



## Comparative Screening of Adaptogenic Activity of *Punica Granatum* (Pomegranate) Peel and Juice

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### ABSTRACT

Stress disturbs the normal physiological condition and has been postulated to be involved in the etiology of variety of diseases ranging from psychiatric disorders such as anxiety and depression, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, peptic ulcer, hypertension and ulcerative colitis. Additionally, the problems of tolerance and physical dependence on the prolong use of synthetic drugs, limits their clinical utility. Therefore, there is a need for effective herbal anti-stress agent in the therapy of stress and stress induced disorders. Keeping this in mind, the evaluation and comparison of the adaptogenic potential of Hydroalcoholic extract of dried peel of Pomegranate (PPE) and fresh Pomegranate juice (PJ) was studied in rats. The present study was undertaken to investigate the influence of forced swimming endurance stress (Acute stress) on swim time, alterations in biochemical parameters and changes in WBC count in rats. This activity was tested at oral doses of 200mg/kg and 400mg/kg for hydroalcoholic extract of dried peel (PPE) and at 4ml/kg and 8ml/kg for fresh juice (PJ) of Pomegranate, using *Withania somnifera* (100mg/kg) as a standard reference drug. Pretreatment with the extract and juice at different doses significantly ameliorated the stress induced variations in the biochemical parameters namely- serum ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), glucose, cholesterol and triglycerides. There was significant increase in swim time in both extract and juice treated groups. The ability of *Punica granatum* extract and juice to prolong the swim time and ameliorate the stress induced changes in forced swimming endurance stress model, therefore, suggests an adaptogenic activity.

**Keywords:** Adaptogenic, Anti-stress, Forced swimming endurance stress, *Punica granatum*.

### INTRODUCTION

Physical exercise is known to be a stressor stimulus, both in humans and animals which leads to countless physiological alterations and results in a state of threatened homeostasis. Therefore acute-chronic exercises in animal models may cause high level of stress<sup>1</sup>. Mild to moderate stress can be motivational (Eustress) and improve performance. However, prolonged stress (Distress) is associated with greater risk of Atherosclerosis, Hypertension, Cancer and Psychological disorders (Anxiety and Depression). Since stress is able to affect the state of health, it is reasonable and straight forward to believe that stress is capable of affecting or influencing the protective function of immune system<sup>2</sup>. Stressors range from physical to psychological as cold, heat, infection, and toxin. In order to adapt to stress for survival, the latter triggers a multitude of changes in the body termed as 'General Adaptation Syndrome (GAS)'. This state of resistance against adverse stimuli is termed as 'Adaptogenic activity', while drugs used are called as Adaptogens. Adaptogens should be such that they should enable a person to withstand stress without altering physiological function of the body<sup>3</sup>. Use of several antistress agents, particularly the benzodiazepines like diazepam show significant antistress activity. But the problems of tolerance and physical dependence exhibited by benzodiazepines, on prolonged use, limit their utility<sup>4</sup>.

Herbal adaptogens have been sold in market as modern nutraceuticals or traditional medicines as "Anti-stress formulae". Some of the adaptogenic herbs include-

*Asparagus adscendens*, *Andrographis paniculata*, *Centella asiatica*, *Panax ginseng*, *Ocimum sanctum*, *Morus alba*, *Embllica officinalis*<sup>5</sup>.

Forced swimming endurance stress test is most widely used test for assessing the anti-stress property of a novel compound. This paradigm is based on the observation that animals when forced to swim in water eventually assume a characteristic immobile posture. This immobility posture therefore reflects a state of tiredness, fatigue, reduced stamina with the end point being the inability of rat to swim further and start drowning<sup>6</sup>.

*Punica granatum* L. (Family: Puniceaceae), known as Pomegranate is a commonly eaten fruit. It is a globose fruit, 5-7.6 cm in diameter, shiny reddish or yellow green in colour when mature. It is filled with crunchy seeds, each of which is encased in a juicy, somewhat acidic pulp which is itself enclosed in a membranous skin<sup>7</sup>. The fruit is of great interest to scientists of pharmaceutical, nutritional and pharmacological research and in new drug development<sup>8</sup>. In Ayurveda, this fruit is regarded as "a pharmacy unto itself", which is used as an anti-parasitic agent, a blood tonic, in diarrhoea and ulcers. It serves as anti-diabetic agent in the Unani system of medicine<sup>9</sup>. The major active constituents reported in peel are- Tannins (Ellagic acid, Gallic acid, Punicalagin, Punicalin, Granatin A, Granatin B, Pedunculagin); Flavonoids (Cyanidin, Delphinidin, Pelargonidin, Anthocyanidin, Quercetin), whereas juice is composed of Gallic acid, Alkaloids (Isopelletierene, Serotonin, Tryptamine, Melatonin); Flavonoids (Epicatechin,



