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Effect of Trichopus Zeylanicus Leaf Extract on Acute Stress Induced Anxiety in Mice

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Abstract

Trichopus zeylanicus Gaertn. (Dioscoreaceae) [TZ] leaf is traditionally used as a general health tonic in tribal regions of south India. In the present study the effect of alcoholic extract of TZ leaves was evaluated on acute stress induced anxiety in mice at oral doses of 100mg/kg, 250mg/kg and 500mg/kg. Acute stress was induced by restraint stress method and the stressed rodents were evaluated in light and dark model and elevated plus maze. The extract at the doses of 250mg/kg and 500mg/kg showed a significant increase in the number of crossings and reduced time spent in the dark chamber in light and dark model. Further, it significantly reduced the time spent in the closed arm in elevated plus maze as compared to stressed mice. Moreover, TZ significantly reduced stress induced increased plasma corticosterone levels and hyperglycemia in rats.

Index terms— acute restraint stress, anxiety and trichopus zeylanicus.

1 Introduction

evere 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-pituitary-adrenal (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 present day life style has increased the physical and psychological demands resulting in an up rise in various stress-related disorders which further necessitates an urgent need to develop agents to overcome these conditions. Traditional medicines are rich in non-specific anti-stress agents which are of increasing clinical significance.

Trichopus zeylanicus, variety Gaertn. (Family: Dioscoreaceae) is an herbaceous, perennial and rhizomatous wild plant grown in Kerala. It is also known as Arogyappacha meaning, greener of health and is used as a health tonic by the tribal population. It is reported that TZ enhancement the swimming performance of rats in forced swimming test. Further, TZ is reported to have several pharmacological activities such as choleretic activity, hepatoprotection, aphrodisiac property and mast cell stabilizing activity (D. A. Evans et al 2002 and A K. Sharma et al, 1989).

In the light of the above information and folklore use, the present study evaluates the effect of Trichopus zeylanicus leaf extract on acute stress induced anxiety in mice.

2 II.

3 Materials and Methods

Shade dried leaves of TZ were purchased from the local market for the whole batch of experiments. The leaves were authenticated by matching with the reference specimen no. 2129 at the Botany Department, Government Science College, Durg, India.

4 a) Preparation of Extract

The powdered leaves, (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 (34.9%) was stored under refrigeration at 4-8 ?C until its use.

5 b) Animals

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 at least 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. The minimum number of animals and duration of observations required to obtain consistent data were employed (IAEC) - SIP/CPCSEA/IAEC/2013/I/02.

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

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 TZ 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 stress the male Swiss albino mice were evaluated for behavioral changes on the Elevated plus maze model, open field test and Light and dark 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 g) Light and Dark model

In Light Dark Model, exploration of rodent is inhibited by bright illuminations. The animals are placed on brightly lit side of a two-compartment chamber and number of crossings between the light and dark side is recorded. One-third of chamber (40 x 60 cm) is darkened with a cover and separated with a wall from otherwise brightly illuminated area. An opening (Diameter 13 cm) allows the animal to pass from illuminated to darkened compartment. At the start of the test, the mouse was placed in the middle of illuminated part of the cage. The number of crossings and time spent in the open arm was registered during 5 minutes (Vogel et al., 1997).

9 h) 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 i) 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 j) 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 k) 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) Light and dark model

The statistical analysis revealed a significant ($P<0.05$) increase in the number of crossings between the light and dark compartments in mice pretreated with TZ at 250mg/kg and 500mg/kg as compared to the stress control animals. The effect of ginseng (100 mg/kg) was not significantly different from that observed after TZ 500 mg/kg. Further, TZ treatment significantly increased the time spent in the light chamber as compared to the stressed rats. The results are showed in the table 1.

16 b) 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 TZ (250 and 500mg/kg) treated animals as compared to the stress control ($P<0.05$). Further, TZ 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 2.

17 c) Open field behavior

The results of open field behavior are depicted in table **??**. Statistical analysis showed a significant increase in the ambulatory behavior at the dose of 250 and 500mg/kg ($P< 0.05$). However no significant changes in the behavior were observed at 100mg/kg of TZ as compared to the stress control animals.

18 d) Effect of TZ 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 TZ extract at a dose of 250 and 500mg/kg significantly countered this elevation in blood glucose level. The results are depicted in the table 4.

19 e) Effect of TZ extract on serum corticosterone level

Exposure to acute restraint stress resulted in a significant elevation in serum corticosterone level. Further, treatment with TZ at 250mg/kg and500mg/kg significantly reduced the elevated levels of serum corticosterone. The results are depicted in the table 4.

IV.

20 Discussion and Conclusion

The elevated plus maze 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 even in other models like open field and light and dark model. Trichopus zeylanicus leaves have shown significant pharmacological effects like enhancement in swimming performance of rats in forced swimming test and aphrodisiac activity (Subramoniam et al, 1997), which further proposes evaluating its effects on stress and stress induced neuropsychological conditions. The present study investigated the effects of hydroalcoholic extract of Trichopus zeylanicus leaves 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 TZ extract significantly countered altered blood glucose and corticosterone levels in animals exposed to acute restraint stress and proves to be a potential antistress agent.

Further, administration of TZ extracts and evaluation of these stress induced animals in models of anxiety revealed a significant lowering of anxiety response such as increase in ambulatory behavior in the open field.

The extract also showed a significant increase in the number of crossings in the EPM and light and dark model. Moreover, the results were comparable to Ginseng at 500mg/kg of TZ.

As reported by Sharma et al, TZ has shown significant adaptogenic activity in forced swimming test and milk induced leucocystosis. Likewise the effect of TZ in alleviating symptoms of stress induced anxiety may be attributed to its adaptogenic potential.

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.

Figure 1:

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