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POMEGRANATE SEED OIL: A COMPREHENSIVE REVIEW ON ITS THERAPEUTIC EFFECTS

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ABSTRACT: Pomegranate, *Punica granatum* L. (Punicaceae), as a medicinal and nutritional ancient fruit, has an outstanding medical history throughout the world. Each of compartments of pomegranate has interesting pharmacological activity. Juice, leaf, flower, and peels of pomegranate possess potent antioxidant properties, while juice, peel and oil are all weakly estrogenic activity. Pomegranate seeds have ethnomedical indication and high conjugated α-linolenic acids (CLn) contents. Pomegranate seed oil (PSO) with high amount of punicic acid (PA), a conjugated isomer of α-linolenic acid, has variety of pharmacological properties. The main properties are as follows; antioxidant, anti-inflammatory, nephroprotective, hepatoprotective, neuroprotective, anti-cancer, enhancing the immune system, enhancing carbohydrate metabolism and reducing insulin resistance. Based on some studies, effects of PSO on lipid profile are controversial and consistency of data is rare to find yet. Therefore, this review is aimed to highlight the PSO's composition and beneficial effects on human health and represent the mechanisms involved in its action.

INTRODUCTION: Natural products as an alternative source of medicinal compounds are interested in the researchers` viewpoint. Nowadays, the use and investigation about medicinal plants as curative and preventive agents against diseases are increasing because of their popularity, low adverse effects, easy to earn and safety. Some important drugs such as digoxine, quinine and vinca alkaloids are formulated from herbal medicines [1-3].

Plants produce chemical compounds with various effects as a part of their normal metabolic activities. About 120 active compounds currently extracted from the medicinal plants.

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All aspects of traditional plants and their close mechanisms are not determined completely but many beneficial effects of them have been proven. Awareness about these characteristics of plants leads the researchers to discover the new dosage forms with best quality and minimum adverse effects [2, 4, 5].

In this review, we try to evaluate and collect the information about chemical compositions, mechanisms, efficacy, adverse effects, benefits and toxicity of pomegranate seed oil (PSO) as a vintage of pomegranate.

Pomegranate:

Pomegranate (*Punica granatum* L.), from family Punicaceae, has been traditionally used for thousands of years as a medicinal fruit **Fig.1**. Mediterranean regions (including Iran, India and Pakistan) have the highest rate of pomegranate cultivation in the world [6]. Based on excavations of the Early Bronze Age (3500–2000 BC), it is

believed that the pomegranate was one of the first cultivated fruits for its beneficial properties. Pomegranate was held sacred by many of the world's major religions and nations. In the Holy Quran, pomegranate has been described as a paradise fruit and a symbol of insurgence and life everlasting in Christian art. In China, pomegranate is widely represented in art symbolizing fertility, posterity, abundance, numerous and virtuous offspring, and a blessed future. It was also a symbol of invincibility in battle by the Persians[7-9]. For over 4,000 years, human beings have culti vated pomegranate for its medicinal properties. Juice, seeds, leaves, flowers, bark and roots of pomegranate have various effects. Lowering fever, treating diabetes, anthelmintic, anti-diarrhea, blood tonic, stopping the bleeding, and healing ulcers are the most important traditional uses of pomegranate [10-14].

FIG. 1: POMEGRANATE FRUIT

Chemical Composition and Pharmacological Properties:

The parts of the fruits of pomegranate have different amounts of chemical compounds including vitamins, polysaccharides, minerals, polyphenols, and carbohydrates that are the most abundant ingredients in pomegranate [15, 16]. Important pharmacological components of pomegranate are summarized in **Table 1**. High antioxidant capacity of pomegranate besides its anti-invasive, anti-proliferative and pro-apoptotic features have been studied in many human and animal models [15, 17-20]. Moreover, phytoestrogenic compounds isolated from seeds, juice and peels of the fruit have many hormonal activity [21, 22]. Treatment of stomach-ache, inhibiting herpes and influenza viruses and suppressing the reproduction of cancer cells are the other pharmacological characteristics of pomegranate juice and seeds extracts. Antimicrobial, anti-inflammatory, protective effects against liver disease, cardiovascular protection, anti-diabetic and anti-obesity effects of pomegranate have been investigated and documented by many researchers [11, 23-26]. The presence of different substances with various chemical structures reveals multiple therapeutic effects of pomegranate.