Isoquercetin, Procyanidin, Epigallocatechin-3-gallate), caffeic acid, chlorogenic acid and p-coumaric acid<sup>8</sup>. The synergistic activity of the pomegranate constituents appear to be superior to that of single constituent. The reported activities include- Antidiarrheal<sup>7</sup>, hypolipidemic and antioxidant<sup>10</sup>, antidiabetic<sup>11</sup>, antineoplastic<sup>12</sup>, antiplasmodial<sup>13</sup>, antiepileptic and anti-inflammatory<sup>14</sup>, in alzheimer's disease<sup>15</sup>, antihypertensive<sup>16</sup>.

As the plant *Punica granatum* has a high potential as an adaptogen, the present study was undertaken to investigate and compare the adaptogenic potential of hydroalcoholic extract of dried peel of Pomegranate (PPE) and fresh juice of Pomegranate (PJ) in forced swimming endurance stress model in rats.

## MATERIALS AND METHODS

### Chemicals

All the chemicals used were of analytical grade. Kits for the estimation of biochemical parameters were purchased from SPAN diagnostics kits ltd.

### Animals

Albino Wistar rats weighing 150-200g of either sex were used for this study. The animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60 % humidity). Standard pelletized food and tap water were provided *ad libitum*. The protocol no. 111202 seeking permission for animal experimentation was obtained from IAEC before starting the experiment.

### Plant material and extraction

Fresh specimen of *Punica granatum* flower alongwith stem was collected from University of Mumbai campus and authenticated at Blatter herbarium of St. Xavier's Research Institute, Mumbai. The sample was found to match with blatter herbarium specimen no. 5588. Fresh Pomegranates were purchased from local market. Peels separated from the seeds were dried under shade and powdered, which was then subjected to soxhlet extraction using hydroalcoholic solvent (70% methanol) at a temperature of 55°C for 5-10 complete cycles. Extract obtained was dried under vacuum to obtain solid crude mass of Pomegranate peel extract (PPE) and stored in desiccator for further experimental use. A solution of the extract was prepared by dissolving it in distilled water for oral administration at a dose of 200 mg/kg and 400 mg/kg to respective treatment group animals. Pomegranate juice (PJ) was freshly prepared by crushing and squeezing the manually separated seeds (arils) and administered orally at doses 4 ml/kg and 8 ml/kg to respective treatment group animals.

### Forced swimming endurance stress<sup>6</sup>

Rats of either sex (150-200 g) were used for forced swimming endurance stress. Group I (Vehicle control) and Group II rats (Stress control) received normal saline, Group III rats (Standard) received 100 mg/kg, p.o. of alcoholic

root powder extract of Ashwagandha, Group IV and Group V rats received 200mg/kg and 400mg/kg of hydroalcoholic extract of dried peel of Pomegranate (PPE) respectively, Group VI and Group VII rats received 4ml/kg and 8ml/kg of Fresh juice of Pomegranate (PJ) respectively. Treatment group animals received respective treatment, once daily for a period of 7 days. On 8<sup>th</sup> day, rats of all groups except vehicle control were subjected to swim test by keeping them in propylene tanks (37x37x30cm) filled with water to a height of 25 cm, allowed to swim till complete exhaustion and the end point was taken when the animals began to drown. The mean swimming time for each group was calculated. Blood (2-4ml) was collected by retro orbital puncture for WBC count estimation and also biochemical parameters such as serum ALT, AST, cholesterol, glucose and triglycerides.

### Statistical analysis

All the values are expressed as Mean±SEM and data was analysed by one-way ANOVA using Graph pad INSTAT. The post-hoc analysis was carried out by Bonferroni's multiple comparison test to estimate the significance of difference between individual groups.

## RESULTS AND DISCUSSION

Pretreatment of animals with PPE and PJ, ameliorated the stress induced alterations in biochemical parameters with significant (P<0.05) reduction in elevated levels of ALT, AST, glucose, cholesterol and triglycerides (Table 1).

