

Evaluations of antidepressant activity of *Punica granatum* peel extract in albino mice

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ABSTRACT

Background: Depression is the most common disorder of mental illnesses and affects excess of 10-15% of population. According to the WHO reports, more than 350 million persons suffer from depression all over the world. The aim of present study is to evaluate anti-depressant activity of *Punica granatum* peel extract (PgPE) in albino mice.

Methods: Male albino mice (20-30 g) were used. Animals were divided into 5 groups with 6 animals in each which were subjected to forced swim test. Group 1 is control, group 2 received (standard) imipramine 10 mg/kg, p.o, group 3 (T1) PgPE 50 mg/kg, p.o, group 4 (T2) PgPE 100 mg/kg, p.o, group 5 (T3) PgPE 200 mg/kg, at first animals were forced to swim for 15 min (trained), and the study was performed after 24 hrs. All the animals were treated with individual drug 60 min prior to study, animals were forced to swim for 6 min and the duration of immobility was recorded. The mouse was considered immobile when it floats motionlessly or made only those moments necessary to keep its head above the water surface. The total duration of immobility of each mouse was recorded after the test in each group.

Results: The duration of immobility is significantly reduced at PgPE 200 mg/kg and results were analysed by one way analysis of variance (ANOVA).

Conclusions: PgPE 200 mg/kg significantly ($p < 0.05$) decreases the duration of immobility in mice.

Keywords: *Punica granatum* peel extracts, Antidepressant, Forced swim test

INTRODUCTION

Depression is the most common disorder of mental illnesses and affects excess of 10-15% of population. Depressive disorders are amenable to pharmacology treatment, developed since the 1950s.¹ Patients with depressed mood experience a loss of energy and interest, feelings of guilt, difficulty in concentrating, loss of appetite, libido, and thoughts of suicide.² Prevalence of the depression is about 10-25% in females and 5-12% in males.³ According to the WHO reports, more than 350

million persons suffer from depression all over the world. Approximately two thirds of depression patient's experience suicidal thoughts 10-15% of them being dead from suicide before the age of 40.⁴ Many of these patients being unaware of their disease do not receive any medications. Several synthetic antidepressant drugs are available in pharmaceutical markets; however, their effectiveness is not satisfactory along with undesirable side effects such as dry mouth, hypotension, fatigue, sexual dysfunction and drowsiness as well as drug interactions as the major restrictions for the clinical utility.⁵ People from different regions of the world have

been used medicinal herbs to alleviate disorders for many years and the entrance of new herbal medicines in the pharmaceutical industry has been hot topics in the last decades.⁶

Over the past decade, pomegranate (*Punica granatum*) is titled as a wonder fruit because of its voluminous pharmacological properties. In 1830, *Punica granatum* fruit was first recognized in United States Pharmacopeia; the Philadelphia edition introduced the rind of the fruit was introduced. There are significant efforts and progress made in establishing the pharmacological mechanisms of peel (pericarp or rind) and the individual constituents responsible for them. However, the medicinal properties of *Punica granatum* as a fruit peel have very scantily studies. The CNS activity of *Punica granatum* peel extract is less touched field and there are rare reports on antidepressant activity of *Punica granatum*. Hence the present study was planned.

This review provides an insight on the phytochemical components that contribute too on nephroprotective by Nidhal et al, hepatoprotective by Eshwaraiah et al, antidiabetic by Enas et al, antioxidant by Gurpreet et al, anti-angiogenesis by Dana et al, analgesic and anti-inflammatory by Mithun et al, anti-cancer activity by Lansky, anti-diarrheal activity by Qnais et al, antibiotic resistance by Shaza et al and antiproliferation by Mehmet, of *Punica granatum* have been reported.⁷⁻¹⁶ These phytochemical components contribute wonderful, economic and eco-friendly effects of pomegranate peel extract.¹⁷ The antidepressant activity of *Punica granatum* is mentioned in the Indian system of traditional medicine, but there is a rare scientific evidence to prove its activity by Shamima et al.¹⁸ Hence, the present study is designed to evaluate the antidepressant activity of *Punica granatum* peel extract using animal models in mice was planned.

Botanical classification

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Myrtales
- Family: Punicaceae
- Genus: Punica L.
- Species: *Punica granatum* L.