TABLE 1: MOST IMPORTANT PHARMACOLOGIC COMPOUNDS OF *PUNICA GRANATUM L.* [10, 11, 27-29]

Chemical class	Compound(s) name	Part(s) of the plant
Simple sugars	Glucose, Fructose, Sucrose	Juice
Aliphatic organic acids	Citric acid, Malic acid, Tartaric acid	Juice
Hydroxybenzoic acids	Gallic acid, Ellagic acid, 3,3_-Di-*O*-methylellagic acid	Juice, Leaf,Flower, Seed
Flavan-3-ols	Flavan-3-ol, Catechin, Epicatechin	Juice, Leaf
Flavonol glycosides	Kaempferol 3-*O*-glycoside, Kaempferol 3-*O*-rhamnoglycoside	Leaf
Anthocyanidins	Delphinidin, Cyanidin	Leaf
Ellagitannins	Punicalin, Punicalagin, Corilagin	Leaf
Amino acids	Proline, Valine, Methionine	Juice
Conjugated fatty acids	Punicic acid	Seed
Non-conjugated fattyacids	Linoleic acid, Oleic acid, Palmitic acid	Seed
Sterols	Daucosterol, Camesterol	Seed
Sex steroids	17-_-Estradiol, Testosterone	Seed
Phenyl aliphaticglycosides	Icariside D1	Seed

Pomegranate Seed Oil (PSO):

Mainly, 12–20% of total seed weight is made by PSO. Conjugated octadecatrienoic fatty acids are responsible for approximately 80% of seed contents and PSO is considered to be a rich source of those fatty acids; in particular, punicic acid(PA) (*cis*9, *trans*11, *cis*13 acid)which is the main fatty acid among them. Other isomers of conjugated linolenic acids (CLnAs) are catalpic acid (C18:3-9*trans*,11*trans*,13*cis*) and α-eleostearic acid (C18:3-9*cis*, 11*trans*, 13*trans*) but the oil content of the seed and fatty acid composition are affected by

cultivation sites, harvesting time, fruit genotypes and climatic conditions [15, 30, 31]. Total lipids in PSO comprise mainly of triglycerides (TG) that their composition is varied and the most important templates are CLnA-CLnA-P and CLnA-CLnA-CLnA[32]. Lipid profile of PSO is different due to its cultivar, environmental growth conditions and ripening stage but the common and important fatty acids found in PSO in various types of pomegranate have been listed in **Table 2** [33, 34].

Also, **Table 3** shows the different fatty acid composition of PSO in the Iranian strains of pomegranate [35]. In addition to fatty acids, minor components of the oil including steroids, sterols, and, cerebroside (a key component of mammalian myelin sheaths), lignins, hydroxycinnamic acids, and the potent antioxidant lignin derivatives are found in PSO. PSO is abundant of phytosterols such as β-sitosterol, campesterol, stigmasterol and tocopherols such as α and γ-tocopherol. [32, 36, 37].

TABLE 2: COMMON AND IMPORTANT FATTY ACIDS FOUND IN PSO IN VARIOUS TYPES OF POMEGRANATE. RESULTS ARE EXPRESSED AS AVERAGE (PERCENTAGE OF TOTAL FATTY ACIDS) ± STANDARD DEVIATION [33, 34].

Fatty Acids	India Strain	China Strain	Turkey Strain
16:0	5.7 ± 4.1	5.07 ± 1.30	2.45 ± 0.19
18:0	2.1 ± 3.1	4.20 ± 1.56	1.52 ± 0.26
18:1 (ω-9)	9.0 ± 5.6	7.86 ± 2.25	4.19 ± 0.61
18:2 (ω-6)	10.8 ± 6.9	8.36 ± 2.36	4.49 ± 0.49
18:3 (9*c*11*t*13*t*)		10.70 ± 4.44	6.41 ± 0.27
18:3 (9*t*11*t*13*t*)		8.78 ± 5.16	1.03 ± 0.16
18:3 (9*t*11*t*13*c*)		15.24 ± 6.17	3.48 ± 0.34
18:3 (9*c*11*t*13*c*)	71.5 ± 17.9	36.98 ± 10.12	74.11 ± 1.55

TABLE 3: FATTY ACID COMPOSITION OF PSO IN IRANIAN STRAINS OF POMEGRANATE [35].

