

Screening of Antidepressant Activity of *Punica granatum* in Mice

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ABSTRACT

Introduction: There are many different mental health issues, including depression, that may be alleviated with the use of the medicinal plants found in India. Acute and chronic administration of *Punica granatum* (pomegranate) whole fruit had an antidepressant effect on rats, which was the aim of this research. We employed an oral regimen of *Punica granatum* aqueous extract (250 and 500 mg/kg daily), imipramine (10 mg/kg), and gum acacia (10 ml/kg) as a carrier. Each of the four animal groupings consisted of six creatures. The acute study required the administration of medicines or vehicles 60 minutes before the experiments began. All medications and vehicles used in the long-term trial were given for a total of 14 days, with the last dosage given 60 minutes before the experiment on day 14. To evaluate the efficacy of antidepressants, researchers used the Forced Swim Test and the Tail Suspension Test. An analysis of variance (ANOVA) was performed on the data, with drug therapy being the independent variable. We used Dunnett's test to do post hoc comparisons. The results showed that the period of immobility was greatly decreased in the acute tail suspension test, chronic forced swim test, and acute swim test by the PG 500 mg/kg group, but not in the 250 mg/kg group. The groups treated with PG 250 mg/kg and 500 mg/kg showed a significant reduction in the duration of immobility in the chronic tail suspension test. At 500 mg/kg, the antidepressant effect was similar to that of 10 mg/kg of imipramine. In conclusion, this research provides further evidence that 500 mg/kg of aqueous extract of entire *P. granatum* fruit has antidepressant effect. Given the nutritional and functional benefits of pomegranate extract, it would be wise to recommend its use to patients suffering from depression.

Key words: Feeling down, tests for tail suspension and forced swimming, pomegranate.

INTRODUCTION

Depression is a long-term mental health condition that may strike anybody at any time. The existing arsenal of treatment is often insufficient, with disappointing outcomes in around one-third of all people treated, despite the availability of numerous powerful antidepressants.¹ This gives researchers a reason to keep looking for better antidepressants. There are now more options for treating depression than the currently available synthetic medications due to their limitations. Herbal medicines are an example of an old therapy that has persisted over the years since plants have always been a source of medications. Pomegranate, scientifically

known as *Punica granatum* L. (PG), is a popular fruit and juice variety. The Punicaceae family includes it. The Himalayas in northern India are its natural habitat. From ancient times, it has been farmed all throughout the Mediterranean.² Valuable chemicals are found in several portions of the pomegranate fruit, including the skin, seeds, and arils. The peel contains a myriad of compounds and minerals, including gallic acid, ellagic acid, catechin, epicatechin, epigallocatechin-3-gallate, quercetin, kaempferol, luteolin, rutin, kaempferol-3-O-glycoside, gallagylidilacton, pedunculagin, tellimagrandin, and many more. The seeds contain punical acid, linoleic acid, oleic acid, palmitic acid, stigmasterol, β -sitosterol, dau-costerol, campesterol, cholesterol, estriol, estrone, estriol, estriol, tocopherols, ursolic acid, oleanolic acid, isoflavones, and phenyl aliphatic glycosides/lignins, among other major chemical components. The components found in the aril include sugars, pectin, polyphenols, anthocyanins, fatty acids, amino and organic acids, indoleamines, sterols, triterpenoids, and α -tocopherol.^{3,4}

In traditional medicine, pomegranate is used to cure a variety of conditions, including parasite infestations, diarrhea, acidosis, dysentery, bleeding, microbiological infections, respiratory disorders, and aphthous ulcers. It is also used as an antipyretic and vermifuge.⁵ Various components of the *P. granatum* fruit have shown anti-inflammatory, anti-cancer, anti-tumor, antihepatotoxic, anti-Diabetic, and antiatherogenic³ characteristics. Reportedly, it also helps with Alzheimer's illness.^{3, 7} Juice, wine,⁸ dried arils⁹, and jam are just a few examples of PG-based products that have been the subject of academic investigation.¹⁰ Despite this, research on the health benefits of *P. granatum* in its entire fruit form is limited. It is possible that the combined effect of the fruit's components is more effective than the sum of its parts. The central nervous system (CNS) effects of *P. granatum* have received little attention, and the antidepressant effects of the whole fruit have not been documented. Therefore, the purpose

of this research was to investigate the antidepressant effects of a water extract of *P. granatum* whole fruit in mice after both short-term and long-term treatment. MATERIALS AND METHODS

Adult Swiss strain albino mice weighing 25-30 grams, bred in our institutional animal house were used and were housed in clean polypropylene cages in groups of three. A 12:12 hour dark/light cycle at an ambient temperature of $24 \pm 2^{\circ}\text{C}$ were followed. Food and water were available *ad libitum*. Animals were acclimatized for seven days before exposure to the behavioral experiments. Experiments were performed during the light phase of the cycle (10:00-17:00). The study was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the recommendations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Preparation of aqueous extract

The whole fruit powder of PG (Himalaya Drug Company) weighing 208.75 grams underwent 10 cycles of soxhlation following which 69 grams of study drug was extracted. Hence the yield was 33.05%.

