Review Article

A review on phytochemistry and pharmacological profile of punica granatum.

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Abstract

Pomegranate (Punica granatum L), in addition to its ancient historical applications, has been used in several systems of medicine for a variety of diseases and disorders. Pomegranate juice is regarded as a polyphenol-rich juice with high antioxidant capacity. Pomegranate juice has been shown to exert significant antiatherogenic, antioxidant, anti-carcinogenic, and anti-inflammatory effects in several human and marine models. Pomegranate is potent antioxidant, anti-carcinogenic, and anti-inflammatory properties other potential applications include male infertility, infant brain ischemia, Alzheimer’s disease, obesity, arthritis. The aim of the present review is to discuss the cumulative evidence, which suggests that consumption of pomegranate possesses a diverse array of biological activities and may be helpful in the prevention of some inflammatory mediated diseases, including cancer.

Keywords: Anti-cancer, polyphenol, pomegranate, Punica granatum, therapeutic applications.

1. Introduction

The pomegranate tree typically grows at about 12−16 feet long and also having spiny branches. The plant is extremely long-lived, as evidenced by trees at Versailles, France are known to be over 200 years old. The leaves are glossy in appearance and are lance shaped. The bark turns grayish as the age of the tree advances. The flowers of pomegranate are large white, red, or variegated, and they have a tubular calyx and that eventually becomes the fruit [Figure 1]. The ripened pomegranate fruit looks as deep red, leathery skin. It is grenade-shaped, and crowned by the pointed calyx.

The fruit contains various seeds (arils) which are separated by white, membrane that is known as pericarp, and each is surrounded by small amounts of tart, red juice [Figure 2]. The pomegranate is native of the Himalaya region in north India to Iran. However, the plant has been cultivated since a very long times over the entire Mediterranean region. It is also found in India. Pomegranate (Punica granatum L) has been used in several systems of medicine for a variety of ailments. Pomegranate juice is rich in polyphenols with high antioxidant potential. In human studies and murine models, pomegranate juice and various extracts have been proven to exert significant anticancer, analgesic, anti-inflammatory, neuroprotective, sexual stimulant, antiatherogenic, hypoglycemic, antidepressant, antioxidant, hypolipidaemic, antimicrobial, antifungal, antiviral, antialzheimer’s, immunomodulatory, estrogenic, skin protective, cardioprotective,
dental care, musculoskeletal effects, gastroprotective effects, hepatoprotective, antitrichomonal, antiobesity, antidiarrhoeal and nootropic activity.

Phytochemistry
Although most of the fruits are known to possess therapeutic properties, some studies also have reported that even the roots, bark, and leaves of these trees have medicinal value. Pharmacological mechanisms of pomegranate are well established. Pomegranate juice contains anthocyanins, ascorbic acid, glucose, ellagic acid, gallic acid, catechin, caffeic acid, Epigallo catechin gallate (EGCG), rutin, quercitin, iron and amino acids. Pomegranate seed oil is composed of punicic acid and sterols. The pericarp of pomegranate contains punicalgins, flavonones, flavones, and other flavanols. Tannins as punicalin and punicaflolin, and flavonoidal glycosides like luteolin and apigenin, form important constituents of pomegranate leaves. The flowers of pomegranate are composed of ursolic acid, some triterpinoids like maslinic acid, and asiatic acid. Pomegranate roots and bark are rich in ellagitannins and piperidine alkaloids. However, current research work seems to indicate that the most therapeutically beneficial pomegranate constituents include ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavones. Pomegranate aril (seed) juice provides about 16% of an adult’s daily vitamin C requirement per 100 ml serving, and a good source of vitamin B5 (pantothenic acid), potassium, and natural phenols, such as ellagitannins and flavonoids.

Pharmacological Activities
1. Anticancer activity [1-7]
Several in-vitro anticancer assays were carried on prostate cell lines (DU-145, LNCaP, PC-3) using various pomegranate extracts (juice, seed oil and peel), it was found that there was significant reduction in proliferation and invasiveness of cancer cells. Several effects were observed as disruption of cell cycle, induction of apoptosis, and ultimately inhibition of tumor growth. Combination of punica granatum extracts in from various parts of fruit was found to be more effective than any single extract. The mechanism of anticancer activity was cleared as modulation of proteins regulating apoptosis. Pomegranate was proved to be an effective in prostate cancer, breast cancer, colon cancer, hepatocellular carcinoma, leukemia, chemotherapy induced toxicity.