Variety	Fatty acid (%)					
	18:3	18:2	18:1	18:0	16:0	Others
Red Seed Ardestani	79.43	6.95	6.62	2.09	3.16	1.72
Rizdavar`sDorpaye	79.22	6.87	6.30	2.50	3.36	1.73
Ashkzar`s Sour Narak	82.40	5.22	5.71	1.99	2.95	1.70
Taft`s Tokhm-e Mush	78.25	6.59	7.48	2.54	3.41	1.70
Chatrud`s Sour Shahi	78.73	7.08	6.68	2.16	3.57	1.75

Punicic acid:

PA is a conjugated α-linolenic acid (CLnA) molecule, which is found in PSO and it may contribute to a lot of health benefits associated with pomegranate. PA contains a third double bond, is known as *cis*9, *trans*11, *cis*13, and is referred to as 18:3 fatty acids. It is an omega-5 long chain polyunsaturated fatty acid and a positional and geometric isomer of α-linolenic acid (LnA; C18:3-9*c*, 12*c*, 15*c*) **Fig. 2** [38-40].

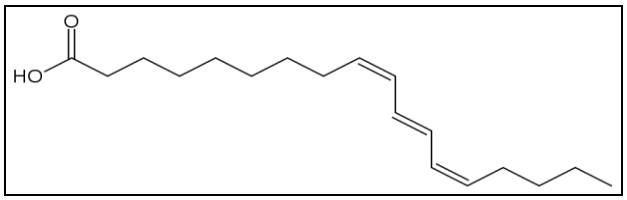

FIG.2: CHEMICAL STRUCTURE OF PUNICIC ACID

PA features *cis and trans*-type double bonds with parentage of 2:1. Numerous studies have shown that conjugated linoleic acids (CLAs) and α-linolenic acids (LnAs) have abundant health benefits and PA has a very high structural similarity with them [41, 42]. Anti-inflammatory, immunomodulatory, anti-cancer, anti-estrogen and beneficial effects on lipid profile are examples of beneficial effects of PA that revealed in many studies [15, 43-45].

Pharmacological actions of PSO:

PSO has been reported to have beneficial effects including cytotoxic and antitumor properties, body fat-reducing and lipid metabolism-normalizing effects, enhancing the immune system *in vivo*, Showing chemo preventive activity against hormone-related (prostate and breast) and colon cancers, reducing the accumulation of hepatic triglycerides and promoting epidermal tissue regeneration [46-50]. Antioxidant and anti-inflammatory activities are the main features of PSO which results from inhibition of lipid peroxidation and neutrophil-activation [37, 39, 51]. Effects of PSO on body weight and serum lipid profile are inconsistent and many studies confirmed

weight reducing effects but the effects of PSO on reducing in serum triglyceride, phospholipid, cholesterol, and LDL-cholesterol levels are contradictory [40, 52]. PSO reduces body weight, leptin and insulin levels, enhances glucose tolerance, improves peripheral insulin sensitivity, increases carbohydrate oxidative capacity, and inhibits the progression of type2 diabetes [31, 53].

PSO is opulent of CLnAs which consists of octadecatrienoic fatty acid isomers with three conjugated double bonds (C18:3). Beneficial health effects of PSO are due to the high level of its CLnAs [30, 54]. The exact mechanisms of action of CLnAs are not completely understood but probable and definite mechanisms are summarized in **Table 4.**

TABLE 4: CLINICAL EFFECTS AND MECHANISMS OF CLnAs

Effect(s)	Mechanism(s)	Reference(s)
Antitumor and anticancer	Induce apoptosis through lipid peroxidation and protein kinase C pathway,	55
	Act as selective estrogen receptor modulators and inhibit estrogen receptors α and β	22
Anti-diabetic	Improve insulin sensitivity,	31
	Suppress NF-κB and TNF-α activation and up regulate PPAR α and γ-responsive genes	53
Reductions in hepatic and plasma TG levels	Increase the levels of PPAR-γ and α mRNA,	33
	Suppress the delta-9 desaturation	56
Antioxidant	Enhance the levels of the antioxidant enzymes,	42, 57
	Reduce lipid peroxidation, oxidative stress	
Anti-inflammatory	Free radical addition to one of the conjugated double bonds of CLnAs	39, 58
	Inhibit TNFα-induced priming of ROS production and MPO,	
	Up regulate colonic PPAR-δ expression,	
	Increase the levels of IL-17 and IFN-γ,	
	Modulates mucosal immune responses,	
	Reducing the expression of TNF-α and IL-6	

NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, **TNF-α:** Tumor necrosis factor-α, **PPAR:** Peroxisome proliferator-activated receptor, **CLnAs:** conjugated linolenic acid, **ROS:** Reactive oxygen species, **MPO:** Myeloperoxidase, **IL:** Interleukin, **IFN:** Interferon

