Effect of Emblica Officinalis on Stress Induced Biochemical and Psychological Changes in Mice

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Abstract

Emblica officinalis Gaertn. (Euphorbiaceae) [EO] fruit is traditionally used as a general health tonic in Ayurveda. In the present study the effect of alcoholic extract of EO fruits was evaluated on acute stress induced biochemical and psychological changes in mice. Acute stress was induced by restraint stress method and the effect of EO at the doses of 100, 250 and 500mg/kg was evaluated on levels of plasma glucose and corticosterone. The level of anxiety induced by acute stress was measured by subjecting the mice to elevated plus maze. The extract at the doses of 250mg/kg and 500mg/kg significantly countered the stress induced elevated plasma glucose and cortisol levels. Likewise, the extract at the doses of 250 and 500mg/kg increased the number of entries and time spent in the open arm in elevated plus maze. In conclusion the Emblica officinalis fruit extract significantly reduces the adverse effects of stress on physical and mental health.

Index terms— stress, anxiety, emblica officinalis.

1 Introduction

he present day life style has increased the physical and psychological demands resulting in an up rise in various stress-related disorders like anxiety and depression. This necessitates an urgent need to develop agents to overcome these stress associated psychopathological conditions (Bhattacharya et al., 2000). Traditional medicines are rich in non-specific antistress agents which can be used clinically in the treatment of stress related maladies.

Emblica officinalis (EO) belonging to family Euphorbiaceae, is an important ingredient of Ayurvedic system of medicine. It is also named as Amla, Phyllanthus Emblica or Indian gooseberry. The fruits of the plant are categorised as rasayanas, a group of plant-derived drugs that are reputed to promote health and longevity by augmenting defence against disease, arresting the aging process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and creating a sense of mental well-being (Weiner and Weiner, 1994). It has its beneficial role in cancer, diabetis, liver treatment, heart trouble, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering cholesterol level (Khan et al., 2009).

Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases, ranging from psychiatric disorders such as depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis (Elliott and Eisdorfer, 1982). The benzodiazepine anxiolytics, despite having significant antistress activity have not proved effective against stress induced adverse effects on immunity, behavior, cognition, peptic ulcer and hypertension (Elliott and Eisdorfer, 1982). Furthermore, these drugs have adverse effects on the fetus during pregnancy and on the neonate during lactation (Trevor and Way, 2001).

Therefore, to find a solution to an effective antistress agent with a broad spectrum of activity and overcoming the adverse effects of benzodiazepines, the present study evaluates the effect of Emblica officinalis fruit extract on acute stress induced biochemical and psychological changes in mice.

2 II.

3 Materials and Methods

Shade dried fruits of EO were purchased from the local market for the whole batch of experiments. The fruits were authenticated by matching with the reference specimen no. 2156 at the Botany Department, Government Science College, Durg, India.

4 a) Preparation of extract

The powdered fruits, (250 g) were loaded in a soxhlet extractor and were defatted with petroleum ether (60-80). The marc was dried and further extracted with 70% ethanol by maceration (Riebling and Walker, 1975). The extract was concentrated on rotary flash evaporator and vacuum dried over anhydrous sodium sulphate. The dried material (48.9%) was stored under refrigeration at 4-8 ?C until its use.

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Male, Swiss albino mice (20-30 g) were used for behavioral studies, whereas biochemical estimation was performed in male Wister albino rats. Each experimental group consisted of atleast six animals. The animals were housed for a minimum of five days prior to the pharmacological experiments, with free access to standard rodent pellet diet (Lipton India Ltd) and tap water, and maintained on a 12/12 h light-dark cycle.

All experiments were conducted in accordance with institutional Animal Ethics Committee guidelines. The experimental protocols were approved by the institutional animal ethics committee (IAEC) - SIP/CPCSEA/IAEC/2013/I/0. The minimum number of animals and duration of observations required to obtain consistent data were employed.

6 c) Extract and standard drug

The hydroalcoholic extract was formulated as suspension using 0.1% Sodium carboxymethyl cellulose (CMC). Ginseng 100mg/kg (Revital?) was used as reference drug. The extract was adjusted to give a fixed volume of 10 ml/ kg orally in doses of 100mg/kg, 250mg/kg and 500mg/kg.

7 d) Acute restraint stress model

In the present study stress was induced using acute restraint stress (Masood et al., 2003) model with minor modifications. The mice were divided into six groups of six animals each of either sex. Stress was induced by restraining the animals in PVC restrainers for a period of 4 hours. Group 1 animals served as normal control were administered 0.5% Sodium CMC in water and were not exposed to stress. Group 2 animals served as negative control as untreated stress induced; Group 3 animals were administered ginseng 100mg/kg orally. While, Group 4, 5 and 6 were administered EO extract orally at the doses of 100mg/kg, 250mg/kg and 500mg/kg respectively. The animals were pretreated with the extracts and the reference drug for a period of seven days before the induction of stress.

Following the induction of albino stress the male Swiss mice were evaluated for behavioral changes on the Elevated plus maze model. A different set of male Wistar rats, treated as above, were used for biochemical analysis. The animals were sacrificed post stress induction by cervical decapitation, the blood was withdrawn from the jugular vein and serum glucose and corticosterone levels were determined.

