such as bleeding, perforation and finally death if associated with decompensating of coexisting medical conditions [1,4].
Gastric ulcer are induced as result of combination of many harmful agents such as stress, smoking, ingesting non-steroidal anti-inflammatory drugs, corticosteroids, food ingredients. Moreover, the disruption of the homeostasis between the defensive (mucin, prostaglandin, bicarbonate, nitric oxide and growth factors) and the offensive factors (increased gastric secretion of pepsin and acid) and the most commonly by Helicobacter pylori (H. pylori) infection. It affects 10-15% of the population worldwide and its prevalence rate is associated with age and sex, as well as lifestyle [5,6].

The prevention and therapy of gastric ulcers are a medical defy. The approaches to the treatment of gastric ulcers include inhibition of gastric acid secretion by histamine receptor antagonists, antacids, proton pump inhibitors (PPIs), anticholinergic, moreover annihilation of H. pylori. However, all the current therapies are not always effective, have adverse effects, and are expensive with high recurrence rate of ulcer. For this reason, there is a need to search alternative drug sources or natural products which are more effective and safe against gastric ulcers [3,7]. Nowadays a specific attention has been directed to the employ of dietary antioxidants of natural products as a promising an alternative therapy and may have several health benefits [1]. The pomegranate (Punica granatum L.) is
vastly cultivated in Mediterranean countries as Turkey, Egypt, Tunisia, Spain, and Morocco [8,9]. It is consumed fresh, but also the other forms as juice, oil, and extract supplements can used [10]. Pomegranate is rich in antioxidant of polyphenolic class which includes tannins and anthocynins and flavonoids [8]. Its antioxidant capacity three times the antioxidant capacity of the same amount of green tea or red wine also the antioxidant capacity of pomegranate peel extract is 10 times higher than pulp extract [8,10,11,12]. Moreover, pomegranate is an valuable source of many vitamins as A, C, and E and folic acid. It also exerts antiatherogenic, anticarcinogenic, anti-inflammatory, antidiarrheal, antimicrobial effects and antidiabetic activities. In addition, the pomegranate has significant role on spermatogenesis and ovulation [8,10,13].

Mesenchymal stem cells (MSCs) provide a promising alternative approach for the curing of human disease. They primarily originate from the mesoderm and the ectoderm during early embryonic development and are present in several types of tissues and organs as bone marrow, fat, muscles, lungs, liver, pancreas, and synovial membrane [2,14,15]. MSCs are of great values on clinical proposal because of their easy isolation, multipotency, and high proliferative potential in vitro. They can not only work in the hematopoietic system but migrate into damaged tissues and organs and self-renew and differentiate into corresponding
cells. Moreover, MSCs have long-term storage without major loss of their potency [15,16].

The present study was conducted to evaluate the effect of pomegranate and bone marrow mesenchymal stem cells (BMSCs) on experimentally induced gastric ulcer in adult male rats.

Material and Methods
In this study, 54 adult male rats of average weight 180-200 g were used. Animals were settled in the animal research laboratory unit of Kasr Al-Ainy Faculty of Medicine, Cairo University. Firm nursing and cleaning procedures were employed to keep the animal in a typical well condition, the animals were settled in animal coop at room temperature (25±1°C), relative humidity (55±5) with 12h light/12h dark cycle, fed standard basal diet and water ad-libitum. Rats were acclimated to these conditions for two weeks before beginning the experiment. All morals rules for animal management were monitored. The experimental protocol was advised by the Institutional Animal Care Committee.

Used reagents
Absolute ethanol were obtained from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO, USA) respectively.

**Isolation and Labeling of MSCs**

Following strict aseptic procedures, BMSCs prepared and labeled with green fluorescent protein (GFP) (Promega, Madison, WI, USA) in stem cell research unit at the Biochemistry department of the Faculty of Medicine, Cairo University (Cairo, Egypt) according to the method described by Soleimani and Nadri 2009 and Niki et al., 2004 [17,18]. Cultured BMSCs were confirmed by morphology (fibroblast like cells) using an inverted microscope; Leica DM IL LED with camera Leica DFC295 and using fluorescence microscope for tracking of intravenous MSCs (Leica Microsystems CMS GmbH, Ernst-Leitz-Straße 17-37, Wetzlar, D-35578, Germany). Fluorescent analysis Cell Sorting (FACS) used for BMSCs which show negativity of CD34+ and positivity of CD105+ specific to MSCs.

**Pomegranate peel extracts preparation**
The peels of pomegranate fruits were separated manually and washed with excess water for removal of sugars and adhering materials from seeds. The peels were air dried for 48 hours in a ventilated oven at 40°C then ground in a grinder to a fine powder and passed through a 24- mesh sieve. Pomegranate peel extract was obtained by alcoholic extraction. Sample of 100 grams fine powdered was extracted with 800ml ethanol at room temperature for 24 hours in shaking water bath. The mixture filtered through a Whatman No. 2 filter paper for removal of peel particles. The extract were concentrated under reduced pressure in a rotatory evaporator then freeze-dried and stored at 4°C in refrigerator while waiting for use [19].

**Induction of gastric ulcer**

Gastric ulcer induced by administering intragastric 100% ethanol (1 ml/200 g) on the second day of starvation using an oral feeding stainless steel 20G, 1.5-inch needle. Only one dose of ethanol and after one hour of its administration the gastric ulcer was induced [20].