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The Antidepressant-Like Effects of *Punica granatum* (Pomegranate) Extract in Mice

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Abstract

The aim of the present work is to evaluate the putative antidepressant-like effects of pomegranate fruit extract including seeds (PFE) on the performance of male mice in the forced swimming test (FST), after acute administration, after short-term treatment (7 days) and, after repeated administration in a 24-h period (24, 12 and 1 h before swimming test). A single dose (20 ml/kg p.o.) of PFE, in male mice provoked a significant reduction of the immobility time. Such effect was also observed with short-term treatment (7 days) with doses of 1 and 10 ml/kg/day of PFE. Moreover, it was noted that there were important differences in the onset of the antidepressant-like effect in the FST, depending on the modality of treatment with PFE. Both efficacy and potency were higher when repeated administration of PFE was used, and surprisingly the dose of 10 ml/kg (24, 12 and 1 h before swimming test) was as effective as Fluoxetine. In the same way, the short term administration (7 days) improved significantly efficacy and potency of the PFE in comparison to a single dose treatment. These results indicate an antidepressant-like profile of action for PFE which deserves further research.

Keywords

Pomegranate; Antidepressant Effect; Forced Swimming Test

1. Introduction

Depression is a major disease affecting nearly 13% - 20% of the population [1]. Roughly, 90% of patients with depressive symptoms suffer from mild to moderate depression, while only 10% are severely depressed. In mild

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to moderate depression, in particular, some practitioners and patients are reluctant to use standard antidepressants like tricyclic antidepressants or specific serotonin reuptake inhibitors (SSRIs), because of their side effects. Therefore, additional treatment strategies with fewer side effect profile, credible benefits and moderate costs are of particular interest [2] [3]. In the search for new molecules useful for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [4]. At the present time, forced swimming test (FST) is the most widely used model for assessing potential antidepressant activity in rodents, following acute and short-term treatment [5].

Pomegranate (*Punica granatum*), a small tree originating in the Orient, belongs to the Punicaceae family [6]. It is grown mainly in Iran, India and in most Near and Far East countries. The main use of pomegranate is as table fruit, but large amounts are used in the beverage and liquor industries [7]. In folk medicine, pomegranate preparations have a number of therapeutic actions [8].

The edible part of pomegranate is rich in anthocyanins thus; the major class of phytochemical present in pomegranate is the polyphenols and includes flavonoids, condensed tannins and hydrolysable tannins. Hydrolysable tannins are predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity [9] [10].

The present research was undertaken to study the duration of immobility in mice in the FST, after acute administration, after short-term treatment (7 days) and, after repeated administration in a 24-h period (24, 12 and 1 h before swimming test) following oral ingestion of a standardized preparation of pomegranate fruit extract (PFE).

2. Materials and Methods

2.1. Pomegranate Extract

Pomegranates (*Punica granatum* L.), was collected by one of the colleagues from the agriculture garden (under supervision of Agricultural Research Organization in Fars Province) in Shiraz, a city where known to have one of the best pomegranate native in Iran. Then washed, chilled to 4˚C, and stored. The seeds of the fruit containing the intact juice sacs were manually separated from the pericarp, and the whole juice extracted by the aid of electric juicer so that seeds break. Then filtered and stored in clean jars in fridge.

2.2. Animals

Male BALB/c mice, weighing 25 - 30 g were used in these experiments. They were group housed under the following laboratory conditions: temperature 23˚C ± 1˚C, humidity 40% - 60%, 12 h:12 h L/D cycle, lights on at 07:00 h. Food and water were available *ad libitum*. All the experiments were carried out between 10:00 and 15:00 h in testing rooms adjacent to the animal rooms. Each experimental group consisted of ten mice. Mice were treated in accordance with the current law and the NIH Guide for the Care and Use of Laboratory.

2.3. Drugs

Fluoxetine (Sigma) were suspended in normal saline (0.9% NaCl).