Pretreatment of animals with PPE and PJ significantly (p<0.05) raised the swim time in rats (Fig. 1). In comparison, extract was found to be more effective than juice in increasing the swim time.

The increase in swim time following treatment with PPE and PJ indicates better stress tolerance by *Punica granatum* which is comparable to the changes produced by reference drug *Withania somnifera*.

Similarly, a significant (p<0.05) decrease in elevated WBC count is observed following treatment with PPE and PJ (Fig 2).

However, maximum effect was seen at higher dose of PPE (400mg/kg) in increasing the swim time and ameliorating the stress induced alterations.

When stress is applied for a short duration of time i.e. acute stress (AS), the physiological changes are self-limiting and adaptive in nature. In this stress, the body sets in motion an array of physiological, biochemical and endocrine responses to counter stress effects. Adaptogens put organs into a state of non-specific heightened resistance in order to resist stressor and adapt to extraordinary challenges. Rasayanas of Ayurveda are found to be effective antistress agents, because they appear to prolong Selye's pronounced second phase of the "GAS", the stage of resistance to stress and prevent the final and third phase of exhaustion<sup>4</sup>.

**Table 1:** Effect of hydroalcoholic extract of dried pomegranate peel (PPE) and fresh juice of pomegranate (PJ) on biochemical parameters in forced swimming endurance stress in rats.

Groups	ALT (IU/L)	AST (IU/L)	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Vehicle control	66.39±1.34	88.11±1.16	103.9±3.56	95.01±3.05	111.5±2.79
Stress control	93.00±4.13 <sup>a</sup>	116.1±3.74 <sup>a</sup>	134.9±2.55 <sup>a</sup>	222.1±12.39 <sup>a</sup>	140.8±1.65 <sup>a</sup>
Standard	62.93±1.76 <sup>x</sup>	99.49±3.36 <sup>z</sup>	82.87±1.94 <sup>x</sup>	138.9±11.23 <sup>x</sup>	112.2±1.88 <sup>x</sup>
PPE <sub>(200mg)</sub>	64.61±3.61 <sup>x</sup>	89.85±1.91 <sup>x</sup>	86.70±2.03 <sup>x</sup>	109.5±7.67 <sup>x</sup>	120.7±3.85 <sup>x</sup>
PPE <sub>(400mg)</sub>	60.23±0.74 <sup>x</sup>	67.05±3.16 <sup>x</sup>	76.74±1.80 <sup>x</sup>	74.38±2.20 <sup>x</sup>	115.0±1.58 <sup>x</sup>
PJ <sub>(4ml)</sub>	65.21±1.52 <sup>x</sup>	81.13±4.94 <sup>x</sup>	106.8±2.57 <sup>x</sup>	159.4±4.66 <sup>x</sup>	128.0±2.60 <sup>z</sup>
PJ <sub>(8ml)</sub>	61.33±0.97 <sup>x</sup>	79.41±4.9 <sup>x</sup>	85.43±4.07 <sup>x</sup>	150.0±4.13 <sup>x</sup>	112.7±1.58 <sup>x</sup>

All values are expressed as Mean±SEM, n= 6. One-way ANOVA followed by post hoc analysis by Bonferroni's Multiple Comparison test is applied. All treated groups were compared with stress control group- <sup>x</sup>significant at p < 0.001 and <sup>z</sup>significant at p < 0.05. Stress control group was compared with vehicle control group- <sup>a</sup>significant at p < 0.001.

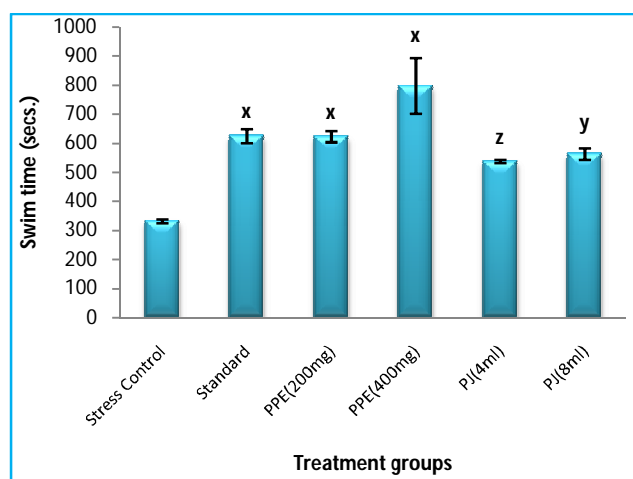
It has been postulated that stress induced changes in biochemical parameters occur rapidly, as increased plasma ACTH level within 5 minutes<sup>17</sup>. Similarly, it has been demonstrated that forced swim test (Acute stress) induced increase in ALT, AST and glucose levels may be the outcome of stimulation of HPA (Hypothalamus-Pituitary Axis). This stimulation may lead to hyperglycaemic response due to release of glucocorticoids and increased glycogenolysis from liver to compensate for initial demand of glucose<sup>1</sup>.