Pharmacological studies of PSO:

Anti-inflammatory activity:

Inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) by PSO have been shown in Schubert study [59]. The results of Jiang *et al.* study showed that, inflammation and its important mediators such as prostaglandin E_2 (PGE_2) have a key role in progression of diseases such as cancer and vascular heart disease. γ- tocopherol in PSO can inhibit PGE_2 formation. They showed that, PGE_2 synthesis in both IL-1β- treated A549 human epithelial cell and lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages was reduced with γ-tocopherol with IC_{50} of 4 and 7.5μM, respectively. γ- tocopherol and its metabolite (γ t, 2,7,8-trimethyl - 2 - (beta-carboxyethyl)-6-hydroxy chroman) can inhibit PGE_2 formation after the explosion for 1 hr to COX-2- preinduced cells followed by addition of arachidonic acid. Also, suppression of inducible nitric oxide synthase expression was reduced by γ-tocopherol [60].

Enhancing B-cell function in vivo by PA was shown in Yamasaki *et al*. study. Diets containing 0%, 0.12%, or 1.2% of PSO was treated to mice for 3 weeks. After this period amounts of immunoglobulin G and M were larger than control group in splenocytes isolated from mice [43]. Ellagic acid is another polyphenolic compound with potent antioxidant and anti-inflammatory activity. Ellagic acid inhibited proliferation and migration of platelet-derived growth factor- BBand it can inhibit IL-1β- and tumor necrosis factor-α (TNF-α)-induced activation of activator protein-1 (AP1) and mitogen-activated protein kinases (MAPK).

In addition, ellagic acid inhibited proliferation and migration of monocyte chemo attractant protein-1 production which have a key role in inflammation [61]. Gallicacid is a polyphenolic compound with anti-inflammatory properties which is found in PSO. Madlener *et al*. showed that gallic acid can inhibit COX-1 and COX-2 in humanHL-60promyelocyticleukemiacells with IC_{50} values of 3.5 and 4.4nM [62]. About 80% w/w of hydrophilic

fraction of PSO is PA. Costantini *et al*. evaluated the anti-inflammatory effect of the aqueous methanolic extract of PSO on some human cell lines.

They showed that the levels of vascular endothelial growth factor and nine pro-inflammatory cytokines was reduced after treating with PSO, dose-dependently [63]. TNF-α is a proinflammatory cytokine that rises in many inflammatory conditions. The expression of NF-kB and AP-1 has been increased in the PSO's effect. The effects of PSO and PA on serum TNF-α concentration is controversial. PSO administration (800mg PSO/day for 4 weeks) to hyperlipidemic patients had no effect on serum TNF-α concentration but in Hontecillas *et al*. study, PA can decrease TNF-α plasma concentration in mice. Changes in serum triglyceride, HDL–C and TNF- α are related to each other. TNF-α promotes lipolysis and can activate the endothelium and cause vascular damage. TNF- α and insulin resistance are related to each other.

It is probable that anti-TNF-α effect of PSO was liable for its anti- diabetic and triglyceride lowering effect [64]. Decrease in TNF-α and IL-6 was reportedby Saha and Ghosh after treatment of diabetic rats for four weeks with CLnAs (α-ESA and PA at 0.5 % total lipids) [65]. PSO decreased intestinal inflammation in rats with necrotizing entrocolitis (NEC). In this model of inflammation, rats fed with 1.5% of PSO orally and then were exposed to asphyxia/cold stress to induce NEC. Decrease in incidence of NEC from 61% in control group to 28% in therapeutic group was seen. Also, PSO normalized the serum levels of proinflammatory cytokines such as IL-6, IL-8, IL-12, IL-23 and TNF-α. Enterocyte proliferation was reduced after administration of PSO [66].

All of above studies confirmed anti-inflammatory properties of PSO. In many studies anti-inflammatory effects of PSO were investigated with its antioxidant and anticancer effects, but in the present paper we mentioned the PSO's beneficial effects separately.

Antioxidant activity: Oxidation dependent mechanisms are very important ways to induce diseases such as many types of cancers and inflammatory diseases [67]. Reduction- oxidation state may activate and deactivate certain genes that promotes many secondary steps in disease conditions [68]. NF-κB and AP-1 are two examples of this one. Reactive oxygen species (ROS) act as 'signal transduction messengers to promote the activity of the cytokines [69]. Genes involved in carcinogenesis, atherosclerotic mechanisms, diabetic changes and HIV replication are near the receptor sites of these cytokines [59]. Thus, antioxidant effects of PSO are effective in anti-inflammatory and cardioprotective activities. Lipid peroxidation and ROS production are involved in many organs toxicity due to various factors such as xenobiotics, drugs, environmental pollutants and etc. [70]. Nephrotoxicity, hepatotoxicity, cardiotoxicity, and neurotoxicity are the main examples of interference of oxidative stress and will be discussed later.

