Review Article

Wound Healing Properties of Pomegranate

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Received: 25 July 2021; **Accepted:** 02 August 2021; **Published:** 05 August 2021

Citation: Valentina Stefanou, Dimitris Timbis, Anastasia Kanellou, Dimitra Margari, Myrto Trianti, Ioannis Tsaknis, Anastasia Azar Naka, Vladimiros Lougovois. Wound Healing Properties of Pomegranate. Archives of Microbiology and Immunology 5 (2021): 263-291.

Abstract

Pomegranate is rich in bioactive compounds such as anthocyanidins, proanthocyanidins, hydrolysable and non-hydrolysable tannins, flavanones, isoflavones, flavanols, flavones, phenolic acids and hydroxy-benzoic acids, with antioxidant, anti-microbial, anti-inflammatory, anti-biofilm, anti-quorum sensing and immunomodulatory properties. Wound healing is a complex process and at the same time in the wound aria is possible to exist microbes, biofilm, reactive oxygen radicals, and to occur quorum sensing, oxidative stress, inflammation, infection, while tissue repair functions are also taking place. Pomegranate through its' various bioactivities is getting involved in various biochemical paths and functions during wound healing process and contribute thus by all these ways into healing process.

Pomegranate juice, plant parts extracts and phytochemicals treatment accelerate wound healing process, increases wound contraction, reduces healing period, manages pain, enhances DNA, protein, hydroxyproline and collagen production, stimulates synthesis of type-1 procollagen and fibroblasts production and migration, leads to excellent epithelialization, neovascularization and granulation tissue organization. In diabetic patients, pomegranate treatment upregulates the levels of VEGF, EGF and TGF-β1 enhancing wound healing. Moreover, pomegranate reduces microbes count that may lead to infection, inhibits biofilm formation and attenuates preformed biofilms, inhibits quorum sensing and swimming motility that contribute to biofilm formation downregulating the expression of

specific bacteria related to swimming motility and quorum sensing. As a strong antioxidant, immunomodulatory and anti-inflammatory agent, pomegranate inhibits oxidative stress, downregulates NOS activity and NO production, inhibits the endothelial activation of genes which predispose to oxidation sensitivity, increases the levels of the antioxidant enzymes glutathione, glutathione peroxidase and catalase, reduces immune cells' count and stimulates keratinocytes proliferation that play a key role in immune defense against infection.

Is important to be mentioned that pomegranate treated effectively acute and chronic wounds such as surgical, incision, excision, burn, oral lichen planus, gingival, aphthous stomatitis, diabetic wounds and gastric ulcers giving results better or comparable to commercial medicines without any adverse effects, while antibiotics and corticosteroids cause side effects that may be severe. These results indicate that pomegranate could possibly be considered as an alternative, safe, multifunctional, wound healing agent.

Keywords: Pomegranate; Phytochemicals; Antioxidant; Anti-inflammatory; Antimicrobial; Immunomodulation; Wound; Biofilm; Quorum sensing; Infection

1. Introduction

Wound healing is a complex process involving antioxidant immunity and epithelialization that by the hemostasis, inflammatory, proliferative and maturation overlapping stages leads to the replacement of the damaged cellular structures and tissue layers [1, 2]. Various types of wounds exist, that can be categorized in two main groups, acute and chronic wounds. Acute wounds are caused due to external damage to the skin and include burns, bites, surgical wounds, abrasions, minor cuts and also severe traumatic wounds caused by gunshot, crush and lacerations Chronic wounds are mostly caused by endogenous mechanisms that are associated with predisposing conditions that compromise the dermal and epidermal integrity. Pathophysiological abnormalities that is possible to predispose to chronic wound formation such as foot ulcers, leg ulcers, pressure sores, include metabolic diseases such as diabetes mellitus, impaired arterial drainage and impaired arterial supply. Moreover, obesity, poor nutrition, smoking, advancing age and immunosuppression associated with diseases such as AIDS, medicine as chemotherapy or other medical treatment as radiation therapy, may also cause chronic ulceration [3,4]. Open wounds are colonized with bacteria that depending on their species, their count, the number of different species, the synergistic action among them and the immune system of the host, may cause infection and delay wound closure-yet in low levels bacteria are beneficial to the process. Patients with trauma, hypertension or arterial insufficiency, systemic diseases such as diabetes mellitus or rheumatoid arthritis have high bacterial invasion risk and often chronic wounds are formed. These wounds are characterized by inflammation [5]. Open wounds for a long time can be a favorable environment for tumor growth. Chronic inflammatory diseases such as oral lichen planus may also involve a risk of developing cancer [6,7].

Herbals that are containing flavonoids and tannins is mentioned to have very strong antimicrobial properties and to be very effective in wound healing [8-12]. Pomegranate is rich in polyphenolic compounds and has been used in traditional medicine of many cultures, for its' strong antioxidant, antimicrobial, anti-inflammatory and wound

healing properties [13]. In Chinese traditional medicine has been used as hemostatic, antiparasitic, anti-inflammatory and is referred also that has been used for wound treating [14]. In ancient Greece, pomegranate juice has been used for mouth ulcer treatment and in ancient Rome it is referred that it has been used to treat diarrhea and tapeworms. In Iranian traditional medicine it has been used to treat various inflammatory disorders, wounds, burn and edema [15, 16]. In ayurvedic medicine is also used as antiparasitic and for wound and ulcer treating [17].

2. Pomegranate Phytochemıcals

Pomegranate contains in all of its' plant parts various categories of bioactive compounds such as flavones, flavanols, flavanones, flavonols, isoflavones, anthocyanidins, proanthocyanidins, phenolic acids, hydroxy-benzoic acids, hydroxy-cinnamic acids, hydrolysable and non-hydrolysable tannins, complex polysacharides and alkaloids. In each plant part are contained different phytochemicals, or same in different concentrations [18].

Pomegranate peel is the 30-40% of the total fruit weight and is very rich in flavonoids as catechin, cyanidin, epicatechin, epigallocatechin 3-gallate, kaempferol, luteolin, naringin, pelargonidin, prodelphinidin, rutin, quercetin, flavan-3-ol and tannins as gallic acid, ellagic acid, casuarinin, methyl gallate, granatin A, granatin B, pedunculagin, punicalin, punicalagin. Pomegranate juice is rich in flavonoids as catechin, catechol, epicatechin, procyanidin, quercetin, pelargonidin 3-O-glucoside, isoquercetin, tannins such as gallic acid, ellagic acid and the alkaloids serotonin, melatonin and tryptamine. In the leaves are found the flavonoids apigenin, apigenin 4´-O-glucopyranoside, luteolin 7-O-glucoside, luteolin 3´-O-xylopyranoside, the tannins gallic acid, ellagic acid, punicalin,punicalagin, brevifolin, brevifolin carboxylic acid, corilagin and in the flowers are contained ellagic acid, gallic acid and also the triterpenes asiatic acid, oleanolic acid, maslinic acid and ursolic acid. Pomegranate seed is rich in punicic acid, linoleic acid, linolenic acid, palmitic acid, phytosterols, stearic acid, catalpic acid and eleostearic acid. The phytochemicals' concentration in pomegranate plant parts differ, depending on the plant variety, climate, ripening stage and storage conditions [2, 19-21].

Pomegranate compounds possess various bioactivities including antioxidant, antimicrobial, antiparasitic, anti-inflammatory, immune stimulating and balancing activities and in mixture as they exist in juice and extracts or isolated, through all these bioactivities, are involved in various biochemical paths and contribute by various ways to wound healing process while are also benefiting overall health [18, 22, 23].

3. Wound Microbiology

There are four basic conditions existing in open wounds that result from the level of the bioburden that is present in the wound aria: i) the bacterial contamination that is a normal short-lives state, ii) colonization, normal state, iii) critical colonization, abnormal state and iv) infection, abnormal state. The last two abnormal states are possible to disrupt the orderly healing sequence and lead thus to chronic wound development [24]. In fact, as chronic wound can be considered a wound arrested in the inflammatory phase that can't progress further and thus it fails to heal [25]. It is referred that chronicity in wounds begins when there is a persistent bacteria tissue level with particular endotoxin production that leads to prolonged elevation of proinflammatory cytokines such as tumor necrosis factor-a

and IL-1 [5]. Over than 90% of chronic wounds contain fungi and bacteria existing in biofilm construct, a membranous tissue that is a protective layer formed by extracellular polymeric substances where pathogens exist with subsequent modulation of their pathogenicity and virulence [26]. Biofilm is commonly found in diabetic foot ulcers, lower extremity arteriovenous ulcer, pressure ulcers and other chronic wounds. Biofilms' main characteristic is that they resist to antimicrobial agents (multidrug resistant), protect microorganisms and prevent wound healing. Up to 80% of infections are caused by biofilms and is referred that they may be 1000 times more resistant to antimicrobial agents than their planktonic counterparts [27-28].

