

Effects of the Nutraceutical, Punicic Acid

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Bedel, *et al.*: Pharmacology of Punicic Acid

Plant extracts and nutraceuticals are the most ancient and widespread form of medication employed by the general population. The pomegranate is a prehistoric, mystical and a highly differentiated fruit. Moreover, pomegranate is found in some medicinal systems as a cure for a variety of illnesses. Pomegranate has been used for a long time for nutraceutical purposes. Current research indicates that the most medicinally useful pomegranate components include ellagitannins, anthocyanins, anthocyanidins, flavonoids, estrogenic flavonols and flavones. Also pomegranate seed oil contains 64-83% puniceic acid. Therefore, this review focussed on the effects of puniceic acid, particularly those that have been reported such as the anticarcinogenic, antioxidant, antiinflammatory and antidiabetic effects. As nutraceuticals appear to play a major role in the prophylaxis of various diseases, puniceic acid could be an important and phytoconstituent among these agents. Nutraceuticals are generally regarded as safe to use with lower incidence of side effects. In spite of all these reports it is obvious that there is a clear need for more clinical studies.

Key words: Puniceic acid, pomegranate seed oil, nutraceutical, therapeutic uses

Plant extracts and nutraceuticals are the most ancient and widespread form of medication employed by the general population^[1]. Phytochemicals, especially antioxidants from vegetables and fruits, have achieved popularity because many epidemiological studies have demonstrated their prophylactic effects against distinct chronic conditions including cancer and cardiovascular diseases. Throughout the last decade, there has been a significantly increased use of dietary supplements such as vitamins, herbals and medicinal foods in the general population^[2].

The pomegranate is a prehistoric, mystical and highly different fruit. Moreover pomegranate is found in some medicinal systems as a cure for a variety of ailments^[3] and has been used for a long time for nutraceutical purposes. Current researches seem to demonstrate that the most medicinal useful pomegranate components are ellagitannins, anthocyanins, anthocyanidins, flavonoids, estrogenic flavonols and flavones^[4]. Studies on pomegranate's benefits have risen remarkably in the previous decade. A PubMed search in Dec 2016 retrieved 1468 studies about pomegranate, 879 of which have been published within the last 5 y. The number of publications concerning puniceic acid (PA) was found to be 68.

Pomegranate seeds contain different components in addition to polyphenols, which may contribute to pomegranate's useful effects^[5]. Pomegranate seed oil (PSO) contains 12-20% of whole seed mass. Recently, PSO has received significant dietary attention. The oil's possible useful effects have been attributed to its major bioactive constituent, PA, a conjugated linolenic acid (CLA), which constituted 64-83% of PSO^[6-8]. Conjugated fatty acid (CFA) is the general term of positional and geometric isomers of polyunsaturated fatty acids with conjugated double bonds. It has been reported that CLA exhibited antiinflammatory, antiatherosclerotic, antiobesity, antitumor and antihypertensive effects^[9-13].

Although the effects of PA on body have been well known, there is only little data about the metabolism of PA. Tsuzuki *et al.* reported that PA is quietly absorbed in rodent intestine. It has been shown that PA can be converted into cis 9, trans 11-18:2 in various

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rat tissues, such as liver, adipose tissue, plasma and brain^[14,15]. Also, Yuan *et al.* have shown that PA can be incorporated into plasma and erythrocytes and a part of PA can also be metabolized into cis 9, trans 11-18:2 in humans. The mechanism of *in vivo* transformation of PA into cis 9, trans 11-18:2 is not precisely known^[16-20].

Patel *et al.* suggested that oral application of pomegranate extract at levels up to 600 mg/kg/d did not produce any side effects in rats of either sex^[21]. In animal studies it has been reported that plasma levels of PA is increased adequately by dietary PA. No toxicity such as mutagenicity was observed in rats bred with PSO for 28 d (0 to 150 000 ppm, which resulted in a mean intake of 0 to 14 214 mg/kg/d). The level for no observable adverse effect was 4.3 g PSO/kg/d in rats^[22]. It was demonstrated that there was no adverse effect in patients treated with PSO capsules during 12 w in a prospective, placebo-controlled, randomized, double-blind trial in women with menopausal symptoms^[23].