In this section, we have an overview the antioxidant action of PSO. Mukherjee *et al*. showed that PA at concentration of 0.6% has maximum antioxidant activity. Peroxidation of polyunsaturated fatty acids in lipids and formation of free radicals showed a reduction. Alternatively, conjugated double bonds of PA and other CLnAs in PSO entrapped free radicals [71]. Membrane lipid peroxidation was decreased by diet containing 0.25 % CLnAs compare to diet without CLnAs [72]. Lowered oxidative stress and enhanced antioxidant enzyme serum levels were observed in Saha and Ghosh study. They treated streptozotocin- induced diabetic rats with α-ESA or PAwith0.5% total lipids. After the procedure, the levels of superoxide dismutase (SOD), gluthatione peroxidase (GPx) and catalase (CAT) were higher in treated group compared tocontrol group [42]. This result was confirmed by other study performed by them in sodium arsenite- induced oxidative stress in rat models [42].

Antioxidant activity of PSO is contributed to tocopherols and polyphenolic compound contents [73]. Oxidative stress reducing agents in PSO have metallic chelating potential, singlet oxygen quenchers and hydrogen donors [74]. Antioxidant effect of PSO will be discussed later.

Effects on insulin resistance:

TNF- α is one of the most important pro-inflammatory cytokines which plays a pivotal role in obesity, inflammation and insulin resistance. Higher serum levels of TNF- α are accompanied with insulin resistance. Anusree *et al*. showed that TNF-α (10ng/mL for 24 hr) decreased 1.7 fold in insulin stimulated glucose uptake with respect to control group in 3T3-L1 adipocytes, but PA with doses of 5, 10, 30μM restored the glucose uptake capacity significantly and dose- dependently. PA at dose of 30μM is comparable to rosiglitazone (100nM) in insulin stimulated glucose uptake. The similar condition was observed in production of ROS with the effect of TNF- α and its restoration with the effect of PA. TNF- α treatment caused significant alterations in mitochondrial transmembrane potential, reduction in ATP production, O_2 consumption, overall increase in cellular ROS generation and a decrease in aconitase activity. The use of PA restored these states to the baseline [75].

Peroxisome proliferator activated receptor γ (PPAR- γ) agonists increase mitochondrial biogenesis and normalize the fission fusion ratio in this organelle that be altered with inflammation and high levels of TNF-α [76]. Agonistic effects of PA on PPAR- γ, lowering oxidative stress and serum TNF-α levels and positive effects of PA on mitochondrial functions are the main anti-diabetic mechanisms of PSO [75].

Effects of PSO on obesity and insulin resistance induced by rich fat diet in mice showed that, rich fat diet with 1% PSO after 12 weeks induced higher peripheral insulin sensitivity in treated group compared to control group (164±52% vs. 92±24% respectively), but liver insulin sensitivity showed no significant diffrence between two groups. In this study, obesity and fat deposition induced by this regimen was ameliorated by PSO, significantly [31]. A similar study was performed by Miranda *et al.* That used diet with 0.5% PA in rats. The results of this study showed no changes in adipose tissue weights and insulin resistance, but the glycemic value in the PA group had decreased, significantly [77]. Improvement in insulin sensitivity is associated with reducing the risk of developing type 2diabetes. Insulin sensitivity was improved with 61.79mg/day PSO in mice which was treated with rich fat regimen compared to control group. In addition, final body weight, body weight accumulation, serum adiponectin, serum leptin, and serum insulin were reduced in treated group [78].

The use of PSO (200 and 400mg/day for 28 days) in streptozocin-nicotinamide induced diabetic rats showed that serum insulin levels increased but the serum glucose level had no change. Up-regulation of PPAR-γ responsive genes by PA may be a mechanism of PSO-induced increase in serum insulin [79]. Anti-diabetic and insulin sensitizing effects of α- linolenic acid were also reported in other studies[53, 78-81].