Depending on the wound type and the clinical condition of the patient, different bacteria are present and their count may vary. There are bacteria that are present in both acute and chronic wounds such as the aerobic bacteria Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus spp., Staphylococcus aureus, Enterobacter cloacae and the anaerobic bacteria Peptostreptococcus sp., Clostridium sporogenes and Porphyromonas asaccharolytica. In acute wounds also may be found the aerobic microorganisms Bacillus sp., Candida parapsilosis and Candida krusei and the anaerobic bacteria Clostridium septicum, Veillonella spp., Prevotella sp. and Bacteroides ovatus. In chronic wounds may also be present the aerobic bacteria Enterobacter aerogenes and Citrobacter freundii and the anaerobic bacteria Clostridium difficile and Peptostreptococcus indolicus [3].

Each clinical condition and disease involves different biochemistry of the body (and the wound) and thus different bacteria are present. In diabetic foot wounds the predominant isolates are *Staphylococcus spp., Enterococcus spp., Peptostreptococcus spp. Prevotella spp., Bacteroides spp. and Streptococcus spp. In burn wounds, mostly are found Klebsiella spp., P. aeruginosa, Bacteroides spp., S. aureus and Peptostreptococcus spp.* Predominant isolates in post-appendectomy infected wounds may be *E. coli, Peptostreptococcus spp. and Bacteroides spp*. In infected postsurgical abdominal wounds are mostly found *E. coli, Clostridium spp., Peptostreptococcus spp., Bacteroides spp.* and in infected postthoracotomy sternal wounds are mainly present *Peptostreptococcus spp., S. aureus, coliforms* and *S. epidermidis*. Cuntaneous absences mainly contain *Peptostreptococcus spp., Bacteroides spp., S. aureus, Fusobacterium spp.* and *Clostridium spp.*, while children's' Cuntaneous absences contain *E. coli, Streptococcus spp., Bacteroides spp.* and *S. aureus*. The predominant isolates of traumatic wounds are *S. aureus, Prevotella spp., Clostridium spp., B. fragilis* group and *Peptostreptococcus spp*. In children's traumatic wounds specimens, were mainly found *Peptostreptococcus spp., S. aureus, Prevotella spp. Fusobacterium spp.*, and *B. fragilis* group.[3] Pseudomonas and Staphylococcus species are present in more than 70% of wounds. [5]

Wound bacteria colonization is changing over time. In chronic wounds, initially are found mostly gram-positive organisms, while in wounds having duration of several months there are found different pathogenic species including anaerobic flora. Aerobic gram-negative species as Pseudomonas spp. are also detected in wounds. These microbes even they do not tend to invade deeper tissues, they can cause severe wound deterioration as they produce tissue destroying enzymes and also due to adherence and antiphagocytic mechanisms and exo/endotoxins [5].
Must be mentioned that bacteria in low counts play important role in wound healing accelerating the process, as they permit a degree of inflammatory response -which is the first step of wound healing but in high levels wound bacteria

cause infection and harm the host. The progression of a wound to an infected state involves and depends on various microbial and host factors as the immune status and general health of the host, the species and count of microorganisms that co-exist, the type, size, depth and blood perfusion level of the wound. Successful wound healing is permitted if the bacteria counts are below 105 organisms per gram of tissue and also void of beta-haemolytic Streptococcus bacteria. Bacteria and exotoxins in high levels cause local inflammation, disrupt epithelialization and contraction and suppress macrophage-regulated fibroblast proliferation. For these reasons, wound infections' prevention and treatment are very crucial aspects of wound healing and antimicrobial agents play important role to that [5, 3, 24, 29].

4. Pomegranate's Antımıcrobıal, Antıquorum Sensıng and Antı-Bıofilm Actıvıty

Pomegranate polyphenols (tannins, phenolic acids and flavonoids, especially anthocyanins) have various biological functions including excellent effectiveness against various pathogenic microorganisms as it is mentioned in several reports. [30, 31, 32, 33] Mostly, the antimicrobial and anti-inflammatory as well properties are attributed to the high hydrolysable tannin content, especially punicalagin [34].

Through its' antimicrobial activity, pomegranate is very effective in preventing infection and aggregation of wounds and in the cases of ulcerogenic microbes it's also preventing ulcer formation by reducing the pathogens' count. For example, Helicobacter pylorus is an ulcerogenic bacteria species that causes duodenal ulcers and it is the primary cause of peptic ulcers in stomach lining and inflammation. *Streptococcus sanguis* is the main microbial factor that causes recurrent aphthous stomatitis. *Streptococcus oralis* and *Streptococcus mitis* are also mentioned as factors implicated in this pathogenesis [35]. Reducing the bacteria's' count, the risk of wound formation is decreased. In case ulcer already exists, pomegranate lowering bacteria's' count (that are ulcer's main reason of formation and conservation) prevents from inflammation, ulcer aggregation and infection. Several researches have shown that pomegranate is very effective in treating of acute and chronic wounds [36,18].

In our previous work we have presented in details the antibacterial activity of pomegranate extracts, juice and isolated compounds against various pathogens, as Aggregatibacter, Helicobacter, Acinetobacter, Bacillus, Porphyromonas, Clostridium, Escherichia coli, Cryptococcus, Citrobacter, Alcaligenes, Prevotella, Bacteroids, Cronobacter, Achromobacter, Prevotella, Pseudomonas, Proteus, Enterococcus, Yersinia, Treponema, Serratia, Lysteria, Klebsiella, Mycobacterium, Shigella, Streptococcus, Salmonella Staphylococcus and Vibrio, several times with results comparable or even better than those of commercial medicines' and it is very important to be mentioned that in contrast with antibiotics that have side effects which may sometimes be severe, pomegranate is safe, as safety tests showed in doses used [18].

Quorum sensing, is an interacting mechanism that bacteria use in order to communicate each other by secreting, sensing and then responding to small diffusible signal molecules. This communication ability makes bacteria to behave as a small community or a multicellular organism and provides significant benefits to bacteria in host colonization and biofilm formation. Moreover, it strengthens defense against competitors and facilitates adaption in

changing environments. Various quorum sensing activities are involved in the pathogenic potential and the virulence of bacteria [37].

As the biofilms are resistant to antimicrobial agents, and also are the reason of chronic inflammation in wound aria preventing from healing, it is crucial to be managed in order to treat chronic wounds. In various studies pomegranate is mentioned to have very good anti-biofilm activity [27, 38, 39].

In a study, the efficacy of pomegranate peel extract to inhibit quorum sensing in *Chromobacterium violaceum* (*C. violaceum* CV026), has been evaluated and the results showed that the extract inhibited by 78% the quorum sensing and the QS-controlled violacein production in *C. violaceum* CV026. Moreover, the quorum sensing associated biofilm formation was decreased while the bacteria growth rate was not affected indicating that the result was due to violacein secretion inhibition and not due to the inhibition of cell growth [40].

Pomegranate peel extract at concentration of 2mg/ml was found to inhibit significantly the biofilm formation showing a decrease of 81±3.6% in biofilm biomass of Yersinia enterocolitica while in lower concentrations, a concentration dependent decrease has been observed. It is noticeable that the extract inhibited the biofilm formation but did not inhibit the bacteria's cell growth, indicating that the biofilm inhibition action of the extract was not due to its' antimicrobial properties [40].

Another important factor in bacteria's biofilm formation is the extent of swimming motility as it is associated to the time needed for the development of biofilm architecture [41]. Examination of the anti-quorum sensing potential of the pomegranate peel extract against quorum sensing-dependent swimming motility in Yersinia enterocolitica, showed a dose- dependent decrease in the bacteria's swimming velocity with the maximum inhibition to be observed at the concentration of 2 mg/ml of the extract, that was the highest used in the experiment [40].

Pomegranate peel extract was found to decrease remarkably the expression of genes fliA, fleB and flhDC that are associated to virulence through motility in Yersinia enterocolitica and this occurred in a dose dependent manner. Moreover, the extract treatment downregulated the expression of the genes yenI and yenR which are involved in N-acylhomoserine lactones synthesis that is associated to biofilm formation [40, 42,43].