Recently, the number of reports on PA has increased. Actually, a new effect was reported by our group, PA exerted nitric oxide-mediated vasodilatory effect in aortic arteries of the rat^[24]. Therefore, in this review we mention about effects of PA, particularly antioxidant, antiinflammatory, antidiabetic and anticarcinogenic effects (fig. 1).

Antioxidant and antiinflammatory effects of PA:

It is well known that oxidative stress plays a considerable role in the pathogenesis of various diseases. There were many clinical and experimental studies on the antioxidant effect of PA. Schubert *et al.* have demonstrated that fermented pomegranate juice and PSO have antioxidant activity^[25]. Regarding the CLA compounds, Saha *et al.* reported that PA possessed hydroxyl radical scavenging activity, reducing properties and metal chelation particularly demonstrated by the trans isomer. Also, they reported that PA was a potent antioxidant agent, caused reduction in lipid peroxidation and scavenged free radicals in arsenic-induced toxicity^[26].

Saha *et al.* suggested that PA has shown higher hypocholesterolemic and antiinflammatory activity due to high cis ingredients^[27]. Moreover, Bialonska *et al.* reported that urolithins, metabolites of PA, exhibited a significant antioxidant activity and growth of human gut bacteria^[28]. Also, in an experimental animal study, Binyamin *et al.* proposed that nano-drop formulation of PSO might be considered for the treatment of demyelinating diseases due to its antioxidant effect^[29]. It has been proposed that consumption of large amounts of CFA by the Asian and Middle East populations appears to be associated with the lower incidence of

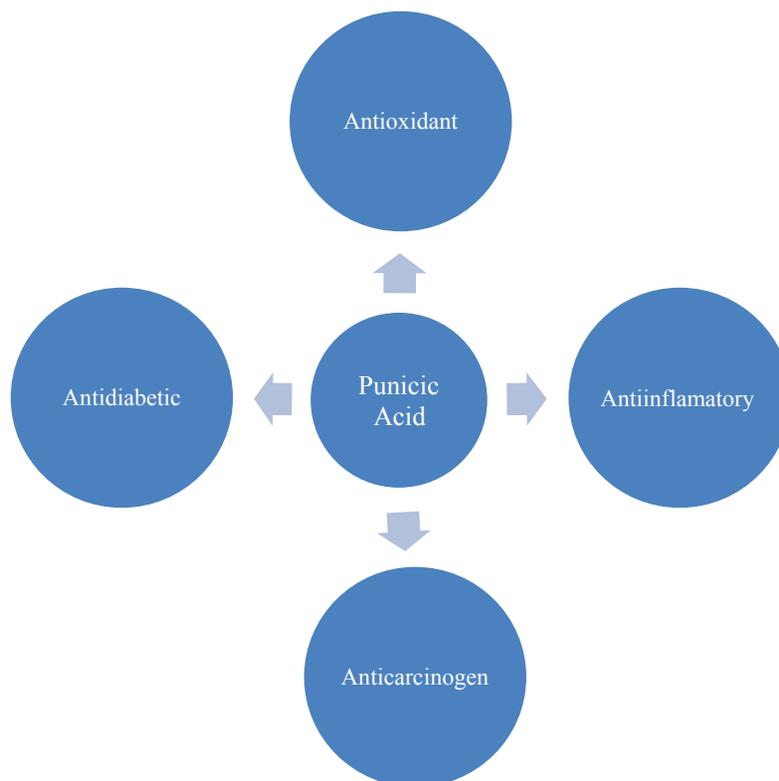


Fig. 1: Effects of punicic acid

inflammatory diseases^[30]. Recent studies indicate that PA has a strong antiinflammatory effect through inhibition of tissue necrosis factor α (TNF- α)-induced elevation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and ameliorates colitis in an experimental rat model^[30]. Likewise, Bassaganya-Riera *et al.* provided *in vivo* molecular proof that PA ameliorated experimental inflammatory bowel disease by modulating T-cell and macrophage function through peroxisome proliferator activated receptors (PPAR- γ) and δ -dependent mechanisms^[31]. It was known that PPAR- γ and δ have potent inhibitor effects in intestinal inflammation in dextran sodium sulfate colitis^[32]. It was demonstrated that in interleukin-10 (IL-10) knockout mice PA ameliorated dextran sodium sulphate-induced inflammatory colitis and spontaneous panenteritis.