Pretreatment with PPE and PJ showed significant (p<0.001) reduction in elevated levels of ALT, AST and glucose which may be due to inhibition of stimulation of HPA (Table 1).

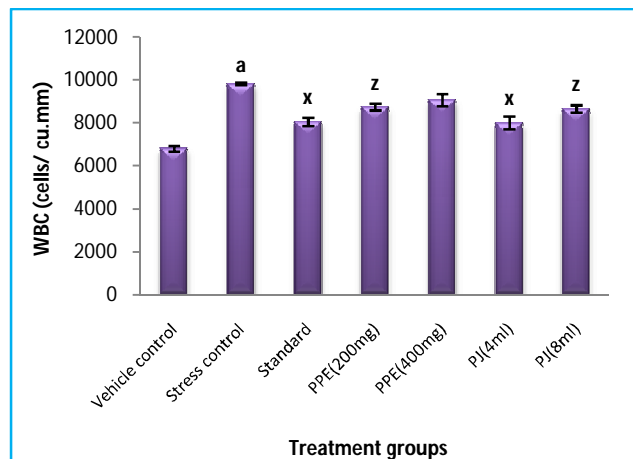
Stress induced increase in cholesterol level was significantly (p<0.001) reduced in PPE and PJ treated animals (Table 1). It is known that corticosterone synthesis by the adrenal cortex is derived from the cholesterol molecule in an oxi-reduction process and it is the parent molecule for the synthesis of all human and animal steroids<sup>1</sup>. Thus significant reduction in cholesterol level hints to simultaneous reduction of cortisol in plasma.

Both PPE and PJ treated animals showed significant (p<0.05) reduction in elevated triglyceride level which may be due to inhibition of sympathetic nervous system<sup>6</sup> (Table 1).

Earlier, it has been demonstrated that moderate stress could raise immune level, by increase in level of WBC. It is believed that under stress, the release of adrenergic stress hormones increase the synthesis and release of cytokines which consequently bring about the production of more WBC and more helper T-cells (CD4 cells) and thus boost the immune system<sup>2</sup>. Both, PPE and PJ treated animals showed significant (p<0.05) reduction in WBC count as compared to stress control which clearly indicates antistress activity (Fig. 2).

**Figure 1:** Effect of hydroalcoholic extract of dried pomegranate peel (PPE) and fresh juice of pomegranate (PJ) on swim time in forced swimming endurance stress in rats.

All treated groups were compared with stress control group- <sup>x</sup>significant at p < 0.001, <sup>y</sup>significant at p < 0.01 and <sup>z</sup>significant at p < 0.05.

**Figure 2:** Effect of hydroalcoholic extract of dried pomegranate peel (PPE) and fresh juice of pomegranate (PJ) on WBC count in forced swimming endurance stress in rats.

All treated groups were compared with stress control group- <sup>x</sup>significant at p < 0.001 and <sup>z</sup> significant at p < 0.05. Stress control group was compared with vehicle control group- <sup>a</sup>significant at p < 0.001.

Various herbal plants are studied for their adaptogenic activity, which are found to be rich in various phytochemicals such as flavonoids, tannins and phenolic glycosides<sup>6</sup>. One of the proposed mechanisms of those active constituents to counteract stress is assumed to be in part due to inhibition of stimulation of HPA and partly due to antioxidant mechanism<sup>4</sup>. It has been proved that both pomegranate peel and juice is rich in bioactives which includes ellagotannins, flavanols, glycosides as cyanidin and delphinidin, polyphenols as quercetin<sup>9</sup> and are probably responsible for counteracting stress and stress induced alterations.

Thus adaptogenic activity of *Punica granatum* extract and juice may be attributed to its highly acclaimed antioxidant activity supported by inhibition of stimulation of HPA.

### CONCLUSION

Present study indicates that *Punica granatum* peel extract (PPE) and juice (PJ) possess antistress and adaptogenic activity.

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