In a research, pomegranate's methanolic extract was found to inhibit the biofilm formation of Candida albicans, E. coli, Staphylococcus aureus and methicillin resistant S. aureus while incubation of the pomegranate extract with already formed biofilms attenuated the biofilms. The effects of pomegranate treatment on the architecture of the biofilms created by *Candida albicans, E. coli, Staphylococcus aureus* and methicillin resistant *S. aureus* were studied by confocal microscopy and results were compared to controls. It was seen that with the pomegranate extract treatment, the surface aria coverage and also the thickness of the biofilms formed by all microorganisms were markedly decreased. Besides, pomegranate extract inhibited germ tube formation that is one of the main virulence traits of *Candida albicans*. The ability of pomegranate methanolic extract to disrupt the pre-formed biofilms of the

microorganisms was found to be significant, with percentages of biofilm disruption to be ~70% for *E. coli, S. aureus* and methicillin resistant *S. aureus* and 90% for *C. albicans*. [39] It is also referred that ellagic acid in concentrations above 75 μg ml−1 inhibits the growth of all species and in concentrations lower than 40 μg ml−1 inhibits the biofilm formation [26, 39].

Periodontal diseases are chronic inflammatory conditions that affect the periodontal tissue, gradually damage the alveolar bone and can lead to tooth loss [44]. In *in vitro* study, monospecies and multispecies biofilms of the periodontal pathogens Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola and pomegranate juice that was diluted in with phosphate saline buffer in concentrations 12.5%, 25%, 50%, 100% were incubated for 1h, 6h and 24h and the optical density of biofilm mass has been measured. The positive control that has been used was biofilm treated with 0.2% chlorhexidine gluconate and the negative control was biofilm without pomegranate treatment. After pomegranate juice treatment the biofilm mass was found to be remarkably decreased compared to the negative control for all incubation times and all concentrations. The best results were observed in *Porphyromonas gingivalis* with 100% juice concentration and biofilm optical density to be 0.34 ± 0.03, *T. denticola* with juice concentration 25% and optical density 0.87 ±0.08, *A. actinomycetemcomitans* with juice concentration 50% and optical density 0.22 ± 0.01. Very good results were also given in the case of the multispecies biofilm, containing all three pathogens with the pomegranate juice to be in concentration 50% where the optical density was 0.09 ± 0.02. These results indicate the strong anti-biofilm activity of pomegranate juice and that possibly it could be used to prevent or treat periodontal diseases [45].

In another study, pomegranate juice that has been diluted into 5 concentrations with brain heart infusion broth (6.25%, 12.5%, 25%, 50%, 100%), was incubated with monospecies and multispecies biofilms of the oral pathogens *Streptococcus sanguinis* and *Fusobacterium nucleatum* and the biofilm mass was measured by microplate reader (490 nm) at 1, 3,6 and 24h. The results showed that at all incubation times and all juice concentrations there was remarkable biofilm reduction. The optimal inhibitory effect on *Fusobacterium nucleatum* has been achieved with pomegranate juice concentration 6.25% v/v, in 24h. For *Streptococcus sanguinis*, the best results were observed with concentration 100% after 6h incubation and for the multispecies biofilm the optimum results were given after 24h incubation with pomegranate juice 6.25% v/v. Phytochemical test revealed that the pomegranate juice was rich in phenolic compounds, flavonoids, tannins, saponin, triterpenoids, steroids and the significant biofilm inhibitory activity of the juice is attributed to these compounds [46].

In a study, the efficacy of punicalagin to inhibit Salmonella virulence factors and quorum sensing systems has been determined. The minimum inhibitory concentrations of punicalagin for various Salmonella strains were found to be 250-1000 μg/ml. Motility assays revealed that punicalagin at concentrations of 1/32 MIC and 1/16 MIC, reduced remarkably the bacterial swarming and swimming motility and this corresponded to the downregulation of the motility-related genes (fliY , fliA, fljB, fimD and flhC) in RT-PCR assays. It was also found that punicalagin downregulated the expression of the most of the selected genes that are involved in Salmonella virulence. Moreover, quorum sensing inhibition assay showed that punicalagin inhibited dose dependently violacein production by

Chromobacterium violaceum and also in Salmonella repressed the expression of genes related to quorum sensing (srgE and sdiA). These findings indicate that punicalagin can probably be used as an alternative agent in order to prevent Salmonella infection [47].

5. Pomegranate Accelerates Tissue Repaırment and Reduces Paın

Diabetes mellitus affects various parts of body and biochemical functions causing vascular, cellular, immune function, neuropathologic and biochemical abnormalities that affect and delay the wound healing process and can lead to chronic wound formation [48]. The Vascular Endothelial Growth Factor (VEGF), known also as vascular permeability factor (VPF), is an angiogenic factor and also a signaling protein which promotes the new blood vessels growth and stimulates their creation in case of injury. Overexpression and also low levels of VEGF may lead to various disorders such as vascular diseases, cancer, pulmonary emphysema. Diminished production of VEGF and decreased angiogenesis contribute into impaired tissue repair in diabetic wounds. It is reported that treatment of diabetic wounds with VEGF accelerates wound healing [49].

Transforming Growth Factor beta 1 (TGF-β1) is a peptide which belongs to cytokines' transforming growth beta superfamily and performs several cellular functions such as the control of cell proliferation, cell growth, cell differentiation, cell apoptosis. It plays important role in wound healing and repair, as through its' effect on mesenchymal cells it acts as a key regulator of remodeling and production of the extracellular matrix [50]. It is reported that TGF-β1 treatment of diabetic wounds leads to significant enhancement of wound healing [51].
Epidermal Growth Factor (EGF) is a protein which by binding to its' receptor (EGFR), it stimulates cell growth and differentiation. It accelerates wound healing significantly and is used to treat diabetic foot ulcers [52].

In a research, the wound healing properties of gel containing Saudi pomegranate peel methanolic extract (5% w/w) have been investigated in diabetic rats with excision wounds. Rats were divided into non-treated group, gel alone treated group and gel with pomegranate peel extract treated group. During entire study period, the rats were monitored for clinical signs, morbidity, body weight and mortality. The efficacy parameters that have been evaluated were hydroxyproline content, percentage of wound contraction, estimation of VEGF, TGF-β1 and Epidermal Growth Factor (EGF) in wound lysates. Nitric oxide (NO) and NO synthase have also been estimated in wound lysates. Moreover, skin histopathology study for inflammation, re-epithelization and neovascularization has been carried out. After 21 days treatment, the pomegranate gel, showed remarkable wound healing activity in comparison with the vehicle group. Pomegranate gel was found to enhance the excisional wound healing as it increased fibroblast proliferation, epithelization, granulation tissue, neovascularization and collagen deposition. Treatment and control group animals had no significant difference in body weight. The pomegranate extract gel's healing mechanism of diabetic injuries can be through its' role in upregulating hydroxyproline content, VEGF, EGP and TGF-β1 expressions in the wounded tissues. Throughout the treatment period, NO levels in pomegranate treated group were found to be remarkably lower than in vehicle group. NOS activity in the wound tissue treated with pomegranate gel were also markedly lower than in vehicle group and these findings indicate that pomegranate contributes into wound healing also by downregulation of NO production and NOS activity [53].

In a study, pomegranate peel polyphenol gel (30% polyphenol mass fraction) has been applied on cutaneous wounds of alloxan-induced diabetic rats for 21 days and histological characteristics and wound closure rates were determined in order to evaluate its' wound treatment efficacy. The results showed that the wound closure process was remarkably shortened in the pomegranate treated rats. Histological examination showed that the pomegranate peel polyphenol gel enhanced collagen regeneration, increased vascularization, fibroblast infiltration and epithelialization in the diabetic wound aria, indicating that probably pomegranate peel polyphenol gel could possibly consist an alternative beneficial medicine for treatment of wounds associated with diabetes [54].