Necrotizing enterocolitis is the main reason of mortality and morbidity in infants and the aetiology is unknown. It was demonstrated that, administration of PA ameliorated intestinal injury in a rat necrotizing enterocolitis model^[33]. Also Caplan *et al.* demonstrated that PA reduced the rate of necrotizing enterocolitis and inflammatory intestinal diseases in the rat^[34]. Also in this model a recent study showed that PSO protected against necrotizing enterocolitis. It was suggested that this effect of PSO might be due to antiinflammatory effects and recovery of epithelial homeostasis by decreasing TNF- α , IL-6 and IL-8 levels. In addition they postulated that PA has considerable useful effects on integrity of gastrointestinal mucosa. In this study it was believed that PA is very important nutritional factor in the future^[35]. Also cold pressed PSO inhibited both cyclooxygenase and lipoxygenase enzymes *in vitro*^[25].

PA AND DIABETES MELLITUS

Type 2 diabetes mellitus is related to defects in insulin release or lipid and glucose metabolisms^[36]. Prevention and treatment of diabetes are among the priority issues. Because of fruit extracts are safe, natural and readily available they have been used broadly in this manner. Also, there are a lot of traditional medicine that have been used for their useful effects in distinct diseases^[37].

As mentioned above, CFAs are striking molecules because of their health benefits in various inflammatory diseases. Recent investigations suggest that oral administration of PSO to mice with dietary PSO improved insulin resistance and high-fat-diet-induced obesity^[38,39]. Vroegrijk *et al.* also showed that body weights were decreased by reducing fat mass in PSO fed animals during 12 w. But they did not find

insulin concentration or a lower fasted glucose in these animals^[38]. Likewise, in another study Nekooeian *et al.* found that PSO ameliorated insulin secretion without changing fasting blood glucose in diabetic animals^[40]. Similarly, it was demonstrated that PA diminished fasting glucose levels and ameliorated glucose normalizing capability^[41]. Koba *et al.* found that PSO dose dependently decreased the perirenal adipose tissue weight at the end of 4 w of feeding with PSO. They also demonstrated that PA diminished hepatic triglyceride concentration and fatty tissue weights in mice, which may be partially related to an increase of fatty acid \hat{a} -oxidation activity^[42]. But some reported data on the effect of PSO on lipid metabolism and concentration have been controversial^[43,44]. Contrary to what has been documented above, Nekooeian *et al.* found that PSO did not decrease blood glucose but enhanced insulin levels and this effect occurred despite any change in blood glucose levels. The mechanism of PSO-induced enhancement in insulin levels is not known. Also in that study it was shown that PSO did not alter lipid peroxidation but diminished the diabetes related oxidant stress. Finally, this study demonstrated that PSO ameliorated insulin secretion without altering fasting glucose levels^[40]. However, Yamasaki *et al.* demonstrated that administration of PSO (0.1 and 1.0% as PA) did not show significant effects on the epididymal and perirenal adipose tissue weight in male mice^[44]. It was found that 4 w treatment with PSO diminished cholesterol, high-density lipoprotein (HDL)-cholesterol ratio and triglyceride concentration compared to the placebo group in a placebo-controlled, double-blind, clinical trial. They also showed no beneficial correlation between PSO consumption and insulin profiles^[44]. Moreover, PSO was demonstrated to have an antioxidant activity and useful effects on lipid profile of dyslipidemic patients^[25,45]. Arao *et al.* demonstrated that feeding of obese Otsuka Long-Evans Tokushima fatty (OLETF) rats with dietary 1% PSO did not affect abdominal adipose tissue weights or blood lipid levels when compared to the normal diet group. But, hepatic triglyceride levels were diminished significantly^[43]. In contrast to this study, Aroa *et al.* suggested that, dietary PSO rich in 9c, 11t, 13c-CLA reduced hepatic triglycerol accumulation in hyperlipidemic OLETF rats^[43]. In another study 1% dietary supplementation with PSO improved glucose tolerance and decreased inflammation in db/db mice^[41].

