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Pharmacological Evaluation of *Punica granatum* Against Forced Swim- and Cold-Induced Stress in Experimental Animals

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Abstract

The present study was carried out to evaluate the effect ethanolic extract of *Punica granatum* (*P. granatum*) Peel on forced swim test and Chronic cold stress in albino rats. The effect was assessed by swimming survival time, estimation of various biochemical parameters like Glucose, Cholesterol, Triglycerides, Plasma cortisol and Blood urea nitrogen (BUN), In cold stress ulcer index, P^H of gastric juice and weight of organ such as liver, spleen, testes, adrenal gland at a dose of 200,400 mg/kg body weight per oral was determined. It was found that extract significantly ($p < 0.001$) increases swimming time. Showed significant ($p < 0.001$) decrease in blood glucose, cholesterol, triglyceride, cortisol and BUN. In cold stress significant ($p < 0.01$) decrease in weight of organs, significant ($p < 0.001$) decrease in ulcer index and increase P^H of gastric juice was observed compared to control group. Thus the obtained results revealed that the extract of *P. granatum* peel has got a significant anti-stress activity.

Keywords: Swimming, Cold stress, ulcer, EPG

INTRODUCTION

Punica granatum Linn. (Pomegranate) is a plant of Punicaceae family locally known as Anar. *P. granatum* is a fruit of great antiquity and is known to have been cultivated in the Middle East more than 5,000 years ago. The plant is found all over India. The fruit of this plant is used as food and as a diet in convalescence, diarrhea, acidosis, dysentery, microbial infections, diarrhoea, helminthiasis, haemorrhage, and respiratory pathologies¹. *P. granatum* revealed various chemical constituents like ellagic acid, ellagitannins, punicic acid, flavonoids, anthocyanidins, anthocyanins, and estrogenic flavonoids and flavones². Flavonoid-rich polyphenol fractions from the *P. granatum* fruit exert antiproliferative. The potential therapeutic properties of *P. granatum* are wide-ranging and include treatment and prevention for cancer³, Cardiovascular disease⁴, Diabetes⁵, Antioxidant⁶, hepatotoxicity⁷. Pomegranate fruit rind possess excellent antibacterial and anti-diarrhoeal and anthelmintic properties⁸. Other potential applications include infant brain ischemia, Alzheimer's disease⁹, male infertility, arthritis¹⁰, dermal wounds¹¹ and anthelmintic¹². All of the above medicinal uses either from fruit, seed, or leaves and peel of *P. granatum* prompted us to investigate the antistress activity of the *P. granatum* fruit peel extract.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL:

Fresh fruit of *Punica granatum* family Punicaceae, were procured from local vendor Hyderabad, Telangana. Fruits are authenticated by authenticated by Dr. Rafiuddin Naser, Associated Professor, Department of Botany, Moulana Azad college of, Aurangabad, (M.S) (PCOG.H-241).

PREPARATION OF EXTRACTION

The fresh fruits peel around 3 kg shade dried for 15 days, fruit peel material was powdered using mixer grinder and passed through sieve no 85. Weight About 150gm of dried peel powder was subjected to soxhlet's apparatus extraction using ethanol solvent for 72 hrs. The extract were concentrated in rotary flash evaporators and stored in refrigerator.

PHOTOCHEMICAL EVALUATION

Ethanol Extracts of *P. granatum* (EPG) were subjected to different phytochemical tests to detect presence of phytoconstituents.

EXPERIMENTAL ANIMALS

Albino mice 20-25gm and Adult rats (Wistar) of both sexes weighing 160–180 g were purchased from Sanzyme lab Pvt. Animal feed was procured from Mahaveera enterprises, Hyderabad. Animals were accommodated in recommended laboratory environment at 25°C under 12 hr light-dark cycle. All the experimental animals had free access to chow and water *ad libitum*.

FORCED SWIMMING TEST

Swiss albino mice of male and female weighing between 20 ± 5g were randomly cauterized into four groups of six rats in each group.