2. Analgesic and anti-inflammatory activity [8]
Punica granatum seed oil in cold pressed condition inhibited both cyclooxygenase and lipoxygenase in vitro. There was 37% inhibition of cyclooxygenase pathway, converting prostaglandins from arachidonic acid and 75% inhibition of lipoxygenase pathway, converting leukotrienes from arachidonic acid. The juice extract resulted in 23.8% inhibition of lipoxygenase in vitro. Punica granatum has been used for its usefulness in number of inflammatory diseases. A dietary supplement that contains pomegranate extract have became popular for the treatment and prevention of arthritis and other inflammatory conditions. For analysis of analgesic and anti-inflammatory activity in animal models, Pet-ether, dichloromethane
and methanolic fractions of flower part are chosen. Carrageenan-induced rat paw edema model was used for testing anti-inflammatory activity of pomegranate. Acetic acid-withriing test was used to screen analgesic activity in mice using Pet-ether, dichloromethane and methanol fractions at the dose of 200 mg/kg showed 75.77% (p<0.001), 68.56% (p<0.001), 54.64% (p<0.001) inhibition of writhing response. In rat paw edema model induced by carrageenan, pet-ether, dichloromethane and methanolic fractions were found significantly reduce (p<0.001) the formation of edema at the 100 mg/kg dose level and that showed 26.92%(p<0.001), 27.97%(p<0.001), 21.85%(p<0.001) inhibition respectively of inflammatory volume at the end of 4 h. These results signify the traditional uses of *Punica granatum* as analgesic and anti-inflammatory.

3. Alzheimer’s disease and dementia:
Neuroprotective activity of polyphenols present in pomegranate (juice) was evaluated in Alzheimer’s transgenic mice. It was found that there was 50% reduction in accumulation of beta-amyloid protein and less hippocampal amyloid deposition when administered with pomegranate juice. It was also observed that animals treated with pomegranate juice have shown improved performance in morris water maze task in terms of swimming speed. Ethanolic extract of pomegranate seed was found to be effective in improving cognitive performance of aged and scopolamine treated young mice. Subacute administration of *Punica granatum* extract (21 days) and vitamin C significantly (p < 0.05) reversed age induced or scopolamine induced deficits in memory. Extract was found potent in reduction of lipid peroxidation value and increased glutathione levels in brain tissues.

4. Antiepileptic activity
*Punica granatum* extract was administered 100, 200, 400, 600 mg/kg before strychnine administration. The parameters such as onset duration and no of convulsions and death time 30 minutes after strychnine administration was measured. Hydroalcoholic extract considerably reduced licking and writhing.

5. Anxiolytic and antidepressant activity
Antidepressant-like activity of ellagic acid was evaluated in Swiss young male albino mice. Mice were immobilized for 150 min period for inducing stress. Ellagic acid in a doses of (8.75, 17.5, 35 mg/kg, po) and fluoxetine (20 mg/kg, ip) were administered to both unstressed and stressed mice; and immobility duration was recorded using forced swim test and tail suspension test. Plasma nitrite levels were also estimated in both unstressed and stressed mice. Effects of aminoguanidine (iNOS inhibitor), 7-nitroindazole (nNOS inhibitor), sulpiride (selective D2-receptor antagonist), prazosin (α1-adrenoceptor antagonist) and p-chlorophenylalanine on antidepressant-like activity of ellagic acid. Ellagic acid at 2 higher doses (17.5 and 35 mg/kg, po) as well as standard drug significantly decreased immobility duration. The involvement of central monoaminergic system involvement was found to be the mechanism of action mediated by ellagic acid.

The administration of 2, 5, 8 ml/kg dose of hydroalcoholic extract of *Punica granatum* was administered to rats during 15 days. The animals were screened for antianxiety and antidepressant parameters such as open field, elevated plus maze and force swim test. In open field there was significant increase in distance travelled, entries in the central zone and the rearings number. Noof open arm entries were increased in the elevated plus maze. In force swim test there was reduction in immobility duration and increased climbing time.

6. Immunostimulatory activity:
Aqueous suspension of pericarp of *Punica granatum* shows both cell mediated and humoral immunity as evidenced by enhanced inhibition of leukocyte migration and increased antibody titer to Hypoid-H antigen. Immunoglobulins are also produced in the
spleen cells of mice and they may be involved in improving function of beta cells in vivo.  

7. Estrogenic activity
Petroleum ether extract of the seeds exhibited potent estrogenic activity which can be antagonized by pomegranate progesterone. Estrogenic compounds like estradiol, estrone and isoflavone phytoestrogens like daidzein and genistein are present in pomegranate seeds. The estrogenic effect of seed extract was investigated in ovariectomized (OVX) animals. Two weeks administration of seed extract in OVX mice prevented loss of uterus weight and shorted the immobility duration. Pomegranate seeds possess estrogenic activity due to amelioration on the depressive state, a clinically important mental profile of women menopausal syndrome, especially due to the uterotrophic action.  