Nephroprotective activity:

Investigating nephroprotective effects of PSO was carried out mainly by Boroushaki *et al.* In five separated studies, four nephrotoxic agents, mercuric chloride [82], diazinon [83], gentamicin [84], and hexachlorobutadien [85] and cis-platin (under publication) were used and protective effect of PSO was shown. Elevated serum levels of urea and creatinine and urinary levels of glucose and protein as well as histopathological changes in kidney, such as severe tubular necrosis and atrophy, represented acute nephrotoxicity by use of these toxic agents. Inducing oxidative stress by these agents was shown in above mentioned studies by elevated kidney malondialdehyde content (MDA, as a biomarker of oxidative stress) and decreased total thiol content in the kidney.

The use of PSO in these models of nephrotoxicity was accompanied by its protective effects and PSO preserved renal functions and decreased histopathological changes in kidney. Restoration of serum urea and creatinine and decrease in urine glucose and protein were established after PSO treatment. Increasing in total thiol content was seen but was not dose dependent in all studies. Kidney was vulnerable to oxidative stress and each factor that induces this state can be a toxic agent on this organ. PSO due to its high content of polyphenolic compounds is a potent antioxidant. Moreover, hydroxybenzoic acid derivatives and sterols in PSO have an antioxidant effect and decrease lipid peroxidation.

The possible nephroprotective mechanisms of PSO mentioned in these articles includes removing peroxy radicals and prevents Cu^{2+}- induced lipid peroxidation, inhibition of TNF- α- induced priming of ROS production and myeloperoxidase, hydroperoxide formation and biohydrogenation of CLnAs and chelating transition metals [86].

Effects on memory:

As mentioned, polyphenols have been found to possess antioxidant properties and recent studies indicated that seed oil extract of pomegranate has the highest concentration of polyphenols. Sarkaki *et al.* in their *in vivo* study demonstrated that, administration of PSO in permanent cerebral ischemia causes a remarkable improvement on memory with criterion condition responses (CCRs) in Y-maze and step-through latency (STL) in two-way shuttle box. The results showed that, both active and passive avoidance memories were meaningfully impaired in rats after cerebral hypoxia-ischemia (CHI) ($P<0.001$) and PSO treatment significantly ameliorated passive and active memory impairments with bilateral common carotid arteries occlusion (2CCAO) ($P<0.05$, $P<0.01$, and $P<0.001$) [87]. Gabizon *et al.* Reported that administration of large concentrations of PSO may postpone the manifestation of disease in young transgenic mice (Tgs), in addition, lower doses of Nano-PSO significantly delayed disease onset in asymptomatic TgMHu2ME199K mice and postponed disease aggravation in already sick mice. Therefore, PSO formulations may be impressive on neurodegenerative diseases [88].

Anti- cancer effects:

PSO has a potent effect on tumor cells. Hora *et al.* investigated the chemo preventive effect of PSO on skin tumor development in CD1 mice. They concluded that, PSO (5%) significantly decreased tumor incidence ($P<0.05$), multiplicity, and TPA (12-O tetradecanoylphorbol 13-acetate) - induced ODC (ornithine decarboxylase) activity during 20 weeks of promotion. The mechanism for this effect can be inhibition of prostaglandin biosynthesis (COX-1, COX-2, and LOX) by punicic acid. In this study, in addition, topical application of 5% pomegranate seed oil remarkably inhibited ($P <0.05$) the TPA-induced epidermal ODC activity. Overally, PSO appears to be a good natural product with a potential chemopreventive effect against skin cancer [89].

γ-Tocopherol is the most important constituents of PSO that is responsible for anti-cancer activity. Jiang *et al.* investigated that, γ- tocopherol, inhibits proliferation of prostate cancer cells but appears to have no effect on the growth of a normal prostate epithelial cell. Their proposed mechanism for this effect was inhibition of sphingolipid synthesis de novo [90]. In another study performed by Jiang *et al.* γ-tocopherol inhibited cyclooxygenase activity in macrophages and epithelial cells. This mechanism is beneficial for human tumor tissues, including human colon cancer, have been reported to contain enhanced COX-2 expression and PGE_2, because PGE_2 has been shown to promote proliferation in certain cancer cells [60]. Another similar study showed a significant role for induction of cell death for all cancer cell lines such as colon cancer, prostate carcinoma cells, and osteosarcoma. According to this study, γ-tocopherol has chemopreventive properties by mechanisms such as reduction levels of C- reactive protein, inhibition of neoplastic transformation, suppression of ras p-21, inhibition of COX-2 activity, down-regulation of cyclins, and up-regulation of PPAR - γ [91].

