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ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITY OF SAPONIN FRACTION OF *TRICHOPUS ZEYLANICUS* GAERTN IN MICE

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ABSTRACT

To evaluate the anxiolytic and antidepressant activity of saponin fraction of *Trichopus zeylanicus* (SFTZ) at 75,150 and 300 mg/kg. Anxiolytic activities of SFTZ were assayed by elevated plus maze method, light- dark test, and antidepressant activities were assayed by tail suspension test and force swimming test on mice. The various doses of SFTZ 75, 150 and 300 mg/kg, i. p have shown significant (***p< 0.001) increase in percentage of entries in open arm and time spent in open arm. Similarly it has also shown increased time spent in light box and decreased time in dark box significantly (***p< 0.001). In antidepressant study SFTZ at various doses of 75,150 and 300 mg/kg, i.p. have shown significantly (***p< 0.001) increased in immobility time in forced swimming and tail suspension method of antidepressant activity in mice. The present study confirmed that SFTZ 75, 150 and 300 mg/kg has shown promising anxiolytic and antidepressant activities.

Keywords: Anxiolytic, Antidepressant, *Trichopus zeylanicus*, Saponins.

INTRODUCTION

Environmental stress is one of the factors of anxiety in daily life. Stress causes disturb sleep, depression, anxiety, irritability, fatigue and lethargy. According to WHO, approximately millions people suffer from mental and behavioural disorder. Anxiety may occur without a cause, or it may occur based on a real situation but may be out of proportion to what would normally be expected. WHO has estimated that 80% of all the global inhabitants rely on traditional system of medicine for their primary health needs and these systems are largely plant based (Reynolds EH, 2003).

The Kannikars are predominant hill tribes of Agasthiyamalai biosphere, Western Ghats and Tamilnadu. Traditionally the Kannikars are well known about the plant uses. *Trichopus zeylanicus* Gaertn is a perennial herb, belongs to the family Trichopodaceae popularly known as "Arogyppacha" or 'Arokyapachilai" in Malayalam literally meaning "the green that gives strength". The plant is found in the Agasthyar hilly forest of Kerala. This plant is used as health tonic. The Kani tribes are using this plant for increasing the stamina (Pushpangadan P *et al*., 1988). *Trichopus zeylanicus* have shown various pharmacological activities. It shows one of the extraordinary Property that *T.zeylanicus* has an ability to increase stamina. Moreover, this plant increases the resistance of rodent against a variety of stress (Sharma AK *et al*., 1989). The methanol extract of *T. zeylanicus* showed hepatoprotective activity (Subramoniam A *et al*., 1998) and stimulates the male sexual behaviour in mice (Subramoniam A *et al*., 1997). The plant also possesses immunomodulatory activity (Pushpangadan P *et al*., 1995). This plant also possessed anti-fatigue, antioxidant and adaptogenic properties (Singh B *et al*., 2001; Tharakan B *et al*., 2005). The leaves of *Trichopus zeylanicus* is used by Kannikars for scabies and ring worm infection (Mohan VR *et al*., 2008). On the basis of knowledge of Kani tribes and literatures, the aim of the present study was to evaluate the anxiolytic and antidepressant effect of Saponin fractions of *Trichopus zeylanicus* in mice.

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MATERIALS AND METHODS

Plant Materials

The plant *Trichopus zeylanicus* Gaertn, (Family: Trichopodaceae) subspecies *travancoricus* was collected from Agasthyar hills of Kerala in the month of September 2008, and authenticated by taxonomist of NIPER nursery, Mohali, Chandigarh, India. A voucher specimen No.NIP-159 has been preserved in their laboratory for Future reference.

Preparation of extract and fractionation

The whole plant material of *Trichopus zeylanicus* (2.0 Kg) was dried. It was ground to coarse powder. The powder materials were defatted three times with 15 L of pet. ether using soxhlet apparatus. The pet. ether extracts were filtered and the solvent was removed under reduced pressure in a rota evaporator (Buchi Laboratories). The defatted materials were stored at 20^0C.The defatted plant material (1.2 Kg) was extracted three times with 10 L of 90% methanol at room temperature. The methanol extracts were filtered, concentrated under reduced pressure and dried in freeze dryer. The 350 g of methanol extract was further analyzed by various phytochemical tests for carbohydrates, proteins, Saponins, glycosides, alkaloids, flavonoids, tannins, steroids and triterpenoids (Harborne JB, 2007; Khandelwal KR, 2008).

Method for Isolation of Saponin fraction

The 210 g of methanol extract was suspended in (3.0 L) of water and extracted with three times with an equal volume of n-butanol. The n-butanol fractions were combined and dried which give 75 g butanol extract contains saponin and flavonoids. The butanol extract precipitated by addition of large excess of acetone (1.0 L). The resulting precipitate was filtered and dried to give (50 g) of crude saponin and flavonoids mixture (Eskander J *et al*., 2011). This extract was referred to as butanol extract (BE) and was used as starting material for the isolation of saponin and flavonoids fraction. The BE was subjected to Sephadex LH-20 column chromatography, washed with water and eluted with methanol at a rate of 4 ml/min and 20 fractions were collected. The fractions were combined into two fractions, Fr.1 (2.3g) was saponin fraction after controlling on analytical TLC plates using a mixture of MeOH: H2O (4:1) as eluent and 5% vanillin-H_2SO_4 reagent for detection of saponin (Harborne JB, 2007). Three different doses of saponins fraction of *Trichopus zeylanicus* (SFTZ) (75, 150 and 300 mg/kg., p.o.) were selected for the anxiolytic and antidepressant studies.