8 e) Elevated plus-maze test (EPM)

This test has been widely used to measure anxiety in rodents **??**Morra et al., 2006). The wooden apparatus, consisted of two open arms (50 cm×10 cm each), two enclosed arms (50 cm×10 cm×40 cm each) and a central platform (10 cm×10 cm), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 70 cm above the floor. Immediately after the induction of stress, each animal was placed at the center of the maze, facing one of the enclosed arms. During the 5-min test period, the number of open and enclosed arms entries, plus the time spent in open arms, was recorded. Entry into an arm was defined as the point when the animal places all four paws onto the arm.

9 f) Blood Collection

A different set of male Wistar rats, treated likewise, was used for biochemical analysis. The animals were sacrificed immediately after acute stress induction. The blood was collected and separated in a refrigerated centrifuge at 4°C. The serum was stored at -80°C until further analysis of corticosterone and glucose.

10 g) Estimation of Corticosterone

Serum corticosterone levels were determined by fluorimetric method (Glick D et al 1964) with minor modifications. Briefly, 500 µL of serum was extracted with 2mL of chloroform. The chloroform was further extracted with 1ml of acid alcohol and the fluorescence was measured at 462 nm and 518 nm.

11 h) Estimation of serum glucose

The serum glucose level was determined using the (GOD-POD method) glucose oxidase-peroxidaseaminoantipyrine and phenol method (Glucose determiNATion kit, Merck) where the quinonemimine dye formed is estimated spectrophotometerically at 540 nm (Philip et al 1994).

12 i) Statistical analysis

The data was analyzed using Prism Graph Pad software and showed as mean±S.D. Comparison between control and drug treated groups were made by one-way analysis of variance (ANOVA) followed by Dunett's test, P values of less than 0.05 were considered to be significant.

13 III.

14 Results

15 a) Elevated plus-maze model (EPM)

The ANOVA revealed a significant increase in the number of entries in the open arm in the normal, ginseng treated and EO (250 and 500mg/kg) treated animals as compared to the stress control (P<0.05). Further, EO treated animals significantly increased (P<0.05) the time spent in the open arm at the doses of 250 and 500mg/kg. The results are shown in table 1.

16 b) Effect of EO extract on serum glucose level

The induction of stress by restraining in mice was confirmed by measuring the serum glucose levels. The animals on exposure to acute restraint stress showed a significant increase in blood glucose levels as compared to the normal mice (P< 0.05). Further, treatment with EO extract at a dose of 250 and

17 Discussion

Severe stressful conditions are responsible for the etiopathogenesis of various psychosomatic disorders. Homeostasis which is maintained by the various neurotransmitters is challenged during stressful conditions. These alterations in neurotransmitter activity result in behavioral changes as well as a cascade of hormonal release from the hypothalamus-pituitaryadrenal (HPA) axis. The imbalance of these monoamines due to prolonged stressful conditions has been associated with a wide range of central and peripheral disorders like anxiety, depression, obsessive compulsive disorder, eating and sleeping disorders, hyperglycemia and decreased immune response (Kalia, 2005; **??**ashid et al 2008).

The elevated plus maze (EPM) is considered to be an etiologically valid animal model of anxiety which uses natural stimuli like fear of a novel open space and fear of balancing on a relatively narrow, raised platform that can induce anxiety in mice (Dawson and Tricklebank, 1995). However it was observed after measurement of anxiety states post acute restraint stress induction that the animals showed further pronounced anxious behavior in EPM. EO fruits have shown significant pharmacological effects like enhancement in swimming performance of rats in forced swimming test **??**Sudhakar et al., 2009) and are used in Ayurveda as a general tonic **??**Deole et al., 2009), which further proposes evaluating its effects on stress induced neuropsychological conditions. The present study investigated the effects of hydroalcoholic extract of EO fruits on the acute stress induced anxiety in mice.

Typically a stress response is characterized by the activation of HPA axis resulting in an increase in blood corticosterone levels which in turn lead to an increase in serum triglycerides levels and hyperglycemia. The study indicated that administration of EO extract significantly countered altered blood glucose and corticosterone levels in animals exposed to acute restraint stress.

Further, administration of EO extracts and evaluation of these stress induced animals in models of anxiety revealed a significant lowering of anxiety response such as a significant increase in the number of crossings in the EPM and time spent in the open arm.

V.

18 Conclusion

Although this study does not suggest anything about the mechanism of antistress potential yet it proves to be a potential lead in this class of drugs and further relates with the works reported by others on its adaptogenic effect which needs to be further evaluated and optimized.

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Figure 2:

[*] *05; in comparison to stress induced animals (n = 6 animals in each group) Table 2 : Effect of EO on serum glucose and corticosterone level following acute restraint stress in mice Groups Serum glucose levels (mg/dl) Serum corticosterone levels*, * . mg/100ml.

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[Table 1 : Effect of EO on Elevated plus maze behavior following acute restraint stress in mice Groups Time spent in the open ar *Table 1 : Effect of EO on Elevated plus maze behavior following acute restraint stress in mice Groups Time spent in the open arm (seconds)*,

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