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Original Article

Screening of Antidepressant Activity of *Punica granatum* in Mice

Rajeshwari Shastry, Aadhya Sharma, Vinay Sayeli, Ullal Sheetal Dinkar*

ABSTRACT

Introduction: India is a rich source of medicinal plants used therapeutically to treat various disorders including depression. This study was undertaken to evaluate the antidepressant effect of acute and chronic administration of *Punica granatum* (pomegranate) whole fruit in mice. **Methods:** We used the aqueous extract of *Punica granatum* (250 and 500 mg/kg per day), standard drug used was Imipramine (10 mg/kg) and vehicle was gum acacia (10 ml/kg), orally. Four groups of animals were used and each group had six animals. In the acute study drugs/vehicles were administered 60 min prior to the experiments. In the chronic study drugs/vehicles were administered for 14 days and the last dose was given on the 14th day, 60 minutes prior to experiment. Forced Swim Test and Tail Suspension Test were used for testing antidepressant activity. Data was analyzed using one-way ANOVA with drug treatment as the independent factor. Post-hoc comparisons were performed using Dunnett's test. **Results:** In acute and chronic forced swim test as well as acute tail suspension test, duration of immobility was significantly reduced in the PG 500 mg/kg, but not in the 250 mg/kg treated group. In chronic tail suspension test, duration of immobility was significantly decreased in PG 250 mg/kg and 500 mg/kg treated groups. The antidepressant activity of 500 mg/kg was comparable to that of Imipramine 10 mg/kg. **Conclusion:** The present study suggests that aqueous extract of whole fruit of *P. granatum* has antidepressant activity at 500 mg/kg. It would be advisable to encourage consumption of pomegranate extract in patients with depression because of its nutritional and functional properties.

Key words: Depression, Tail suspension test, Forced swim test, Pomegranate.

Rajeshwari Shastry, Aadhya Sharma,Vinay Sayeli, Ullal Sheetal Dinkar*

Department of Pharmacology, Kasturba Medical College, Mangaluru,Manipal University, Karnataka, INDIA.

Correspondence

Dr. Ullal Sheetal D, Associate Professor, Department of Pharmacology, Kasturba Medical College, LHH Road, Mangaluru, Manipal University. India

Phone: 91-9448306242

E-mail: sheetal.ullal@manipal.edu

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INTRODUCTION

Depression is a chronic illness that affects people of all ages. Although there are many effective antidepressants available today, the current armamentarium of therapy is often inadequate, with unsatisfactory results in about one-third of all subjects treated.[1] This provides impetus to the search of newer and more effective antidepressants. Limitations to the use of available synthetic drugs open a way for alternative treatments for depression.

Plants have always been a source of drugs and herbal medicines are one of the ancient therapies that have stood the test of time. Pomegranate or *Punica granatum* L. (PG) is widely consumed as a fresh fruit and juice. It belongs to the family Punicaceae. In India it is native of Himalayas in northern India. It has been cultivated over the entire Mediterranean region since ancient times.[2] The pomegranate fruit has valuable compounds in different parts of the fruit- the peel, seeds, and arils. The peel is rich in many compounds such as gallic acid, ellagic acid, catechin, epicatechin, epigallocatechin-3-gallate, quercetin, kaempferol, luteolin, rutin, kaempferol-3-O-glycoside, kaempferol-3-O-rhamnoglycoside, anthocyanins, gallagyldilacton, pedunculagin, tellimagrandin and many minerals including potassium, nitrogen, calcium, magnesium, phosphorus, and sodium. The major chemical components identified from the seeds are punicic acid, linoleic acid, oleic acid, palmitic acid, stigmasterol, β-sitosterol, daucosterol, camesterol, cholesterol, 17-α-estradiol, estrone, testosterone, estriol, tocopherols, ursolic acid, oleanolic acid, isoflavones and phenyl aliphatic glycosides/lignins. The aril contains sugars, pectin, polyphenols, anthocyanins, fatty acids, amino and organic acids, indoleamines, sterols, triterpenoids and α-tocopherol.[3,4]

Pomegranate is commonly used in folk medicine, for eliminating parasites, as an anthelmintic and vermifuge, as an antipyretic and to treat aphthus ulcers, diarrhea, acidosis, dysentery, hemorrhage, microbial infections, and respiratory pathologies.[5] Different parts of *P. granatum* fruit have been displayed to have antioxidant,[3] anticancer,[3] antitumoral,[3] antihepatotoxic,[3]anti-inflammatory,[5] antidiabetic,[6] and antiatherogenic[3] properties. It is also said to be effective in alzheimer's disease.[3,7] Different authors have studied various parts of PG based products such as juice, wine,[8] dried arils[9] and jam.[10] However the medicinal properties of *P. granatum* as a whole fruit

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have been scantily studied. The synergistic action of the constituents of the whole fruit may be superior to that of individual constituents. The CNS activity of *P. granatum* is a less touched field and there is no report on antidepressant activity of *P. granatum* as a whole fruit (peel, aril and seed). Hence the present study was planned to explore the antidepressant activity of water extract of *P. granatum* whole fruit on acute and chronic administration in mice.

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 ± 2°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 test[14] 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 ± SD)	Chronic forced swim test immobility in seconds (Mean ± SD)
Normal control	128 ± 17.24	129 ± 16.62
Imipramine 10 mg/kg	70.83 ± 7.81[a]	75 ± 7.56[a]
PG 250 mg/kg	108.33 ± 17.89	110 ± 17.29
PG 500 mg/kg	92 ± 17.36[b]	92.17 ± 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 ± SD)	Chronic tail suspension test immobility in seconds (Mean ± SD)
Normal control	275.50 ± 24.48	292.83 ± 27.05
Imipramine 10 mg/kg	207.00 ± 17.10[a]	205.50 ± 11.59[c]
PG 250 mg/kg	239.00 ± 9.82	242.17 ± 9.35[d]
PG 500 mg/kg	224.83 ± 28.19[b]	237.67 ± 31.06[e]

[a]p=0.002 vs normal control; [b]p=0.04 vs normal control; [c]p=0.037 vs normal control;[c]p=0.001 vs normal control; [d]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 polyphenolic 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 tail suspension 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 tail suspension model on chronic administration of drug.

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CONFLICT OF INTEREST

Nil

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