PA is reported to possess PPAR γ agonist property. TNF- α mediated insulin resistance played a significant role in

the pathogenesis of diabetes mellitus and is associated with severe mitochondrial impairment. It was shown that PA improved glucose uptake, reactive oxygen species accumulation, mitochondrial biogenesis and energetics in TNF- α treated cell. In addition, treatment with PA was found to ameliorate TNF- α -induced alterations in proteins associated with mitochondrial dynamics like mitochondrial fission 1 protein and optic atrophy type 1. These findings suggest that PA can be considered as an active lead for the management of insulin resistance and associated mitochondrial dysfunctions^[46]. Furthermore, PA was shown to inhibit the expression of TNF- α and the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and upregulate PPAR- α - and γ -responsive genes in the tissues controlling glucose homeostasis in mice. Also in mice model it has been shown that PA activated PPAR γ , enhanced PPAR γ -responsive gene expression to ameliorate diabetes and inflammation activation^[41]. In a clinical trial, Asghari *et al.* suggested that treatment with PSO in dyslipidemic subjects did not affect serum TNF- α content^[47].

In vitro studies reported by Anusree *et al.* found that PA upregulated glucose transporter type 4 (GLUT 4) expression and translocation in adipocytes enhanced adiponectin secretion. They suggested that PA is a potent nutraceutical agent and should be encouraged for use both as a therapeutic and prophylactic agent in diabetes mellitus^[48].

Despite these controversial findings there are many evidences that PSO and PA could be useful in treatment of diabetes mellitus. Therefore, it would be of great interest to further investigate the pomegranate seed extracts for potential antidiabetic activity. However, to clarify beneficial effect of PA and its action mechanisms there is a need for further studies.

PA AND CANCER

There is an increase in the use of nutraceuticals in cancer treatment in last decade. Studies about use of PA in cancer are present. Anticancer effects of molecules present in pomegranate juice were investigated in early studies. Dikmen *et al.* showed the antiproliferative and apoptotic effects of pomegranate juice in breast cancer^[49,50]. There are different types of fatty acids such as long chain polyunsaturated fatty acids known to have potential anticancer activity^[51,52]. PA, a good example for this type of fatty acids, is under investigation for cancer treatment. In a study PA has inhibited breast cancer cell proliferation through its lipid peroxidation

properties and also by affecting the protein kinase C pathway^[53]. Rocha *et al.* found that pomegranate ingredients including PA inhibit both proliferation of cell lines and secretion of proinflammatory cytokines^[54].

PSO has been found to inhibit invasion and proliferation of different cell lines of human cancer. For example, the cytotoxic effects of pomegranate CLA on the MCF-7 mammary cancer cells together with an increased apoptosis of the MDA-MB-435 human breast cancer cells have been recently reported^[55]. The effect of PA on both oestrogen sensitive (MDA-ERa7) and insensitive breast cancer cell line (MDA-MB-231) was investigated by Grossmann *et al.* They found that PA inhibited MDA-ERa7 and MDA-MB-231 cell growth with a percentage of 96% and 92%, respectively, compared to control cells. Furthermore, PA produced apoptotic effect in both cell lines and impaired cellular mitochondrial membrane potential. The breast cancer inhibitor features of PA are related to the protein kinase C pathway and lipid peroxidation^[53].