Each drug solution was freshly prepared just before administration. Drugs and vehicles were administered orally and the doses of each drug were selected on the basis of earlier findings.¹³

Animals were grouped into four and each group had six animals. Group 1 received 1% gum acacia in a dose of 10 ml/kg, group 2: imipramine prepared in 1% gum acacia (Depsol, Intas, Batch no: VNO319) at a dose of 10 mg/kg, group 3 & 4 received aqueous extract of *P. granatum* at a dose of 250 mg/kg and 500 mg/kg per day respectively.¹³ In the acute study drugs/vehicles were administered 60 min prior to the experiments whereas in the chronic study drugs/vehicles were administered daily for 14 days and the last dose was given on the 14th day, 60 min prior to the experiment.

The animal models used for testing antidepressant activity were forced swim test¹⁴ and tail suspension test.¹⁵ In both the models the duration of immobility was measured to evaluate the antidepressant potential of compounds.

The data has been analyzed using one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed by applying Dunnett's test. $p < 0.05$ was considered as statistically significant.

RESULTS

In the acute and chronic forced swim test duration of immobility was significantly reduced in the imipramine treated and PG 500 mg/kg treated groups. There was no significant decrease in immobility in the PG 250 mg/kg treated group (Table 1).

In the acute tail suspension test, there was a significant decrease in the duration of immobility in the imipramine

treated and PG 500 mg/kg treated groups. There was no significant decrease in duration of immobility in the PG 250 mg/kg treated group. In the chronic tail suspension test, there was a significant decrease in the duration of immobility in the imipramine, PG 250 mg/kg and PG 500 mg/kg treated groups (Table 2).

Both animal models of depression used in our experiment showed that the antidepressant effect of PG at the dose of 500 mg/kg was comparable to that of imipramine.

Table 1: Forced swim test

Groups	Acute forced swim test immobility in seconds (Mean \pm SD)	Chronic forced swim test immobility in seconds (Mean \pm SD)
Normal control	128 \pm 17.24	129 \pm 16.62
Imipramine 10 mg/kg	70.83 \pm 7.81 ^a	75 \pm 7.56 ^a
PG 250 mg/kg	108.33 \pm 17.89	110 \pm 17.29
PG 500 mg/kg	92 \pm 17.36 ^b	92.17 \pm 20.15 ^c

^a $p=0.001$ vs normal control; ^b $p=0.026$ vs normal control; ^c $p=0.037$ vs normal control Original

Table 2: Tail suspension test

Groups	Acute tail suspension test immobility in seconds (Mean \pm SD)	Chronic tail suspension test immobility in seconds (Mean \pm SD)
Normal control	275.50 \pm 24.48	292.83 \pm 27.05
Imipramine 10 mg/kg	207.00 \pm 17.10 ^a	205.50 \pm 11.59 ^c
PG 250 mg/kg	239.00 \pm 9.82	242.17 \pm 9.35 ^d
PG 500 mg/kg	224.83 \pm 28.19 ^b	237.67 \pm 31.06 ^e

^a $p=0.002$ vs normal control; ^b $p=0.04$ vs normal control; ^c $p=0.037$ vs normal control; ^d $p=0.001$ vs normal control; ^e $p=0.023$ vs normal control; ^e $p=0.04$ vs normal control Original

DISCUSSION

In the present study, the antidepressant activity of

aqueous extract of whole fruit of *P. granatum* were studied in two classical models for screening animal models for depression, the forced swim test and tail suspension test.

The whole fruit of PG consists of peel (50%), seed (10%), and arils (40%) which are rich in flavonoids, proanthocyanidin, ellagitannins and poly-phenolic compounds. Aqueous extract of the whole fruit (and not only the edible part of the fruit - seed and arils) was considered for the experiment so that the chemical compounds present in the peel are not left out.

In depression treatment is required for a prolonged period to get an optimal response; hence it is important to perform not only acute but chronic administration of the drugs in animal models. The results of the present study indicate that acute and chronic administration of aqueous extract of *P. granatum* at a dose of 500 mg/kg has significant antidepressant activity compared to normal control. This antidepressant effect is comparable to that of imipramine. *P. granatum* 250 mg/kg has shown significant antidepressant activity only on chronic administration in tailsuspension model.

A few authors have studied the antidepressant activity of some of the chemical components present in *P. granatum*. Dinesh Dhingra *et al*¹¹ and Naveen S *et al*¹² reported the antidepressant-like activity of ellagic acid, polyphenols and omega-3 fatty acid respectively from pomegranate peel, in mice. Sokinder Kumar *et al* studied the central nervous system activity of PG seed alone, which has shown antidepressant activity similar to the results of our present study.¹³ All these studies reported that individual components of the *P. granatum* fruit had antidepressant activity. The present study suggests that aqueous extract of whole fruit of *P. granatum* has antidepressant activity. This activity might be due to synergistic actions of various chemical components present in peel and seed.