In a study the wound healing efficacy of diethyl ether extracts of pomegranate (Punica granatum Linn) flowers and Malva sylvestris Linn flowers have been investigated on wounded diabetic male Wistar rats.6 groups of rats have been used. In group I, healthy wounded rats were administrated with simple ointment base. In group II (control group) diabetic rats were treated with simple ointment base. In groups III and IV there were diabetic rats which were treated each by one of the natural extracts (pomegranate or malva). In group V diabetic animals received simple base ointment containing a 1:1 mixture of the two extracts and in group VI diabetic animals were administrated with nitrofurazone, a standard drug. Histopathological studies showed that all the natural extract treated rats showed remarkable decrease in wound aria, compared to the control and compared to the nitrofurazone group. Among the natural extract groups the best results were given by pomegranate flower extract and malva flower extract as on day 18 the wounds were completely healed. On day 18, the wound arias of the animals treated with the 1:1 extract mixture and with nitrofurazone were 0.112 ± 0.033 cm2 and 0.711 ± 1.004 cm2 respectively. These results indicate that pomegranate flowers extracts can be used in order to manage diabetic wounds [16].

Recurrent aphthous stomatitis is a chronic oral mucosa inflammation characterized by recurrent ulcerations and is primarily affecting the non-keratinized mucosa [35]. There are various predisposing and risk factors that contribute in recurrent aphthous stomatitis pathogenesis such as microbial or immunologic factors, hematologic abnormalities, genetic predisposition, hormonal state, stress and trauma [55].

In a research, aphthous stomatitis patients with no systemic diseases and without any other medication, were treated with pomegranate muco-adhensive gel, with commercial oral paste and placebo gel and the results were compared among the groups. It was found that in the pomegranate gel group the pain relief time was significantly shorter than the placebo group and slightly shorter than the commercial oral paste group. Besides, the wound healing time was remarkably shorter than placebo and commercial paste group [8].

In a research, 20 recurrent aphthous stomatitis patients were treated daily with gel containing pomegranate extract 10%, while other 20 patients were treated with placebo gel. The results showed that there was remarkable difference between two groups in the mean time of complete healing (the period from day 0 to the day which the ulcer was completely healed without any scar and the fibrinopurulent membrane is disappeared). For the pomegranate treated group were needed for complete healing 5.3 ± 0.81 days and for the placebo group were needed 8.6 ± 0.99 days. The pain elimination time for pomegranate group was 3.4 ± 1.09 days and for placebo group 5.9 ± 0.6 days [55].

In another research, 28 recurrent aphthous stomatitis patients applied twice a day for a week pomegranate peel extract gel 10% while 28 other patients were treated with placebo gel. Pomegranate peel extract was found to be remarkably effective in decreasing the ulcer size, pain and the healing duration over the period of a week. These results indicate that pomegranate peel extract in oral gel form, could be used for aphthous ulcers' management [13]. The pomegranate varieties' Pleniflora, Sweet Alak and Saveh Black therapeutic effect on minor recurrent aphthous stomatitis has been evaluated and was found that the best results were given by Pleniflora variety as its' water and alcoholic extracts reduced significantly the pain, the complete treatment time and the patients who participated the experiment characterized the treatment meaningfully satisfactory [56].

Oral Lichen Planus (OLP) is a chronic inflammatory disease and is characterized by ulceration, mucosal erythema, alleviate symptoms and there is risk of cancer if it is not treated [57]. In a study, patients with atrophic OLP were divided into 3 groups which were treated with a commercial corticosteroid gel (control group), topical pomegranate seed extract gel and topical pomegranate peel extract gel. In each group, were checked before and after application the pain, the sign scores and the health impact profile (OHIP-14) [6]. OHIP-14 is a questionnaire that is used in several branches of dentistry and it is a reliable tool in order to measure oral health and to predict clinical deterioration in the patients [58]. There was remarkable decrease in OHIP-14 values in seed and peel group compared with corticosteroid group that happens probably due to high concentration of tannins and polyphenols in pomegranate, that enhance DNA, protein and collagen production. It is referred that the high concentration of these phytochemicals in pomegranate accelerate the wound healing as it is stimulating the production and movement of fibroblasts and enhances angiogenesis. Patients in the peel and seed groups had similar results in pain decrease which were also better than those of the commercial corticosteroid gel [6]. Corticosteroid therapy is important to be mentioned that has various side effects such as hyperglycemia, hypertension, hematologic, immunologic, while pomegranate has no side effects and also is benefiting these affected factors [59, 19].

In a study, ointments containing 2.5%, 5% and 10% of whole fruit pomegranate extract standardized with 40% ellagic acid, were topically administrated to Rattus norvegicus with deep second-degree burn wounds in order to evaluate their wound healing efficacy. In microscopic observation of the wound healing process on the collagen was seen that the ointment containing 10% of the extract gave the better results characterized by high density of collagen with a good arrangement and complete and mature epithelium, angiogenesis and low number of inflammatory cells. These results are probably due to phytochemicals with antioxidant, anti-bacterial and anti-inflammatory properties The pomegranate results were better than those of the silver sulfadiazine group, the positive control group, where were observed delayed and incomplete epithelialization, not very good collagen formation and retardation of wound contracture. More over must be mentioned that silver sulfadiazine causes some systemic complications as side effects, such as neutropenia, crystalluria, erythema sultiforme and methemoglobinemia [60]. It also causes allergic reactions, [61] pathogens growth, [62] ulcers, sores or white spots on the lips or in the mouth, stomach pains, unusual weakness and tiredness, [63] while pomegranate is found to have antiallergic and antimicrobial properties [64, 18] to treat mouth ulcers, gastroenterological problems and also it strengthens immune system and treats weakness and tiredness [65, 66, 67].

In another study, the wound healing efficacy of pomegranate peel extract on second-degree burn wounds on Wistar rats has been evaluated and compared to the 1% silver sulfadiazine healing impact on rats with second-degree burn wounds. After 21 days treatment the results showed that at the pomegranate group the average wound region was 0.21 ± 0.07cm2, much smaller than the silver sulfadiazine group with 1.15± 0.1cm2, while the average wound aria at the control group was 2.42± 0.2cm2. Antibacterial test showed that the Minimum Inhibitory Concentration (MIC) in pomegranate treated rats on *Escherichia coli, Pseudomonas aeruginosa* and *Staphyloccocus aureus* was 6.25, 12.5 and 6.25 mg•mL-1 respectively. Histological results showed that with both pomegranate and silver sulfadiazine treatment the inflammatory cells were remarkably reduced and exchanged by new granulation tissue while at the control group there was still high inflammatory cell infiltration [68].

Creams containing pomegranate flower extract 5% and 10% were administrated to Wistar rats with thermal burn injuries in order to evaluate the wound healing activity and the results were compared to those of 1% silver sulfadiazine cream. All formulations were administrated once daily until complete healing. It was found that the wounds' average size decrease on the 15th day of treatment was higher in the pomegranate treated groups than in in silver sulfadiazine group. The genesis of horizontally oriented collagen fibers having the appropriate strength and tension in the scar tissue that has been observed in both pomegranate flower treated groups (5 and 10%), was better than this in the silver sulfadiazine group, indicating that pomegranate flower extract could be used as an alternative drug in treating burn injuries [69].

In an *in vivo* study, the wound healing properties of standardized pomegranate rind extract powder (5 and 2.5%w/w) and its' major antioxidant compound, ellagic acid in equivalent amount as it exists in the extract (0.65 and 0.325%w/w) have been investigated in three different types of wounds, linear incision, excision and burn wounds of Wistar rats. Both the extract and ellagic acid in doses used were found to increase the tensile strength of the incision wound by a maximum of 35.43 and 31.82% respectively. The rind extract at 5 and 2.5% w/w was found to accelerate the wound contraction of the burn and excision wound, while the ellagic acid was effective only at 0.65% in these two wound models. Further assays showed that the rind extract enhanced collagen synthesis by a maximum of 21.83 mg/g and dose-dependently inhibited the neutrophil infiltration. The isolated ellagic acid on the other hand was not effective in increasing collagen accumulation and its' inhibitory properties on neutrophil infiltration were found milder [70]. The wound healing properties of the standardized rind extract were found superior to the isolated ellagic acid's properties indicating that synergic action among the extracts' constituents occurs.