Costantini *et al.* showed that PA remarkably inhibited cell viability in different breast cell lines (MCF-7 and MDA-MB-231) but not cancer cell lines of liver and colon^[56]. Toi *et al.* previously showed, PSO and fermented juice polyphenols to retard oxidation and prostaglandin synthesis, to inhibit breast cancer cell proliferation and invasion and to promote breast cancer cell apoptosis. They also firstly showed an antiangiogenic potential of pomegranate fractions^[57]. PA decreased tumour activity in mice skin cancer cells and played the role of a preventive agent by inhibiting ornithine decarboxylase enzyme, which is active in mouse skin cancer cell lines^[58].

PA has also been studied in colon and prostate cancers. PA diminished incidence of chemically induced F344 rat colon carcinogenesis and expression of PPARc in colon mucosa, not dose dependently^[59]. It is well known that prostate cancer is the second reason of cancer deaths in man. It is necessary to find less toxic but more effective therapeutic agents in the treatment of prostate cancer. Various oil acids including PA inhibit the expression of prostate specific antigen and steroid 5R-reductase type and dihydrotestosterone-induced androgen receptor nuclear accumulation. Also, they showed that PA stimulated DNA fragmentation and internal apoptotic activity through caspase-dependent pathway^[60]. Recently, there has been a renewed push to identify natural remedies such as pomegranate extract to fight prostate cancer^[61]. It was shown

that pomegranate extracts have pro-apoptotic and antiproliferative effects via stimulation of apoptosis and cell cycle arrest^[62,63]. A considerable amount of evidence has shown that pomegranate extracts could suppress the growth of human prostate cancer cell lines *in vitro*. Useful effect of pomegranate extracts on the entire different prostate cancer cell lines have been demonstrated in *in vitro* studies. Many studies showed that pomegranate extracts inhibit proliferation, invasion through Matrigel and induced apoptosis of LNCaP, PC3 and DU145 prostate cancer cells^[64].

More recently, pomegranate extracts has been shown to inhibit testosterone and dihydrotestosterone levels in LNCaP and prostate cancer cell lines (22RV1)^[65]. Also, *in vivo* studies showed that pomegranate extracts inhibit prostate cancer development and progression, possibly via targeting inhibition of PI3K/Akt/mTOR signalling pathways in transgenic mouse model for prostate cancer^[66]. Also, PA may suppress prostate cancer cell invasion through blocking the arachidonic acid metabolism pathway in metastatic cell lines^[67].

Anticarcinogenic properties of PSO could be related to its antiangiogenic activities and inhibition of prostaglandin synthesis. Also, dietary PSO was also shown to diminish growth of human prostate cancer LNCaP and DU 145 cells and to significantly reduce the invasiveness of the PC-3 cell line^[64]. Wang *et al.* have been suggested that pomegranate extracts including PA inhibit prostate cancer metastasis through targeting the CXCL12/CXCR4/AKT signalling axis^[68]. In recent years it has been demonstrated that combined administration of pomegranate extracts including luteolin, ellagic acid and PA have an increased anticancer activity. Anticancer activity of PA is mediated by decreasing cell migration and CXCL12 chemotaxis, increasing cell adhesion, inhibiting epithelial-mesenchymal transition and inhibiting angiogenesis and proliferation^[69]. In a 2 y phase II clinical trial on prostate cancer patients, pomegranate juice consumption normalized prostate specific antigen^[70]. More recently, another phase II clinical trial showed positive effects of PA in patients with prostate cancer^[71].

Role of PA has also been investigated in metastatic prostate cancer. Pomegranate prevents metastasis by decreasing cell migration, increasing cell adhesion. But PA shows its antimetastatic activity in different mechanisms. Hyaluronan is mostly localized in the solid tumour's stroma, stimulating tumour invasion,

cell migration and metastasis^[72]. Antimetastatic effect of PA and pomegranate extract occurs via targeting hyaluronan signalling pathways in prostate cancer cells. Cytokines and chemokine's can be mentioned among other possible mechanisms. PA inhibits this pathway and exhibits useful effects^[69,73].

From the above documented information, it can be concluded that nutraceuticals have a significant role in the prevention of various ailments and PA in particular is an important constituent with great potential. These agents are safe to use and show low incidence of side effects. However, more clinical studies are necessary to realise their true potential.

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