Group I- Forced swimming test [FST] control,

Group II –FST + EPG 400 mg/kg (p.o) for 3weeks.

Group III- FST + EPG 600 mg/kg (p.o) for 3 weeks

, Group IV - FST + Gerifort syrup 2ml/kg (p.o) respectively for 3weeks.

FST methods was described is modified in our laboratory. Above treatment was given to mice for 3 weeks. On 1st week, 2nd week and 3rd week 1 hr. after treatment, all the mice (except normal control) were subjected to swimming endurance test. The mice were allowed to swim individually in swimming tank (30 cm height with 20 cm diameter) containing water of 25 cm height maintained at 25 ± 1°C temperature. The mice were allowed to swim till exhausted and moment when animal drowned is considered as the endpoint. The mean swimming time for each group was calculated.¹³⁻¹⁴

CHRONIC COLD STRESS

Albino rats 160-180gms, male and female were cauterized in to five groups of six animals each.

Group-I Control

Group-II Chronic Cold stress [CCS],

Group-III -CCS + EPG 300 mg/kg p.o,

Group-IV - CCS + EPG 500 mg/kg p.o and

Group-V - CCS + Gerifort 2ml/kg p.o respectively for all 10 days.

Chronic Cold Stress was induced in 2nd, 3rd, 4th and 5th groups on animals, by exposing. 4 ± 1°C temperature every day for 1 hrs for 10 days. On 10th day animals were sacrificed blood were collected for estimation of Cholesterol, Glucose, TG, Plasma cortisol and BUN¹⁵⁻¹⁸. The animals were dissected and the stomach carefully keeping the esophagus closed opened along the greater curvature the gastric contents were collected in a tube and centrifuged at 3000 rpm for 5 min, the volume of

supernatant was expressed as ml/100g body weight. The mucosa was flushed with saline finally the ulcers were observed macroscopically. The observation was made for any ulceration or inflammation in the stomach. The stomachs were opened along the greater curvature and the mucosa was exposed for evaluation. Ulcer index, percentage protection and the P^H of gastric juice was determined statistically¹⁹⁻²⁰.

STATISTICAL ANALYSIS

The results obtained from the pharmacological experiments were statistically analyzed using SPSS Version 19.0. The data is presented as mean of $\pm$ SEM. To compare the values and to establish the significance ANOVA was used and P value are

reported as mean $\pm$ SEM. *a $p < 0.001$, *b $p < 0.01$ and *c $p < 0.05$.

Results

Primilary phytochemical study shows *P.granatum* peels extract presence of Alakloids, Glycoside, Phytosteroids, Flavonoids, Terpinoids, Vitamins and Tannins.

FST

In FST swimming animal increased swim time treatment with EPG at the dose of 400 and 600mg showed dose dependent significant increase in swimming time ($p < 0.01$ at 2nd week and $p < 0.01$ at 3rd week). Gerifort has shown significant ($p < 0.001$) increase in 2nd and 3rd week of swimming time compared to control (figure1).