In a research, the wound healing potential of pomegranate, myrrh, henna extracts and their blend ointments were assessed in excision and also dead space wound models in rats and the results were compared to those of a commercial gentamycin ointment. Each natural extract and their blend were used in ointment formulations with total concentration 15% w/w. Results showed that the percentage of wound contraction from days 16-20 was 93.55–100%, 88.35–99.52%, 85.90–98.5%, 97.30–100% and 90.25–100% for pomegranate, myrrh, henna, extracts blend and gentamycin respectively. Pomegranate seems to be more potent than myrrh and henna and comparable to commercial gentamycin in wound healing. The natural extracts' blend showed the highest efficacy among all natural

extracts indicating synergistic action and also gave better results than the commercial gentamycin. Moreover, the blended formulation shortened the epithelization period more than the other natural extracts and similarly to gentamicin formulation. Histological study of excision biopsy at day 24, showed healed skin structures with normal epithelization and fibrosis within the dermis in groups treated with natural extracts and gentamycin formulations. Besides, all formulations showed antimicrobial activities against Candida, Staphylococcus aureus and Escherichia coli [71]. The side effects that are referred for gentamycin are transient rise of creatinine, acute kidney injury that in some few cases are non-reversible, while pomegranate is found to be safe in the safety test that have been carried out [72, 18].

Methanolic extract of dried pomegranate peel containing 44% phenolic compounds (34.03% gallic acid and 3.31% catechin), has been used to form 2.5% and 5.0% w/w gels that were daily topically administrated for 15 days on excision wounds of Wistar rats and the wound healing activity was evaluated by measuring the skin contraction percentage and by collagen content estimation in terms of hydroxyproline content. The negative control did not get any gel treatment, the positive control got blank gel and there was also a group that was treated with silver sulfadiazine at a dose of 2.5 mg/day. The results showed that both 2.5% and 5% pomegranate gels gave better wound contraction results than the silver sulfadiazine as at day 9, the wound contraction was 58.42%, 69.42% and 48.52% for pomegranate 2.5%, 5.0% and silver sulfadiazine respectively. Negative control had 22.5% and positive 46%. In 15th day, the pomegranate 2.5, 5.0 and sulfadiazine groups had wound contraction 96.33%, 100.00% and 90.05%. Histopathological studies on 8th day showed that there was remarkable healing of the wounds of rats administrated with the extracts. The hydroxyproline amount was increased by twofold in the group that received the 5.0% pomegranate gel and the complete healing in this group was after 10 days, while in the 2.5% pomegranate gel group there was complete healing after 12 days and in the positive control group that received the blank gel, after 16-18 days [73].

In an *in vivo* study, ointment containing 5% w/w methanolic pomegranate peel extract was daily, topically administrated to guinea pigs with excision wounds, in order to evaluate its' wound healing efficacy. DNA and protein content of granulation tissues show the protein synthesis levels and cellular proliferation. The pomegranate ointment treated animals were found to have higher DNA and protein content and similar to cetrimide cream, compared with untreated controls indicating that pomegranate extract peel stimulates cellular proliferation. Histopathological study showed that by day 12, both pomegranate ointment and cetrimide cream have led to well-advanced granulation tissue organization and epithelialization. In both pomegranate and cetrimide treated animals, the original tissue regeneration was found to be markedly faster and the contraction of wounds was significant compared to controls, without any inflammation, oedema or congestion [74].

Eucerin cream containing pomegranate seed hydralcoholic extract (2%, 5%, 10%) and also purified extract 75% were topically administrated twice daily in wounded rabbits and the results were compared to those of non-treated group (control), eucerin ointment group (negative control), 1% phenytoin group (positive control) and 0.1% betamethasone ointment. The results showed that Eucerin cream containing 10% pomegranate extract had

comparable wound healing activity to the commercial phenytoin. Treatment with 10% pomegranate cream and phenytoin cream was found to lead to the optimum hydroxyproline formation, while in the other groups low effect on hydroxyproline constitution has been observed. In both 10% pomegranate and phenytoin groups, the highest tissue strength and resistance to stretching have been observed, compared to other groups. Betamethasone showed the least resistance to stretching. The healing time for the 10% pomegranate extract cream was 12 days while for Eucerin group was 21 days. Pomegranates wound healing efficacy is attributed to tannins, flavonoids, punicic acid and phytoestrogen [75]. While pomegranate has no side effects as safety tests have shown, betamethasone and phenytoin cause various adverse effects. Betamethasone ointment is referred to cause skin dryness, irritation, stinging, itching, small white or red bumps on the skin, unwanted hair growth, acne, redness, swelling, or other skin infection signs, severe rash, elevated blood pressure, tiredness, vertigo [18, 76, 77]. Phenytoin may cause various side effects and some of them are severe. Common side effects include hirsutism, gingival hyperplasia and coarsening of the facies. Rarer cutaneous adverse effects are purple-hand syndrome, drug-induced lupus, IgA bullous dermatosis and pigmentary alterations. Besides, it may cause generalized cutaneous eruptions such as Stevens-Johnson syndrome, maculopapular exanthem, toxic epidermal necrolysis, generalized exfoliative dermatitis, fixed-drug eruptions and vasculitis. It is also referred to alter mineral and vitamins levels. Moreover, phenytoin is associated with Pseudolymphoma or rarely malignant lymphoma and also with hypersensitivity syndrome that is manifested by lymphadenopathy, rash and fever [78].

In a study, 60 male Wistar rats with full-thickness incision wounds were divided into 5 groups of 12 rats each and they were topically administrated with hydralcoholic extract of top layer pomegranate peel, hydralcoholic extract of pulp, extract of peel and pulp, Eucerin (control group) and phenytoin. In all pomegranate and phenytoin treated groups, remarkable wound healing has been observed in comparison to the control group. Among all groups, the best wound healing results were given by pomegranate peel extract and phenytoin. Different plant parts, having different chemical composition, they also have different effects in wound healing. The peel top layer extract enhanced remarkably the wound healing process while the pulp was less effective Histological examination showed that the pomegranate peel extract accelerated the wound healing process by decreasing the immune cells' count and by accelerating the fibroblast migration to the wounded tissue. The peel and the pulp-peel extract (to a lesser extent) improved significantly the wound's visual attributes and also the general health of the rats [15].

The antioxidant activity and gingival wound healing efficacy of punicalagin and pomegranate extract alone and also combined with Zn(II) has been evaluated in a study and punicalagin was found to be superior antioxidant than the rind extract with IC50 DPPH 6.04±0.29 and 10 ± 0.44 for punicalagin and rind extract respectively and also for punicalagin with Zn(II) and rind extract with Zn(II) IC50 DPPH values were found to be 8.1±0.27 and 6.99±0.20 while for ascorbic acid (control) IC50 was 8.31±0.64. Zinc combination improved the antioxidant activity of the rind extract, yet no essential difference has been noticed in the case of punicalagin. The results showed that rind extract and punicalagin combined with Zn(II) (0.1mM) decreased markedly fibroblast viability, while 0.1 mM Zn(II) alone did not reduce viability. Fibroblast treatment with rind extract or punicalagin alone decreased cell viability dose dependently at high concentrations, yet in low concentrations neither punicalagin nor rind extract influenced it [34].

An open wound for long time may be an environment that is favoring growth of tumors, so wound healing is important. In research has been shown that pomegranate aqueous and lipophilic fractions facilitating skin repair, by different ways as aqueous fractions promote regeneration of dermis and lipophilic the regeneration of epidermis. On monolayers and human skin organ cultures and were evaluated for their wound healing ability aqueous fractions from pomegranate peel and fermented juice and also lipophilic fractions from pomegranate seeds. The seed oil's use on the monolayer cultures resulted in stimulating of the proliferation of keratinocytes which guided in mild thickening of epidermis and it didn't have any effect on the fibroblast function. The pomegranate peel extract mostly and lesser the fermented juice and seed cake extracts were found to stimulate the synthesis of type I procollagen. Besides they inhibited the production by dermal fibroblasts of matrix metalloproteinase-1 (MMP-1) interstitial collagenase. They didn't stimulate keratin proliferation [7].

Indomethacin is a non-steroidal anti-inflammatory drug with analgesic and antipyretic activity that induces gastric ulcers as side effect [79]. In a study, in order to evaluate the gastroprotective activity of aqueous methanolic pomegranate flowers' extract, Wistar rats were orally administrated with the extract at doses of 490 and 980 mg/kg bw and 1 hour later ulcers were induced with indomethacin, aspirin and alcohol. The extract was rich in tannins, flavonoids and saponin. The results showed that at both doses the extract reduced markedly the ulcer lesion index at ulcers caused by aspirin, indomethacin and alcohol. Total acidity also decreased. The pomegranates' gastroprotective properties are attributed to flavonoids, tannins and saponin. Tannins are referred to form complexes with polysaccharides and proteins and create thus a protective layer on epithelial tissues that prevents from bleeding and accelerates healing process [80, 36].