8. Prevention of skin damage
Pomegranate derivatives represent a considerable prospect as cosmeceutical because of their protective effect to UV-induced damage. A study undertaken carried out the inhibition of increased activity of melanoproteinases in both reconstituted human skin and also human skin fibroblasts. Ellagic acid, one of the active phytoconstituents of pomegranate may provide a photoprotection. It has been proved to relieve UV-induced skin wrinkles and inflammation. Also it prevents photo aging. Antwrinkle activity of ellagic acid was tested in hairless mice exposed to UV-B radiation, in which it decreased UVB-produced wrinkle formation on the skin and epidermal thickness. Topical application of ellagic acid attenuated the production of pro-inflammatory cytokines as IL-1beta and IL-6, and blocked the infiltration of inflammatory macrophages in integuments of SKH-1 hairless mice, which is exposed to UV-B, for eight weeks. Furthermore, this compound alleviated inflammatory intracellular cell adhesion molecule-1 expression in UV-B-irradiated keratinocytes and photoaged mouse epidermis. These results suggest that ellagic acid prevents collagen destruction and inflammatory responses caused by UV-B.  

9. Antidiabetic, hypolipidaemic and antioxidant activity
Anti-diabetic, hypolipidemic and antioxidant activity of hydroalcoholic extract from leaves and fruit peel of Punica granatum was investigated. Diabetes was induced in Wistar rats using Streptozotocin. Animals were grouped in seven groups of six animals each. Groups-I-normal control, Group-II- diabetic control, Group III-leaves extract-100 mg/kg b.w. of P. granatum, Group IV- leaves extract 200 mg/kg b.w. of P. granatum, Group V-fruit peel extract 100 mg/kg b.w. of P. granatum, Group VI- Hydroalcoholic peel extract 200 mg/kg b.w. of P. granatum and Group VII-glibenclamide respectively. Fasting blood bold glucose level was recorded on 1st, 7th, 14th, 21st and 28th day. Lipid profile and levels of antioxidants were determined at the end of the study. Safety profile of both extracts was evaluated using acute and chronic toxicity studies. Higher dose of fruit peel extract of P. granatum (PEPG) and glibenclamide significantly lowered blood glucose level from 7th day onwards however glibenclamide was found to be more effective in comparison to the extract. Fruit extract at lower dose and leaves extract at higher dose significantly lowered blood glucose level from day 14th onwards. Leaves extract at lower dose also significantly lowered blood sugar level from 21st day onwards. Treatment of Glibenclamide and higher dose of fruit PEG extract significantly lowered the total cholesterol, triglyceride levels and significantly and increased the high density lipoprotein cholesterol level. Treatment with glibenclamide followed by higher dose of hydroalcoholic extract was found more effective in reducing plasma thiobarbituric acid reactive substances and increasing levels of antioxidant enzymes (superoxide dismutase and catalase). Concludingly, Leaves and fruit PEGG possesses significant anti-diabetic, hypolipidemic and antioxidant properties. The underlined study supported the traditional use of P. granatum as antidiabetic.  

10. Cardioprotective
Pomegranate juice contains potent antioxidants as soluble polyphenols, tannins, and anthocyanins possessing appreciable anti-atherosclerotic activity. Sumner et al., 2005,
investigated that daily consumption of pomegranate juice for successive three months could improve myocardial perfusion in 45 patients who had Coronary Heart Disease (CHD) and myocardial ischemia. The study was reported in a randomized, placebo-controlled, double-blind study. After three months, it was observed that, the amount of stress-induced ischemia was treated in the pomegranate treated group, but increased in the control group Thus, it was concluded that daily consumption of pomegranate juice improves stress-induced myocardial ischemia in patients with CHD. [20]

11. Musculoskeletal
Dietary supplementation of polyphenols as ellagitannins, may prevent muscle damage experienced after the heavy exercise, producing delayed-onset of muscle soreness. Dietary supplementation with ellagitannins significantly improves the recovery of isometric strength of muscles two to three days after a damaging eccentric exercise. [21] Pomegranate fruit consumption reduced composite Disease Activity Index in rheumatoid arthritis patients, and this effect could be related to the anti-oxidative property of pomegranates. Dietary supplementation with pomegranates may be a useful complementary strategy to attenuate clinical symptoms in rheumatoid arthritis patients. [22]

12. Gastroprotective
Aqueous methanolic extract of pomegranate fruit when orally administered significantly reduced the ulcer index produced by alcohol, indomethacin and aspirin administration in rats. Furthermore, in pylorus-ligation model of rats, extract significantly reduced the ulcerative lesions, gastric volume, and total acidity. It prevented ulceration by increasing the gastric pH and mucus secretion in pylorus-ligated rats. Pomegranate tannins play a protective role against gastric ulcer. [23]