2.4. Behavioral Tests

This test was performed according to the procedure described by Porsolt *et al*. [11]-[13], with slight modifications [14]. Briefly, 1 h after dosing, the animals were individually forced to swim in a transparent glass vessel (25 cm high, 15 cm in diameter) filled with (12.5 cm high) water at 21˚C - 24˚C. The total duration of immobility (in seconds) was measured during the last 4 min of a single 6 min test session. Five groups of 10 mice were treated acutely with single dose of vehicle, PFE (1, 10 and 20 ml/kg) p.o. (Gavage), Fluoxetine i.p. (10 mg/kg) and 1 h later mice were individually forced to swim in the glass vessel. Other five groups of mice were short-term treated (7 days) with a single daily dose of vehicle, PFE (1, 10 and 20 ml/kg) p.o., or Fluoxetine i.p. (10 mg/kg), and 1 h after the last administration they were individually forced to swim in same conditions. In a different set of experiments, five groups of 10 animals were subjected to repeated administration of three doses of vehicle, PFE using 1, 10 and 20 ml/kg p.o., Fluoxetine i.p. (10 mg/kg), 24, 12 and 1 h prior to the swimming test. Behaviour was monitored from the frontal side by a video camera for subsequent analysis. Mice were consid-

ered immobile when they made no further attempts to escape except the movements necessary to keep their heads above the water.

2.5. Statistical Analysis

The results are expressed as mean ± S.D, and statistical analysis of the data was performed by Origin VI. $^{***}p < 0.001$; $^{**}p < 0.01$ were considered significantly different from vehicle, after non-parametric ANOVA**.**

3. Results

Effects of PFE on the Immobility Time

A significant shortening of the immobility time was observed when an acute dose of either 20 ml/kg p.o., of PFE ($p < 0.01$) or fluoxetine (10 mg/kg i.p.) ($p < 0.001$) was administered to mice compared to vehicle treated animals. The reduction of immobility time, between these two mentioned groups was at the same level, statistically (**Figure 1**). Short-term treatment (7 days) induced a decrease of the immobility time when the animals were exposed to the FST after dosing orally, once a day, with 1 ($p < 0.001$) and 10 ($p < 0.001$) ml/kg of PFE (**Figure 1**). In addition, repeated administration in a 24 h period showed a significant reduction of the immobility time with 1 ($p < 0.01$) and 10 ml/kg ($p < 0.001$) of PFE in different groups of mice submitted to FST in comparison to control group (**Figure 2**).

4. Discussion

The present study characterized the effects of the administration of PFE on mice performance in FST following acute, short-term and repeated treatment. In many previous papers the antioxidant and anti-inflammatory properties of pomegranate fruit juice in animal labs has been emphasized [15]-[18]. Also, it has been reported that Pomegranate extract improves a depressive state and bone properties in menopausal syndrome model ovariectomized mice [19]. Besides that, it was demonstrated that PFE exhibits low toxicity, no lethality, was well tolerated, did not induce significant changes in several behavioural and physiological parameters and is devoid of any hypnosedative activity, when administered to mice [15]. Recently, the antidepressant action of some herbal medicines has been reported focusing on polyphenols as their major component [3] [4] [14]. The edible part of pomegranate is rich in anthocyanins thus; the major class of phytochemical present in pomegranate is the polyphenols.

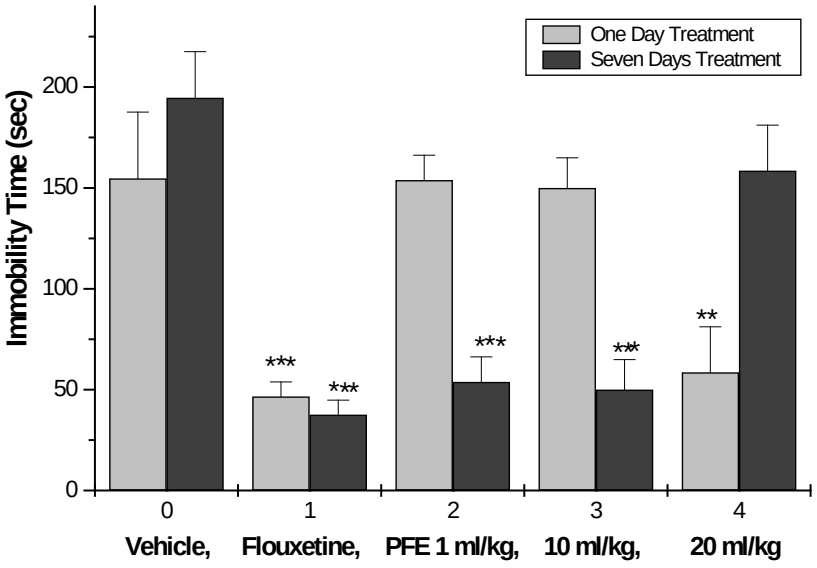

Figure 1. Effect of the vehicle, fluoxetine (10 mg/kg, i.p.) and increasing single doses of PFE (1, 10 and 20 ml/kg p.o) on the forced swimming test (FTS) in mice. Each bar represents the mean ± SD of 10 animals. $^{***}p < 0.001$; $^{**}p < 0.01$, significantly different from vehicle, after non-parametric ANOVA.