In another similar study, Wistar albino rats were orally pretreated with methanolic extracts of pomegranate peel of three Iranian cultivars (Poost-Sefid-Shirin, Tabestani Torsh, Poost Siyah Torsh), at doses of 25, 50 and 100 mg/kg for 15 days, and the treatment was followed by indomethacin administration (50mg/kg), in order to test the pomegranate extracts' antiulcerogenic activity. It was found that with the peel extract treatments at all doses the gastric mucosa was protected against the indomethacin induced damage, while in the control group the incidence of ulceration was 100%. The best results among all extracts were given by the Tabestani Torsh cultivar, at dosage of 50mg/kg, that inhibited significantly the peptic ulcer formation compared to the control group. Tabestani Torsh (50mg/kg) gave the lower ulcer index (5.4±0.55) and also reduced hemorrhage and the polymorphonuclear leucocytes infiltration that cause tissue damage during the inflammation precess. These results indicate that pomegranate peel extract and especially of Tabestani Torsh cultivar can be used for prevention of ulcers induced by indomethacin [81].

Pomegranate seed extract has been orally administrated in adult New Zealand rabbits with surgically caused full thickness skin wounds and the progress of epithelization and presence of infection were evaluated macroscopically. The dose used was 100 mg/kg body weight and the treatment lasted 20 days. The results showed that on the 5th, 10th, 15th and 20th days the average wound region was considerable decreased in the pomegranate treated animals contrasted to the control group. On the 20th day the wound contraction and epithelialization were higher in the

pomegranate group. All the pomegranate treated rabbits were found to have entire collagen amassment, granulation tissue creation and re-epithelialization in contrast to the controls. Inflammatory cell infiltration in the control group was higher than pomegranate group. Moreover, in the pomegranate treated groups there was increase of the antioxidant enzymes' glutathione and glutathione peroxidase levels and enhancement of catalase's activity has also been observed [82].

12 male albino Wistar rats with wounds on dorsal thoracic region (size 400 mm2) were divided into 3 groups of 4 rats each one. Group 1 rats were administrated with dried powder of pomegranate rind, group 2 with commercial antibiotic containing fusidic acid and group 3 with Vaseline. Pomegranate rind powder was found to exhibit highly significant activity in comparation with commercial medicine and Vaseline group. All rats of the groups 1, 2 and 3 were healed by 15, 17 and 21 days respectively. TLC analysis of the pomegranate rind extract revealed the presence of gallic acid, salicylic acid, rutin and quercetin and the significant wound healing activity may be because of the presence of these phenolics [83]. Fusidic acid in ointments and creams usually does not have side effects, except some few cases that skin irritation is reported, [84] yet its' efficacy in wound healing was lower than pomegranate's. In a research, methanolic pomegranate peel powder extract rich in polyphenolic compounds was combined with Manuka honey or lyophilized multiflora honey, bee venom and also with polyvinyl alcohol, in order to develop a new nanofibrous wound dressing. The formations were prepared at the following concentrations: (Manuka honey/peel extract/polyvinyl alcohol) ((10 %/1 %/12 %), (20 %/2 %/10.5 %) and (25 %/2.5 %/9.7 %), (Manuka honey/peel extract/bee venom/polyvinyl alcohol) ((25 %/2.5 %/0.01 %/9.7 %) and lyophilized multiflora honey/peel extract/bee venom/polyvinyl alcohol) (25 %/2.5 %/0.01 %/9.7 %). All formulas showed remarkable antibacterial activity against E. coli and S. aureus, while no cytotoxicity has been observed. In the in vivo wound healing study that has been carried out, the increased percentage of wound closure at the test groups compared to the negative control and also the histological examination showed that there was significant enhancement of healing process in all groups, with the Manuka honey/pomegranate/bee venom nanofibers to give the best results and to be considered thus as very promising alternative wound healing agent [85].

In a study, the wound healing efficacy of bio interactive membranes containing standard pomegranate extracts on surgical wounds of adult male Rattus norvegicus albinus Wistar rats has been evaluated and compared to the wound healing efficacy of simple gelatin-based membranes. 60 rats were divided into 3 groups of 20 rats each, the control group with undressed wounds, the group with wounds dressed by gelatin-based membranes and pomegranate extract gelatin-based membranes and healing process was observed in 3, 7. 14. 21 days. The standard extract, containing 41.67 mg/g ellagic acid and 32.24 mg/g gallic acid showed remarkable antioxidant activity with IC50 value to be 1.715 µg/mL. Significant differences in inflammatory response have been observed with pomegranate group to give the best results compared to others. In comparation with gelatin-based membranes, pomegranate membranes showed higher maximal tension and swelling index and also lower water vapor permeability. Besides, the pomegranate membrane group showed significant increase in wound contraction that the control groups. However, in the elongation and elastic modulus of both membrane types no difference has been observed. Pomegranate membranes compared to controls promoted important histological processes in the dynamics of wound healing, such as better

collagen deposition and arrangement, improvements in the granular tissue formation and also earlier cutaneous appendages development. These results indicate that biointeractive gelatin-based pomegranate extract membranes have a possible potential for use as wound healing agents [86].

6. Pomegranate Enhances Antıoxıdant Immunıty and Shows Antı-Inflammatory Actıvıty

Inflammation is the first stage of wound healing process and its' overall function is to destroy and neutralize toxic agents found at the site of the wound and restore the tissue homeostasis. Persistent inflammation though, may contribute to various diseases and disorders and thus is important to be treated [36].

During the wound healing process, oxidants play important role as they provide signaling and defense against microbes. Various inflammatory cells as neutrophils, fibroblasts, macrophages and endothelial cells generate big amounts of superoxide and its' derivatives through the phagocytic isoform of NADPH oxidases. Presence of big amounts of free radicals induces oxidative stress that is responsible for tissue damage and various health issues and thus antioxidants are essential in order to reduce levels of free radicals. It is referred that antioxidants enhance the healing process of infected and non-infected wounds as they reduce the damage caused by free radicals [87].

T-lymphocytes play important role in wound healing as they modulate the fibroblast activity and attenuate dermal scaring during normal healing process [88] the inflammatory levels, influence the differentiation and activation of T-lymphocyte populations. Reactive oxygen species (ROS) have a dual effect on the T-lymphocytes population. Exposure to high ROS levels reduces the T-lymphocyte activation and proliferation, yet intermediate oxidation levels are necessary for the activation, differentiation and also effector functions of lymphocytes. Pomegranate through its' antioxidant nature contributes into reducing inflammatory and prevents the decrease of T-lymphocyte proliferation and their inactivation [89].

Antioxidant supplementation improves immune responses and antioxidant pomegranate is found to be very effective in stimulating and also balancing the immune system, benefiting thus general health and contributing to body's homeostasis [23, 90, 22].

In a research, the effect of pomegranate juice on indomethacin gastric ulcer has been investigated. In the study were used 40 male albino rats that were divided into 4 groups of 10 rats which were treated with pomegranate, vehicle, pomegranate and indomethacin, vehicle and indomethacin. Group I, consisted of healthy rats treated with vehicle for 4 weeks, group II, consisted of healthy rats that consumed 0.5 ml of pomegranate juice (100mg/kg bw) daily for 4 weeks, group III, consisted of healthy rats that were treated with vehicle for 4 weeks and then was injected with indomethacin and group IV, consisted of healthy rats that were receiving 0.5 ml of pomegranate juice (100mg/kg bw) daily for 4 weeks and then was injected with indomethacin. Stomach paraoxonase activity and NO and plasma asymmetric dimethylarginine were determined. Under normal conditions, NO is produced by NOS from its' substrate-L-arginine that is metabolized and produce L-citrulline and amino acid. Yet under pathological conditions, L-arginine is possible to be involved in different pathway, which is catalyzed by protein arginine methyltransferase.

In the presence of proteins that contain methylated arginine residues, the activity of protein arginine methyltransferase leads to the formation of asymmetric dimethyl arginine and also of symmetric methyl arginine. The asymmetric dimethyl arginine is referred to act as an endogenous NOS inhibitor, and the inhibition of this enzyme leads to decrease NO production. The excessive accumulation of asymmetric dimethylarginine may reduce the bioavailability of NO in various cells and cause multiple systems' impairment, including gastrointestinal tract. Pomegranate juice administration was found to increase markedly paraoxonase's activity in the treated group in comparation with the indomethacin group and also reduced the levels of asymmetric dimethylarginine that was affecting NO. The antioxidant activity of the juice reduced oxygen radicals' production and release in the vascular wall, inhibited the endothelial activation of sensitive in oxidation genes and also improved the biological action of NO. Data comparation among the groups showed that pomegranate juice decreased ulcer index to 2.16±0.12 showing 69.4% prevention and offering thus remarkable protection against indomethacin induced gastric ulcers[79].