As mentioned, PA as an important component of PSO has various anti- cancer effects. In a research performed by Lansky *et al* .PA was tested as a potential inhibitor of in vitro invasion of human PC-3 prostate cancer cells in an assay employing Matrigel ™ artificial membranes. Results showed a considerable inhibition of PC-3 prostate cancer cell invasion [15]. In another study PSO exhibited an enhancing B-cell function in vivo that can be effective in some cancers [43]. In a study PSO via lipid peroxidation mechanism showed a cytotoxic effect against leukemia cells [92].

It is known that dietary phenolic compounds can elicit vital cellular responses such as cytotoxicity, cell cycle arrest and apoptosis by activating a cascade of molecular events. Hydroxybenzoic acids, such as ellagic acid and gallic acid are other important components of PSO. In LI *et al.* study, ellagic acid had shown an anti-cancer effect with flow cytometric assay, polymerase chain reaction (PCR) and determination of caspase-3 activity

methods. Ellagic acid significantly Induced p53/p21 expression, G1 arrest and apoptosis in bladder cancer cells [93]. In another study, gallic acid showed a strong dose and time-dependent growth inhibition and apoptotic death of human DU-145 prostate cancer cells [94]. In a study provided by Madlener *et al.* Gallic acid induced a dose-dependent apoptosis in HL-60 cells and attenuated G0/G1 to the S phase, and COX in HL-60 leukemia cells [62].

Other constituent of PSO, ursolic acid, with mechanisms such as apoptosis in MCF-7 via p-53 up regulation [95], apoptosis in endometrial cancer cells via caspase-3 pathway [96], and apoptosis in melanoma cells via the intrinsic cell death pathway and caspase 3 activation [97] showed anti- cancer activity.

Furthermore, sterols (daucosterol, campesterol, stigmasterol, beta-sitosterol) have shown an anti-cancer effect via; Inhibition of pro-inflammatory cytokines [98], PC-3 apoptosis and cell cycle arrest via ROS changes and prostaglandin release [99, 100], and Reverts impaired glutathione/oxidized glutathione ratio via estrogen/phosphatidylinositol 3-kinase pathway [101].

Effects on lipid profile:

Conjugated fatty acids are polyunsaturated fatty acids in which at least one pair of double bonds are separated by only one single bond, as in conjugated linoleic acid. These fatty acids have useful biological effects. Arao *et al.* investigated the effects of PSO rich in PA (9*cis*, 11*trans*, 13*cis*-conjugated linolenic acid; 9c, 11t, 13c-CLNA in obese, hyperlipidemic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Results showed that, feeding with 1% pomegranate did not affect serum lipid levels and abdominal white adipose tissue weights.

However, 2 weeks feeding of the diet supplemented with 5% PSO showed a significant reduction of omental white adipose tissue weight. Also, the accumulated hepatic triacylglycerol was significantly decreased by PA regimen. In addition, the activity of hepatic enzymes was not altered by PA regimen. Also, PA contained diet could decrease level of monounsaturated fatty acid (MUFA) in OLETF rats. Furthermore, this in vivo study demonstrated that PA can inhibit the delta-9 desaturation which leads to reduction in hepatic triacylglycerol (TAG) accumulation [102]. In contrast, yang *et al* .reported that PSO does not alter serum cholesterol concentration [103]. Vroegrijk *et al.* in their study demonstrated that PSO regimen ameliorates rich-fat diet induced obesity and causes a reduction in body fat mass independent of changes in food intake or energy expenditure [31]. Anti-atherogenic effects of PA, as a type of conjugated fatty acid and the main constituent of PSO, has been investigated by Mirmiran *et al*. This double-blind placebo-controlled clinical trial with 400 mg PSO exhibited the mean concentration of TAG and the TAG: HDL cholesterol (HDL-C) ratio, were significantly decreased after 4 weeks. Also, the results showed a diminution of cholesterol HDL-C ratio while, the serum cholesterol, LDL cholesterol remained unchanged. Therefore, this study could demonstrate a beneficial effect of lipid profile including TAG and TAG:HDL-C ratio [52]. Mc Farlin *et al.* demonstrated the regimen with PSO (approximately 61mg/d) during 14 weeks can reduce the body weight with the mechanism mediated by a leptin/adiponectin pathway.