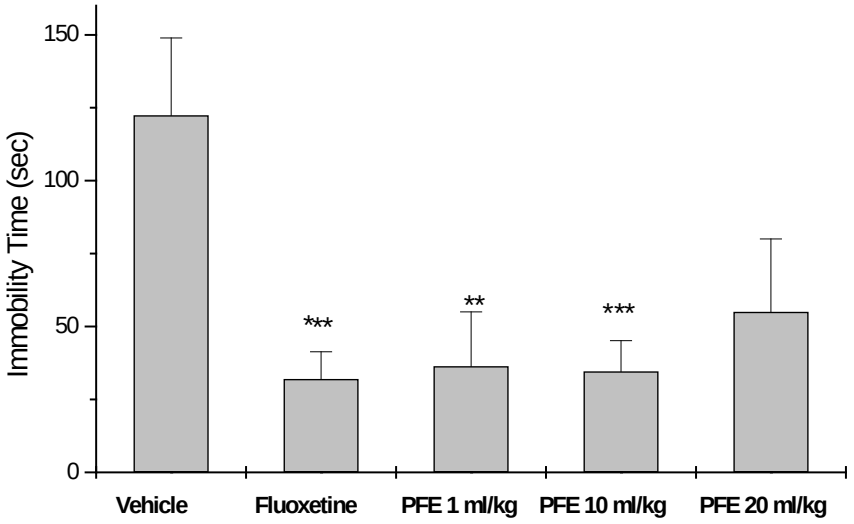

Figure 2. Effect of repeated administration of three doses in 24 h of vehicle, fluoxetine (10 mg/kg i.p) and increasing doses of PFE (1, 10, and 20 ml/kg p.o) on the forced swimming test (FTS) in mice. Each bar represents the mean ± SD of 10 animals. $^{***}p < 0.001$; $^{**}p < 0.01$, significantly different from vehicle, after non-parametric ANOVA.

Because the pharmacotherapy of depression typically requires chronic drug treatment to obtain a full response in terms of antidepressant effect, it is critical to perform, not only acute, but also short-term, chronic and repeated treatments in the FST mice model. Certainly, the administration of PFE produced a diminution of immobility time of mice exposed to the FST under acute, short-term or repeated modalities. Indeed, single dose of 20 ml/kg p.o., of PFE provoked a highly significant reduction of immobility time ($p < 0.01$). Similar results were also observed with short-term treatment (7 days) with single dose of 1 ($p < 0.001$), and 10 ($p < 0.001$) ml/kg/day of PFE, but not with higher dose. Additionally, in a different set of experiments, repeated administration in a 24-h period (24, 12 and 1 h before swimming) doses of 1 ($p < 0.01$) and 10 ($p < 0.001$) ml/kg p.o., of PFE provoke significant reduction of the immobility time of male mice subjected to the FST, when compared to control group (vehicle) but not with higher dose. It is important to note that although single acute high dose, showed antidepressant effect as fluoxetine, no significant effects observed in high dose of 20 mg/kg of PFE in repeated administration. This can be interpreted as acute tolerance or tacyfilaxy that might have happened in receptor responses. Moreover, depending on the modality of treatment with PFE (acute, short-term or repeated) in the FST, it was observed important differences in the onset of the antidepressant-like effect. In fact, both efficacy and potency of PFE as antidepressant were higher when repeated administration was used, and interestingly 10 mg/kg (24, 12 and 1 h before swimming) was as effective as fluoxetine (10 mg kg i.p.) used as positive control. In the same way, the short term administration (7 days), improves significantly the efficacy and potency of the PFE, in comparison to single dose treatments. These behavioural effects were similar to those seen in mice treated with conventional antidepressant drugs, such as tricyclic, monoamine oxidase inhibitors and selective serotonine reuptake inhibitors agents [5] [11] [20].

5. Conclusion

In conclusion, the results of this preclinical study provide evidence about the antidepressant effects of the pomegranate fruit extract administered either acute or repeatedly in mice. These results contribute to the scientific validation of the indications of this plant in Iranian folk medicine. However, further experiments are needed to identify its active compounds and the corresponding mechanisms of action. Our results encourage us to pursue the identification of the molecules associated to the effect observed in PFE.

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Note List of Abbreviations

Pomegranate fruit extract (PFE)
Forced swimming test (FST)
Specific serotonin reuptake inhibitors (SSRIs)