In another study, the role of pomegranate rind ethanolic extract has been investigated as antibacterial agent against 6 bacteria types (*Salmonella typhi*, *Pseudomonas aeruginosa*, *Staph aureus*, *Streptococcus spp.*, *E. coli* and *Klebsiella pneumonia*), as antifungal agent against 4 fungi types (*Aspergillus niger*, *Aspergillus valvas*, *Aspergillus fumigates* and Candida) and as wound healing agent in fungi infected incision wounds. 30 adult rabbits were divided into 3 groups, the control and the treated groups. The control group had clean wounds (that have been induced in aseptic conditions) and it was treated with the ethanolic extract of pomegranate rind. The second group wounds were fungi infected and were treated for 14 days with 0.5g of ointment formulated of the extract. The third group wounds were infected by fungi and were treated for 14 days with 0.5g lotion of the pomegranate extract. The results showed that the extract had remarkable antifungal and antibacterial activity against almost every tested fungi and bacteria. In all pomegranate treated groups, remarkable improvement in wound contraction percentage and healing has been observed. The percentage of wound healing on day 14 in control group (not infected wounds) was 100%, in second group (infected wounds treated with ointment) was 98 ±1.26 and in the third group (infected wounds treated with lotion was 94 ±3.12). Moreover, the pomegranate rind extract prevented remarkably oxidative stress by increasing the antioxidant enzymes' glutathione and catalase levels in serum [33].

In our previous work we have presented in detail the anti-inflammatory activity of pomegranate constituents, isolated or in mixture as they are found in extracts and juice where synergistic action may occur. The main pomegranate phytochemicals that possess strong anti-inflammatory properties are urolithins that are produced by gut microbiota while metabolizing ellagic acid and ellagitannins. Other compounds with potent anti-inflammatory activity are procyanidin, kaempferol, luteolin, gallic acid, granatin B, gallagyldilacton and melatonin. Phytochemicals and extracts were found to downregulate significantly the expression of pro-inflammatory cytokines IL-5, IL6, IL-10, IL-8, IL-18, IL-1β, IFN-γ and TNFα. Moreover, the levels of NF-κB, COX, NO, MPO and MMPs are significantly downregulated reducing thus the risk of chronic inflammation. The results of experiments where pomegranate's anti-inflammatory activity was investigated were in several cases similar to those of commercial medicines and sometimes were better. It is very important to mention that while commercial anti-inflammatory medicines as corticosteroids or non-steroidal anti-inflammatory drugs (NSAIDs) may cause various side effects,

pomegranate is safe and is benefiting functions and improving biochemical parameters that the commercial medicines are harming due to adverse effects [36].

7. Antibiotics' and Corticosteroids' Side Effects

The medication that is used in order to treat wounds, includes mostly antibiotics, antiseptics and corticosteroids. Generally, medications may be topical, oral or intravenous. Antibiotics and antiseptics are needed in order to kill the microbes that cause infection or slow their growth and thus prevent the wound infection induce or aggregation [91, 92, 93].

For superficial mild wounds topical antibiotics are used. For mild and moderate wound infections are used oral antibiotics. More severe wound infections are treated with initial parenteral antibiotics. Oral administration is also mentioned for prevention of wound infections [93, 94]

Antibiotics may cause various side effects that may be mild and reversible once medication treatment is over, or severe and possibly irreversible. Some of the side effects referred are stomach pain, gastrointestinal discomfort, nausea, diarrhea, rashes, fever, vomiting, anemia, arthropathies and allergic reactions [95]. Besides, antibiotics are affecting the beneficial gut microbiota, causing dysbiosis that is associated to various serious disorders and diseases[18]. Irreversible side effects include Stevens-Johnson syndrome, aminoglycoside-induced ototoxicity and toxicity secondary to nitrofurantoin. Lipid abnormalities, lipodystrophy, Impaired platelet aggregation, nephrotoxic effects, hepatitis, acute pancreatitis, clinical bleeding, neutropenia, eosinophilia, neuromuscular blockade, muscular tremors and myalgias are also referred to be antibiotic's side effects. The most common acute fatal adverse effects of antibiotics are fatal hepatitis necrosis secondary to trovafloxacin and hypersensitivity reactions that may lead to Stevens-Johnson syndrome or anaphylaxis [95].

Topical use of antibiotics affects mostly the aria that is applied and thus it is less possible to affect the whole body, yet is referred that topical antibiotics' use on clean surgical wounds has not been found to decrease the rate of infection and also it is possible to aggravate open wounds preventing the normal wound healing process. Frequently, the adverse effects of topical antibiotics are irritant in nature, with feeling of burning or stinging. Contact dermatitis is also mentioned as side effect [84, 96, 97, 98].

Short term use of corticosteroids may cause mild to severe adverse effects including cutaneous effects (acne, acanthosis nigricans, ecchymoses after minor trauma), truncal obesity, hypertension, myopathy, weakness, electrolyte abnormalities, pancreatitis, immunologic, neurophysiologic and hematologic effects. Moreover, corticosteroids during treatment may inhibit collagen synthesis and fibroblast growth as they are reducing hydroxyproline production and lead thus to deceased connecting tissue structural stability but this is reversible when the treatment stops. Long term use of corticosteroids may cause more severe side effects such as adrenal insufficiency, ophthalmologic, hepatic and gastrointestinal effects, aseptic joint necrosis, osteoporosis, growth suppression, hyperlipidemia and possibly congenital malformations [99].

Topical steroids that are mostly used for wound treatments, are referred to cause epidermal effects, such as epidermal thinning, melanocyte inhibition, dermal effects such as striae, easy rupture on trauma, blot hemorrhage, stellate scars, prematurely aged skin appearance and also combined epidermal and dermal effects such as atrophy, telangiectasia, striae, purpura, stellate pseudoscars, ulceration, easy bruising. Vascular effects are also referred such as fixed vasodilatation, rebound phenomenon, perioral dermatitis, facial erythema, Rosacea. Moreover, contact allergy can be induced and also ocular effects, decreasing healing of traumatic ulcers, exacerbation of herpetic ulcers and increased susceptibility to bacterial and fungal infections. Hypopigmentation, hyperpigmentation, steroid addiction, rebound flare (psoriasis), delayed wound healing, acneiform eruption, allergic contact dermatitis, alteration of fat distribution (Cushingoid appearance), steroid rebound, urticaria, miliaria are also referred as topical steroids' adverse effects [100].

8. Pomegranate Safety

Pomegranate extracts, in the doses needed to manifest medicinal properties and were used in traditional medicine, were found to be safe in safety tests that have been carried out [18, 74]. The oral lethal dose 50 (LD50) of pomegranate fruit extract standardized to 30% punicalagins was found to be higher than 5g/kg b w. Intraperitoneal LD50 in mice and rats was 187 and 217 mg/kg b w respectively. In a research Wistar rats were administrated daily by gavage with 0 (controls), 60, 240, 600 mg/kg bw of pomegranate extract standardized to 30% punicalagins. The treatment lasted for 90 days followed by recovery phase of 28 days. Compared to the controls, the extract treatment did not cause any important toxicological changes in the clinical observations, body weights, ophthalmic examinations, feed consumption, body weight gains, organ weights and clinical pathology evaluations. Serum and hematology parameters showed that there were within the normal laboratory limits statistically significant changes compared to controls, which are considered as biological variations and not as toxic effects. In the terminal necropsy were not observed any treatment related gross or histopathology findings. According to these results, the NOAEL (no observable adverse effect level) of the extract is 600mg/kg bw (which is the highest dosage that has been tested). It is also referred that daily oral administration individual weighting 60 kg with punicalagin at dose of 180 mg/kg for 90 days does not cause any side effects [101].

Safety test that has been carried out for pomegranate seed oil showed that there were not noteworthy results for 2g pomegranate seed oil/kg body mass. The cut off worth of LD50 could be considerate as higher than 5g/kg body mass. According to the assessment OECD 423, no marking or categorizing of oral toxicity is needed for the pomegranate seed oil. Punicic acid NOAEL was found to be 50,000 ppm, equivalent to 4.3g pomegranate seed oil/kg body mass/day [2].