This study showed that weight gain was associated with an increase in biomarkers of cholesterol profile, adipose tissue accumulation ($P<0{\cdot}05$). PSO resulted in a decrease in total weight gain, leptin and insulin, and an increase in plasma adiponect in cocentration ($P<0{\cdot}05$) [78]. Miranda *et al.* demonstrated that, dietary supplementation of 0.5% PA did not cause to decreased fat accumulation in adipose tissue, liver, or skeletal muscle and there was no significant difference between the experimental groups in serum TGs, HDL cholesterol, or non-HDL cholesterol. An interesting result from this research was hypoplasia induced in the liver due to the anti-proliferative effect on hepatocytes increased transaminase levels. Also, this issue should be considered before proposing PA as a functional ingredient [77]. Another study performed by Yamasaki *et al.* with diets containing 0%, 0.12%, or 1.2% PSO for 3 weeks, showed a meaningful increase in serum triacylglycerol and phospholipid levels but not in total cholesterol in the PSO treated groups [43].

Other beneficial effects of PSO: As mentioned, PSO has many pharmacological effects, other beneficial effects of PSO is summarized in the **Table 5**.

TABLE 5: OTHER BENEFICIAL EFFECTS OF PSO.

Pharmacological effect	Mechanism(s) and clinical evidence(s)	Reference(s)
Anti- osteoporosis	Down-regulation of expression RANK-RANKL downstream signaling targets and osteoclast differentiation markers in osteoclast-like cells, increasing alkaline phosphatase activity, mineralization of matrix and transcriptional levels of major osteoblast lineage markers involving the Wnt/β-catenin signaling pathways	104
Anti- pancreatitis	Anti- inflammatory and antioxidant mechanisms of PSO caused reduction in amylase and lipase activity in serum, pancreatic MPO activity, edema, leukocyte infiltration and vacuolization.	105
Hepatoprotective	Decrease in MDA, DNA fragmentation, caspase- 3 and GSR activities, elevation in levels of GSH, SOD, GST and t-GPx activities. Consequently reduction in oxidative stress and apoptosis.	106
Improving in insulin secretion	increasing serum insulin and glutathione peroxidase activity	79
Anti-atherogenic	Decrease in TAG and the TAG:HDL cholesterol (HDL-C) ratio,	52
Neuroprotective	Neutralize ROS or enhance the expression of antioxidant gene, decrease in lactate/pyruvate ratio, extracellular nitric oxide, and lactase dehydrogenase generation Reduction in lipid oxidation and neuronal loss	107 108
Anti-menopausal symptoms	Reduction in the number of hot flashes per day, Reduction in sum score of the Menopause Rating Scale II parameters in the treated group after 12 weeks but not significant in comparison to control group	109
Cosmetic	Stimulate keratinocyte proliferation, mild thickening of epidermis, stimulating keratinocyte proliferation, stimulating type I procollagen synthesis, inhibiting matrix metalloproteinase-1	110

RANK: Receptor Activator of Nuclear Factor κ B, **RANKL:** Receptor Activator of Nuclear Factor κ B ligand, **MPO:** Myeloperoxidase, **MDA:** Malondialdehyde, **GSR:** Glutathione reductase, **GSH:** Glutathione, **SOD:** Superoxide dismutase, **t-GPx:** Glutathione peroxidase, **TAG:** triacylglyceride, **HDL:** High-density lipoprotein, **ROS:** Reactive oxygen species

Toxicity:

In our study on PSO toxicity, only one study had been found. In this study, in vitro toxicity was done with Ames and Chromosomal aberration test and *in vivo* toxicity was done with acute and chronic (28 days consumption) test. No mutagen city of PSO was observed with Ames test and Chromosome aberration test with 5000 and 333µg/ml, respectively.2000mg/kg PSO did not show any significant side effects in rat. Use of 0, 10,000, 50,000 and 150,000ppm of PSO per day in a 28 days period did not show any adverse effects but in 150,000ppm, that was much higher than routine anti-diabetic and anti-inflammatory dose, increasing in hepatic enzymes and weight was deduced and in this study this dose was considered as no observable adverse effect level (NOAEL) [111].

CONCLUSION: The use of pomegranate has been increased due to its reported health benefits. Pomegranate and its derivatives, especially PSO, are rich source of several chemical compounds with potential physiological activities. The information presented in this review article has shown some pharmacological and toxicological mechanisms and properties of PSO. It has been demonstrated that monotherapy or supplementation therapy with PSO may haveprotective effects against several diseases, including cancers, diabetes, cardiovascular disease, inflammation, neurotoxicity, mouth and skin disorders, pancreatitis, and osteoporosis. These effects act possibly due to highly antioxidant activity of PSO. Therefore, it is recommended that, PSO can be a very beneficial medicine for treatment and protection of certain diseases and disorders, although probably underlying mechanisms of this protection need further explorations.

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