13. Hepatoprotective
Abnormal liver function tests represent a condition commonly known as fatty liver. The mechanism of action of pomegranate flower (PGF), a traditional anti-diabetic medicine, on fatty liver was studied. The findings suggested that this medicine ameliorates diabetes and obesity-associated fatty liver disease, at least or in part, by activating the hepatic gene expression that is responsible for fatty acid oxidation. [24] Pretreatment with pomegranate flower extract, at a dose level of 50–150 mg / kg body weight, for 7 days significantly reduced nitritetriacetate (Fe-NTA)-induced oxidative stress dose-dependently. This resulted in protection from hepatic injury. The extract showed protective effect up to 60%. Extract treatment showed elevation in the glutathione (GSH) levels and activities of the antioxidant enzymes, namely, glutathione peroxidase (GPX), catalase (CAT), glutathione-S-transferase and glutathione reductase (GR), and (GST), by up to 28.5, 36, 40.2, 28.7, and 42.5%, respectively. [25]

14. Effect on fertility
Effects of pomegranate juice (PJ) consumption on antioxidant activity, sperm quality, spermatogenic cell density, and the serum testosterone level of male healthy rats was investigated. There was a significant decrease in malondialdehyde (MDA) level observed after the treatment with PJ. Additionally, a marked increase in glutathione peroxidase (GSHPx), glutathione (GSH), catalase (CAT) activities, and also the vitamin C level, was observed in rats that are treated with different doses of PJ. Treatment with PJ showed increased the epididymal sperm concentration, motility of sperm, sperm cell density, diameter of seminiferous tubules, and the thickness of germinal cell layer. It also decreased the abnormal sperm production rate when compared to the controlled group. These results suggest that PJ administration improves the sperm quality and antioxidant activity in rats. [26]

15. Anti-trichomonial
*Trichomoniasis vaginalis* is a significant and very serious worldwide health problem. The drug metronidazole has so far been used as the treatment measure, but some metronidazole resistant strains have been produced and unpleasant adverse effects have been developed. Treatment of patients with vaginal trichomoniais constitutes a major therapeutic challenge and treatment options are very limited. Natural plant extract which is purified from pomegranate was studied *in vitro* for its efficacy against *T. vaginalis* on Diamond media. Infected women who accepted to be treated with pomegranate juice were found to
be completely cured. The pomegranate extract have shown promising anti-trichomoniasis vaginalis.\[27\]

16. Antiobesity
Several infusions or decoctions of medicinal plants are used in traditional system of medicine to reduce obesity. Positive effects on fat reduction have been shown using pomegranate extracts. Most of the beneficial effects that are achieved are related to the presence of anthocyanins, tannins, very high levels of potent antioxidants, including polyphenols and the flavonoids. Many studies have reported confirming use of different extracts of pomegranate as anti-obesity, and various mechanisms have been proposed as to how these different extracts help in fat reduction.\[28\]

17. Antidiarrheal
The anti-diarrheal activity of alcoholic and aqueous extracts of pomegranate fruit rind was investigated in experimental animal models, using albino rats. The extracts showed significant antidiarrhoeal activity in rats, when compared to a standard antidiarrhoeal drug.\[29\]

18. Erectile dysfunction
Pomegranate juice was used for the treatment of mild-to-moderate erectile dysfunctions in men. The randomized, placebo-controlled, double-blind, crossover trial, enrolled 53 men with mild-to-moderate impotence were included. The subjects were consumed pomegranate juice, or placebo, for four weeks. After a two-week washout period, they switched off the treatments. Study concluded that the subjects were more likely to have improved scores when pomegranate juice was consumed.\[30\]

19. Antiviral effects
Haidari et al., 2009, evaluated four major polyphenols present in pomegranate extracts i.e. ellagic acid, caffeic acid, luteolin, and punicalagin. They suggested that the phytoconstituent punicalagin had an anti-influenza component, because this compound was found to be having potential of competitively blocking the replication of the viral RNA and inhibited agglutination of chicken RBCs by the virus as well and showed viricidal effects. Indeed, it inhibited the replication of human influenza A/Hong Kong virus (H3N2) in vitro\[31\]

20. Antifungal activity
The pomegranate peel was separated, dried and powdered. Candida Albicance was inoculated at 37°C and after inoculation, was seeded on Sabourauds agar medium. Sterilized filter papers saturated with 30 μl of extracts are placed on the seeded plates and inoculated at 24 and 48 h. Zones of inhibition on all four sides were measured around the filter paper with a vernier caliper. The highest inhibition of Candida albicans was with a mean zone of inhibition of 22 mm. The results concluded that, potential use of this extract as cheap and convenient adjuvant to pharmaceutical antifungal products.\[32\]

21. Antimicrobial activity
Punica granatum L., is reported to have antimicrobial activity against a range of gram positive and gram negative bacteria. Some of the formulations of pomegranate containing ferrous salts. It was seen that several phenolic compounds and flavonoids show antimicrobial activity.\[33\]

References
5. Bishayee A, Bhatia D, Thoppil RJ, Darvesh AS, Nevo E, Lansky EP.


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