The dried peel of *Punica granatum* L. is extensively used in Ayurvedha, Siddha and Chinese medicinal systems. Different parts of the tree like Roots, Bark, Flowers, Fruit, Fruit peel, Pulp and Seeds.

Aim of the study

The aim of present study is to evaluate anti-depressant activity of *Punica granatum* peel extract in albino mice.

METHODS

Animals

Male albino mice of weighing 20-30 grams were used in this study; laboratory bred albino mice were obtained from M/S Sainath Agencies, Hyderabad and placed in individual cages in central animal house of department of Pharmacology, Chalmeda Anand Rao Institute of Medical sciences, Bommakal, Karimnagar, Telangana, the animals were stabilized for 1 week under standard conditions at temperature 25±1°C, 60±5% relative humidity and 12 hrs dark light cycles. They had been given free accesses to standard pellet diet and water ad libitum. The study was conducted according to the ethical norms approved by the IAEC, and was carried out in accordance with the recommendations of CPCSEA. Ref: CAIMS/IAEC/Research Project/Lr/04/2016/CPCSEA.

Acute oral toxicity study

The *Punica granatum* peel ethanolic (PgPE) extract were found to be safe up to the dose level of 2000 mg/kg, p.o., and did not produce any toxic symptoms. The survived animals were sacrificed, and complete absorption of the drug through gastrointestinal tract was observed. Hence, 1/20th and 1/10th of maximum therapeutic dose (2000 mg/kg) were selected for the pharmacological models.

Solvents: Normal saline and distilled water were used.

Plant material: *Punica granatum* peels extract (PgPE).

Drug: Imipramine (Depsonil).

Instruments

Syringes (2 ml and 5 ml), tuberculin syringes, infant feeding tube and beakers.

Apparatus: Aquarium (25×18×25 cm), weighing machine and stop watch.

Miscellaneous: Measuring jar, marking pens and cotton.

Preparation of extract

Pomegranate fruit were collected from local market of Karimnagar, and they were identified by the HOD, Department of Botany, S.R.R. Degree and P.G. College of Sciences, Karimnagar. Pomegranate fruit peel were manually separated, shade dried and grounded to coarse particles weighing 250 grams underwent 8 cycles of extraction which was carried out with Soxhlet apparatus using 50% ethanol. The extracts were pooled and concentrated under vacuum at 45°C.¹⁹

Experimental design

Male albino mice of weighing 20-30 grams were used. Animals were divided into 5 groups with 6 animals in each which were subjected to forced swim test (FST). Group 1 (control) received distilled water 10 ml/kg, p.o, group 2 (standard) received imipramine 10 mg/kg, p.o, group 3 (test 1), group 4 (test 2) and group 5 (test 3) received ethanolic peel extract of *Punica granatum* i.e., 50 mg/kg, 100 mg/kg and 200 mg/kg, p.o. respectively.²⁰ All group animals were individually forced to swim in an open aquarium (25×18×25 cm) containing 18 cm of water at 25°C. At first animals were forced to swim for 15 min (trained), and the study was performed after 24 hrs. All the animals were treated with individual drug 60 min prior to study, animals were forced to swim for 6 min and the duration of immobility was recorded. The mouse was considered immobile when it floats motionlessly or made only those moments necessary to keep its head above the water surface (Figure 1). Animals were removed from water, dried with a clean towel and placed back in cages. The water was changed after each test. The total duration of immobility of each mouse was recorded after the test in each group.^{21,22} After completion of forced swim test animals were transferred back to the central animal house.

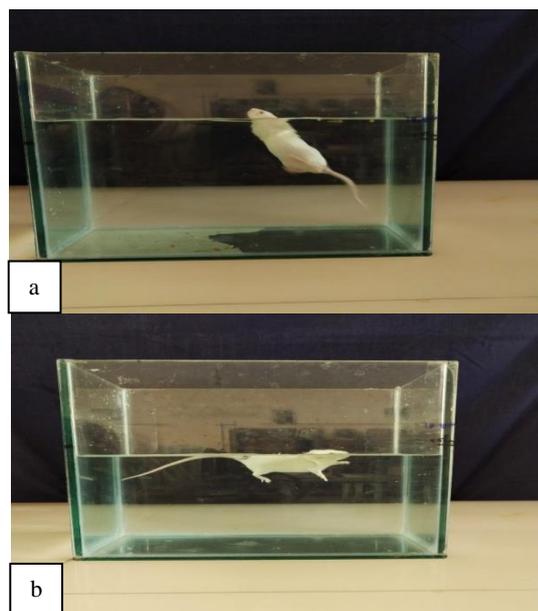


Figure 1: (a) Mobility and (b) immobility of mouse in aquarium.