In a safety test, female Balb/c mice were administrated by gavage for 22 days with pomegranate peel extract at doses of 0.5, 1.9 and 7.5 mg/kg and also was done a single intradermal injection of 224 mg/kg in one dose. Toxicological studies showed that there were no toxic effects in the epithelial cells layer of larynx, tongue and trachea. No behavioral and no side effects or mortality have been observed on the mice. Repeated extract administrations did not cause any local irritations or alterations of oral mucosa. These results show that pomegranate

peel extract has no toxicity and it is suggested to be used for its' medicinal properties [102].

9. Discussion

Pomegranate juice and plant part extracts (peel, rind, whole fruit, pulp, seed, flower) are found to possess excellent wound healing activity and this high efficacy is the result of the combination of the pomegranate's phytochemicals multiple bioactivities which allow them to get involved at the same time in various biochemical paths and functions that occur in wound aria, and accelerate by multiple ways the healing process. Pomegranate isolated compounds are also mentioned to enhance wound healing as well.

Extracts and phytochemicals are referred to benefit wound closure process in several types of acute and chronic wounds such as surgical, burn, excision, incision, gingival, diabetic, aphthous stomatitis, oral lichen planus wounds and gastric ulcers. Pomegranate extracts and phytochemical treatment accelerates significantly the wound healing process, increases the wound's contraction, shortens the healing duration, enhances DNA, protein, hydroxyproline and collagen production, stimulates type I procollagen synthesis and fibroblasts' production and migration to the wound tissue, leads to very good epithelialization and granulation tissue organization, high density of collagen with good arrangement - genesis of horizontally oriented collagen fibers with the appropriate strength and tension and neovascularization. Pomegranate lipophilic and aqueous fractions are facilitating skin repair by promoting the regeneration of epidermis and dermis respectively. Oral administration protects gastric mucosa and prevents gastric ulceration due to gastroprotective film that is formed by tannins and proteins / polysaccharides on epithelial tissues which prevents from bleeding and accelerates healing process. Moreover, pomegranate administration reduces pain and shortens the time needed for pain relief. The extracts are mentioned to give better results than isolated compounds indicating that synergic action occurs.

Pomegranate treatment of diabetic wounds enhances wound healing process by upregulating the Vascular Endothelial Growth Factor (VEGF), an angiogenic factor, Transforming Growth Factor (TGF-β1) that performs various cellular functions and plays important role in wound healing and the Epidermal Growth Factor (EGF) which also accelerates wound healing.

Apart from tissue regeneration processes, pomegranate phytochemicals are also contributing to the healing process through their antimicrobial properties, as they are highly effective against various microbes that are present in the wounds (acute and chronic) and reducing their count reduce the risk of infection development. Moreover, reducing the count of ulcerogenic bacteria the risk of ulcer formation is also decreased.

Except its' microbicidal activity, pomegranate affects microbes by different ways too, accelerating wound healing process. Over than 90% of chronic wounds contain microbes in biofilms, antibiotic resistant formations that protect bacteria setting them even 1000 times more resistant than their planktonic counterparts, cause chronic inflammation and delay wound healing. Pomegranate is very effective in inhibiting biofilm formation and also disrupts remarkably pre-formed biofilms, decreasing biofilm size, thickness and biomass. More over it is inhibiting quorum sensing and

bacterial swimming motility that are involved in biofilm formation and this is done through downregulation of specific genes of bacteria that are related to quorum sensing and swimming motility. More specifically, pomegranate treatment is found to decrease significantly the expression of genes associated to virulence through motility, genes involved in N-acylhomoserine lactones that are associated to biofilm formation, motility related genes, quorum sensing related genes. Pomegranate is referred to downregulate the expression of most genes involved in Salmonella virulence. Pomegranates anti-quorum sensing and anti-biofilm activity is referred to be achieved by gene expression downregulation mechanisms and not because of its' antimicrobial activity, as the cell growth and count at the experimental conditions were not affected.

Pomegranate also contributes into wound healing with its' anti-inflammatory, immune stimulating and regulatory properties through its' antioxidant activity. It reduces reactive oxygen radicals and inhibits oxidative stress by increasing the levels of the antioxidant enzymes catalase, glutathione and glutathione peroxidase or it is treating oxidative stress in case it already occurs. It is balancing NOS activity and downregulates NO production. Is also referred that pomegranate inhibits the endothelial activation of genes that predispose to a sensitivity in oxidation. Moreover, pomegranate treatment is found to decrease immune cells count. It is reducing leucocytes infiltration which causes tissue damage during inflammation process. It is also referred that seed oil is stimulating proliferation of keratinocytes that play important role in immune defense against infection.

This remarkable wound healing activity of pomegranate is attributed to its high content in several phytochemicals such as flavanols, flavones, flavanones, isoflavones, flavonols, proanthocyanidins, anthocyanidins, hydroxy-benzoic acids, phenolic acids, hydroxy-cinnamic acids, hydrolysable and non-hydrolysable tannins and alkaloids that possess various bioactivities. Pomegranate in the most cases gave better or comparable results to the commercial medicines, without showing any side effects while antibiotics and corticosteroids cause various side effects that in some cases may be severe.

It is very important to mention that the healthy condition of an organism can be considered as a complex equilibrium among various factors, biochemical parameters and functions and what exactly determines health is not the presence or absence of any factor, parameter or function but their co-existence in an equilibrium that proceeds the functionality of the organism. Inflammatory responses are normal part of wound healing process, yet if inflammation is too excessive, the wound repair is prolonged and this may lead to chronic inflammation that is associated with various disorders and diseases. Oxidants play important role in the wound healing process as they provide signaling and defense against microorganisms; however in high concentrations they cause damage to host cells and may induce or aggravate severe diseases. Pomegranate constituents possessing strong antioxidant, anti-inflammatory, immunostimulant and immunomodulatory properties are involved in these biochemical paths contributing to the wound healing process overall.

Pomegranate is referred various times as a regulator. Immunoregulator, and also regulator in other functions and parameters such as lipids in blood or diabetes.[19,23,59] An agent that is affecting the pathological condition and

not affecting the normal healthy condition. Regulation is superior to simply changing any parameter's value towards any desirable direction, as in the first case there is a tendency to achieve or is achieved the balance needed for homeostasis maintenance and in the second case, parameters' values may change towards desirable direction, yet as there is no "sense" and tendency towards the healthy balanced condition the change may be not beneficial while, depending on dosage, the change may be excessive, leading again to abnormalities while a normal condition can also be affected and disturbed as well.

It is a hypothesis, that as the wound healing process is a complex process with directly and indirectly interdependent (because they consist a functional system) biochemical processes, functions, paths and parameters, it possibly could be managed more sufficiently by agents with more than one bioactivity, or even with more than one compounds possessing different bioactivities (as the functions that occur are also more than one and they differ each other)
The use of a simple one compound with one or limited bioactivities, possibly can affect towards the desired direction a function and parameters associated with it, but as the functions are interdependent, it is possible some other functions consequently to be disturbed and that could possibly be manifested as side effects. Yet the opposite is not valid. Pomegranate indeed doesn't have any side effects but other natural products and extracts do.
Further experiments and investigation could show if this hypothesis is true, or in the case it's not, provide us with important information that can be useful in determination on how things really occur.

10. Conclusion

Pomegranate constituents in extracts or isolated with their various activities, playing the role of immunomodulatory, tissue repair, anti-oxidant, anti-microbial, anti-inflammatory, anti-quorum sensing, anti-biofilm formation agent and also acting as gene expression modulators, are getting involved in various biochemical paths and functions that occur during the wound healing process (and in fact consist healing process), accelerating thus the treatment. Pomegranate juice, plant part extracts and phytochemicals were found to be effective in healing of both acute and chronic wounds such as burn, surgical, incision, excision, gingival, oral lichen planus, aphthous stomatitis, diabetic wounds and gastric ulcers. It is important to be mentioned that pomegranate extracts and phytochemicals, have given most of the times better or comparable to commercial medicines results, without showing any side effects while corticosteroids and antibiotics cause adverse effects that may be severe. These results indicate that pomegranate can be considered as an alternative, very effective and safe wound healing agent.

11. Sources of Funding: None

12. Conflicts of Interest: None.

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