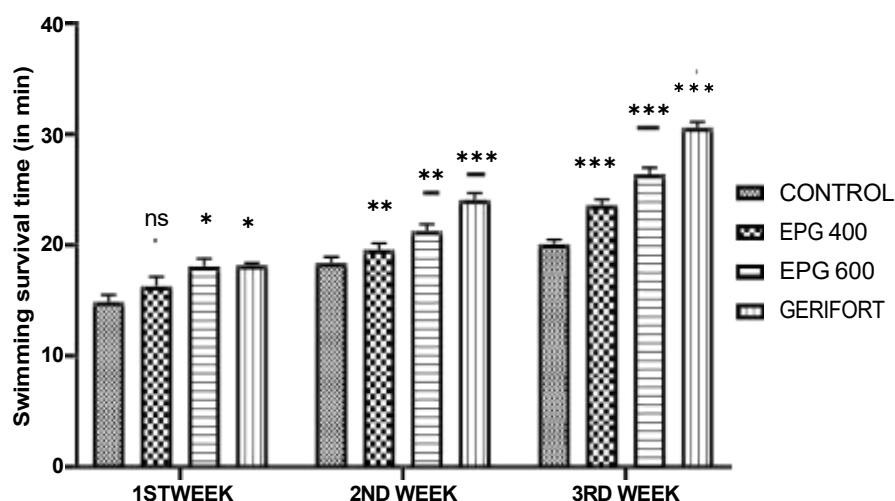


Figure1: Effect of *P. granatum* on Force Swimming test

Effect of *P. granatum* on Biochemical Parameters in CCS.

In CCS induced significant increases the levels of biochemical parameters such as glucose, cholesterol, BUN, triglyceride and plasma cortisol. Treatment with EPG 400 and 600 significant ($p < 0.001$) decrease in glucose, cholesterol and triglyceride levels and significant ($p < 0.05$) decrease in BUN and plasma cortisol levels. Whereas gerifort has significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) restored the elevated levels of biochemical parameters glucose, cholesterol, BUN, TG and plasma cortisol when compared with stress control group (figure2-6).

In CCS increases the incidence and severity of gastric ulcers % protection and P^H of Gastric juice, treatment with EPG and Gerifort showed ulcer protection by significant reduction in Ulcer incidence(%), increase in P^H of Gastric juice (figure7).

Discussion

Stress and stressor-related disorders are the global illnesses²¹. The most common stressors could be physical (extreme environmental conditions, external force), emotional (tragedies, sadness, academic, etc.), and chemical stress (due to medicine, solvents, and exposure to the laboratory or industrial chemicals).