PG is a good source of polyphenols and other antioxidants. In recent years oxidative stress has been implicated in a variety of diseases and these polyphenolic compounds present in different parts of PG act synergistically to prevent oxidative stress induced damage such as in depressive condition. The various phytochemical and bioactive compounds present in the PG fruit in combination are useful in the management of depression. Since the beneficial health effects of pomegranate are increasingly proved, it would be advisable to encourage consumption of pomegranate extract in patients with depression as it provides nutritional supplementation as well as has functional properties.

CONCLUSION

P. granatum 500 mg/kg has significant antidepressant activity on acute and chronic administration, which was comparable to imipramine.

P. granatum 250 mg/kg has shown antidepressant activity only in tailsuspension model on chronic administration of drug.

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CONFLICT OF INTEREST

Nil

REFERENCES

1. While A, Keen L. The effect of statins on mood: a review of the literature. *Eur J Cardiovasc Nurs.* 2012;11(1):85-96. PMID:20875773.
2. Meerts IATM, Verspeek-Rip CM, Buskens CAF, Keizer HG, Bassaganya-Riera J, Jouni ZE, van Huygevoort AHBM, van Otterdijk FM, van de Waart EJ. Toxicological evaluation of pomegranate seed oil. *Food Chem Toxicol.* 2009;47(6):1085-92. <http://dx.doi.org/10.1016/j.fct.2009.01.031>; PMID:19425183.
3. Viuda-Martos M, Fernandez-Lopez J, Perez-Alvarez JA. Pomegranate and its many functional components as related to human health: a review. *Compr Rev Food Sci Food Saf.* 2010;9(6):635-54. <http://dx.doi.org/10.1111/j.1541-4337.2010.00131.x>.
4. Al-Muammar MN, Khan F. Obesity: the preventive role of the pomegranate (*Punica granatum*). *Nutrition.* 2012;28(6):595-604. <http://dx.doi.org/10.1016/j.nut.2011.11.013>; PMID:22342388.
5. Larrosa M, Gonzalez-Sarrrias A, Yanez-Gascon MJ, Selma MV, Azorin-Ortuno M, Toti S, *et al.*, . Anti-inflammatory properties of a pomegranate extract and its metabolite urolithin-A in a colitis rat model and the effect of colon inflammation on phenolic metabolism. *J Nut Bio-chem.* 2010;21(8):717-25. <http://dx.doi.org/10.1016/j.jnutbio.2009.04.012>; PMID:19616930.
6. Bagri P, Ali M, Aeri V, Bhowmik M, Sultana S. Antidiabetic effect of Punicagrana- tum flowers: effect on hyperlipidemia, pancreatic cells, lipid peroxidation and antioxidant enzymes in experimental diabetes. *Food Chem Toxicol.* 2009;47(1):50-4. <http://dx.doi.org/10.1016/j.fct.2008.09.058>; PMID:18950673.
7. Campos-Esparza MDR, Torres-Ramos MA, "Neuroprotection by natural polyphenols: molecular mechanisms," *Central Nervous System Agents in Medicinal Chemistry.* 2010;10(4):269-77. <http://dx.doi.org/10.2174/187152410793429728>.
8. Zhuang H, Du J, Wang Y. Antioxidant capacity changes of 3 cultivar Chinese pomegranate (*Punica granatum* L.) juices and corresponding wines. *Journal of Food Science.* 2011;76(4):606-11. <http://dx.doi.org/10.1111/j.1750-3841.2011.02149.x>; PMID:22417342.

9. Singh DB, Kingly ARP, Jain RK. Studies on separation techniques of pomegranate arils and their effect on quality of anardana. *Journal of Food Engineering*. 2007;79(2):671-74. <http://dx.doi.org/10.1016/j.jfoodeng.2006.02.026>.
10. Melgarejo P, Martínez R, Hernández F, Martínez J-J, Legua P. Anthocyanin content and colour development of pomegranate jam. *Food and Byproducts and Processing*. 2011;89(4):477-81. <http://dx.doi.org/10.1016/j.fbp.2010.11.004>.
11. Dhingra D, Chhillar R. antidepressant-like activity of ellagic acid in unstressed and acute immobilization-induced stressed mice. *Pharmacological Reports*. 2012;64(4):796-807. [http://dx.doi.org/10.1016/S1734-1140\(12\)70875-7](http://dx.doi.org/10.1016/S1734-1140(12)70875-7).
12. Naveen S, Siddalingaswamy M, Singsit D, Khanum F. Anti-depressive effect of polyphenols and omega-3 fatty acid from pomegranate peel and flax seed in mice exposed to chronic mild stress. *Psychiatry Clin Neurosci*. 2013;67(7):501-8. <http://dx.doi.org/10.1111/pcn.12100>.
13. Kumar S, Maheshwari KK, Singh V. Central nervous system activity of acute administration of ethanol extract of *Punica granatum* L. seeds in mice. *Indian J Exp Biol*. 2008;46(12):811-16. PMID:19245177.
14. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: screening test for anti-depressants. *Archives of International Pharmacodynamics*. 1977;229(2):327-36.
15. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology*. 1985;85(3):367-70. <http://dx.doi.org/10.1007/BF00428203>; PMID:3923523.