Observations were recorded and results were analysed by one way analysis of variance (ANOVA).²³

RESULTS

In this current study, the results obtained in the FST are mild significant at a dose of 100 mg/kg where as it is highly statistically significant at dose of 200 mg/kg (Table 1 and Figure 2). Animals after antidepressant

treatment struggle more even in desperate situation, and they spend less time with immobility. Results showed that *Punica granatum* peel extract produces a diminution of the duration of immobility time of animals when exposed to FST.

Punica granatum peel extract significantly ($p < 0.05$) decreases the duration of immobility in mice.

Table 1: Result of FST.

Groups	Parameters	% of immobility
Normal (control)	Mean±SD	2.27±0.64
	SEM	-
Standard (imipramine)	Mean±SD	1.76±0.40**
	SEM	0.166
Test 1	Mean±SD	2.49±0.28
	SEM	0.11
Test 2	Mean±SD	2.13±0.31*
	SEM	0.12
Test 3	Mean±SD	1.96±0.23**
	SEM	0.09

All the values are expressed as mean±SEM of all group animals. *P value <0.05, **P value <0.01.

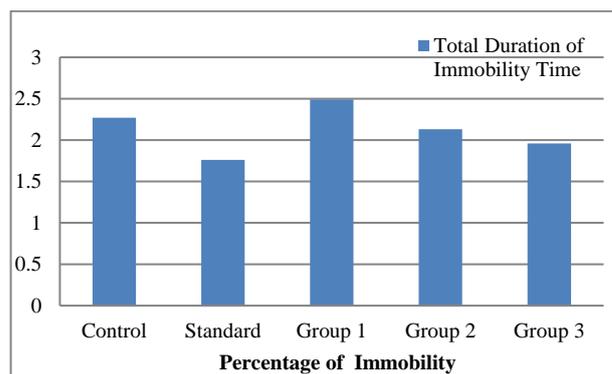


Figure 2: Total duration of immobility time.

DISCUSSION

The current study is a behavioral studies where when a normal animal is submitted to a difficult or inescapable situation; it alternates between two kinds of behavior that is agitation and immobility. Intense motor activity and expense of energy such as struggling and body jerks are named as searching-behavior. Whereas, immobility and energy saving, in studies i.e., when the animal stops struggling is characterized as waiting-behavior, and it is assumed that antidepressant effect is in favor of the searching behaviour.²⁴ Present study, FST model play an important role in the evaluation and development of antidepressant drugs.²⁵ In the present study, the PgPE (200 mg/kg, p.o.) administered to mice produced significant antidepressant effect in FST and its efficacies were found to be comparable to imipramine (10 mg/kg, p.o.) (Table 1 and Figure 2). From all the above, the antidepressant activity of PgPE was found to be

significant at 200 mg/kg, p.o. A few authors have studied the antidepressant activity of some of the chemical components present in *Punica granatum* peel. Dhingra et al and Naveen et al reported the antidepressant-like activity of ellagic acid, polyphenols and omega-3 fatty acid respectively from pomegranate peel, in mice.^{26,27} Abdul et al reported the antidepressant like activity of polyphenols respectively from *Punica granatum* methanolic extract in mice.¹⁸

From the discussion it is suggested that *Punica granatum* peel extract flavonoids and polyphenols components might produce antidepressant effect by interaction with adrenergic receptor there by increasing the level of noradrenaline along with reduction in level of serotonin and dopamine in brains of mice. This showed that PgPE has an antidepressant like effect but further molecular study should be investigated to confirm it.

CONCLUSION

The ethanolic extract of *Punica granatum* peel extract at the dose of (200 mg/kg) has shown significant antidepressant activity when compared to the control. Amongst all the groups imipramine has good stability and activity. Peel extract of *Punica granatum* has shown significant antidepressant activity in forced swim test (FST).

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Ethical approval: The study was approved by the Institutional Ethics Committee

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