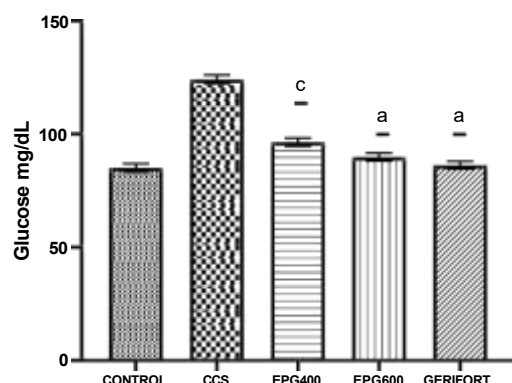


Figure 2: Effect of EPG on Glucose in CCS

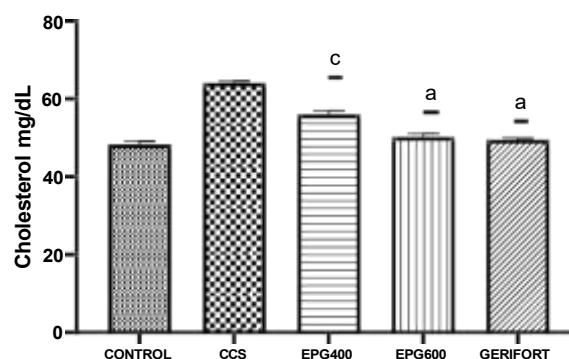


Figure 3: Effect of EPG on Cholesterol in CCS

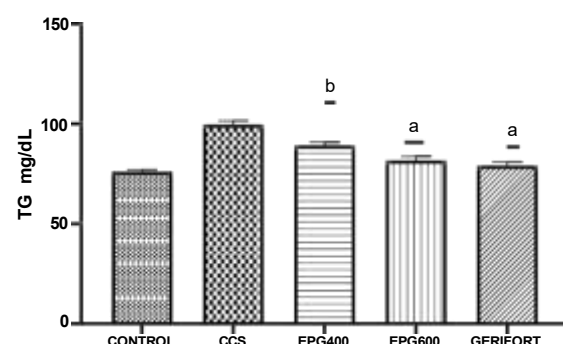


Figure 4: Effect of EPG on TG in CCS

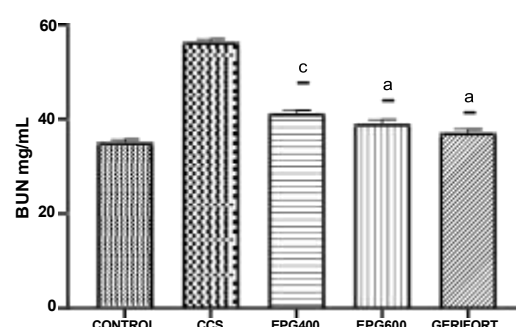


Figure 5: Effect of EPG on BUN in CCS

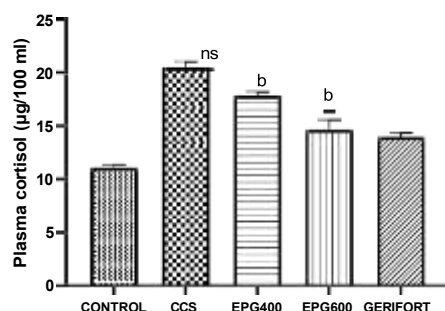


Figure 6: Effect of EPG on Plasma cortisol in CCS

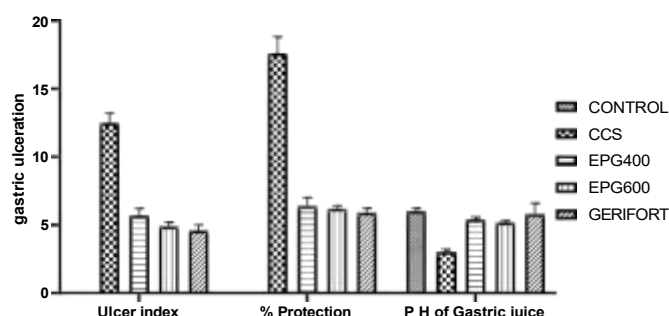


Figure 7: Effect of EPG on Gastric ulceration in CCS

²²FST is most widely used paradigm for the evaluation of anti-stress and antidepressant property. This paradigm is based on the observation that animals forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity²³. The appearance of immobility therefore, reflects a state of tiredness, fatigue, reduced stamina or a lowered mood (hopelessness)²⁴. These signs represent the core symptoms observed in depressed patients and in individuals under severe stress. It is well known that drugs with anti-stress properties

reduce the duration of immobility in animals.

Chronic cold Stress-induced ulcers are caused by number of factors both physical and psychological. Increase in gastric motility, vagal overactivity, mast cell degranulation, decreased mucosal blood flow, and decreased prostaglandin synthesis are reported to be involved in the genesis of stress-induced ulcers¹⁹. The result showed the ability and

the efficacy of the ethanolic extract of *P.granatum sativa* in preventing gastric ulceration in cold stress induced rats suggesting its anti-ulcer activity.

CONCLUSION

The phytochemical results suggest that the Ethanolic extract of *P.granatum* peel contain poly phenols are gallic acid, chebullic acid and ellagic acid, hydroxyl cinnamic acid, caffeic acid, chlorogenic acid, Anthocyanins like cyaniding, pelargonidin, delphinidin and other fatty acids, catechin, flavonoids like EGCG,, punicalagin, punicalin, quercetin, rutin, kaempferol, naringin, kaempferol-3-O-glycosides, kaempferol-3-O-rhamnoglycosides and other Anti-stress activity and antioxidant molecules. The main active component in punicalagin, punicalin, quercetin and poly phenols a natural product of the group of secondary metabolites, Several studies have shown that possesses a wide range of anti oxidant pharmacological activity. So we concluded the anti-stress activity due to these constituents' presence, whereas standard Geriforte, an established anti stress/adaptogenic drug.

FUNDING

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CONFLICT OF INTEREST

We have no conflict of interest to declare

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