Acute toxicity

Acute toxicity assay was performed as per OECD guidelines 423(limit test). Six female Wistar albino rats (three animals in each step) were randomly selected. The animals were kept fasting for overnight providing only water. The test drug was administered orally at one dose level of 2000 mg/kg b. w. after that rat were observed continuously for the first 4 hours and then periodically up to 24 h for toxic symptoms and mortality (OECD, 2000).

Drugs

Diazepam (DZP, 1.0 mg/k, i.p, Ranbaxy Pvt, Ltd.) was used as a positive control in anxiolytic study, picrotoxin (PCT, 2.0 mg/kg i.p, Sigma) as anxiogenic agent. Fluoxetine (FLU 20 mg/kg, i.p, Sun Pharmaceuticals, Baroda) used as a positive control in antidepressant study and saline isotonic solution (NaCl 0.9%) was used in control group.

Animals and Treatments

Male Swiss albino mice weighing 25-30 g were selected for the study. All the experiments were carried out between 10:00 h to 16:00 h in standard laboratory conditioned maintained at 25 ± 1^0C and 60% humidity, 12h light and 12 h dark cycle, with free access to food and water *ad libitum*. The study was conducted in noise free room with controlled condition. The study protocols were approved by Institutional Animal Ethical Committee (IAEC) as per CPCSEA guidelines.

The mice for study were randomly selected and divided in different groups. For anxiolytic study mice were divided into six groups each group contains six animals. Different dose of saponin fraction of *Trichopus zeylanicus* (SFTZ) 75, 150 and 300mg/kg., p.o. was used for treated groups prepared in saline isotonic solution. The doses of SFTZ, DZP, PCT, FLU and saline isotonic Solution (SI) were administered 1hr before a test.

Elevated plus maze (EPM)

This test has been widely used for behavioural model to measure the anxiogenic and anxiolytic effect of drug in rodents (Lister RG, 1987; Pellow S *et al*., 1985). This apparatus was made of wooden an arm consisted two open (25×5 cm) crossed with two closed arm (25×5×15 cm). The arms are connected with Central Square (5×5 cm) in dimly illuminated room. The maze was elevated 25cm from the ground. Each animal was placed at the centre of the maze, facing one of the enclosed arms. The number of entries and the time spent in enclosed and open arms were recorded for 5 min. Entry into an arm was defined as the animal placing all four paws onto the arm. Total exploratory activity (number of entries) were observed and noted. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

Light-dark test (LD)

The apparatus consisted of a wooden box with

two compartments (20×20cm each), one of which was illuminated with a white light while the other remained dark. Each animal was placed at the centre of the illuminated compartment, facing one of the dark areas. The time spent in light and dark places, as well as the number of entries in each space, was recorded for 5 min (Carnevale G *et al*., 2011; Crawley J *et al*., 1980).

Forced Swimming test (FST)

This test is widely used as animal model for evaluation of antidepressant activity (Porsolt RD *et al*., 1978). The development of immobility when mice are placed in an escapable cylinder filled with water reflects the cessation of persistent escape–directed behaviour. The apparatus consist of glass cylinder (20 cm high ×12 cm diameter) filled to 15 cm depth with water (24± 1^0C) in the pre test session, every animal was placed individually into the glass cylinder for 15 min, 24 h prior to the 5 min swimming test. After 5 min, they were removed and dried with towel. During test session the following behavioural responses were recorded by trained observer, climbing behaviour, swimming behaviour and immobility time, that was considered when the mice made no further attempts to escape, and makes only the movements to keep its head above the water.

Tail Suspension Test (TST)

Tail suspension test is behaviour despair model of depression. The tail suspension test was based on the method (Steru L *et al*., 1985; Yu ZF *et al*., 2002). Mice were suspended on the edge of table 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. Testing was carried out in darked room with minimal background area. The total duration of immobility induced by tail suspension was recorded during 5 min. the animal was considered immobile when it did not show any movement of the body except for those required for respiration and hang passively.

Statistical Analysis

All statistical procedure was performed by using the Graph pad PRISM statistical software package, version 5.04 (PRISM). All data are expressed as Means ± SEM and values obtained from the different tests were compared against the control group by using analysis of variance (ANOVA) and followed by post hoc Dunnett's test. Differences between experimental groups were considered statistically significant (*p<0.05, **p<0.01, ***p<0.001).

RESULTS

Acute toxicity studies

The SFTZ did not show any mortality and toxicity up to dose of 2000 mg/kg b.w. p.o.in female rat.

Phytochemical screening

Phytochemical screening of the methanolic extract of *Trichopus zeylanicus* showed the presence of carbohydrate, proteins, saponin, alkaloids, flavonoids, steroid and triterpenoids. The TLC analysis of collected fraction from butanol extract shown presence of colored spot with Rf value 0.45, after spraying of vanillin–sulphuric acid reagent. The preliminary thin layer chromatography study confirmed that the presence of saponins in butanol extract of *Trichopus zeylanicus.*

Elevated plus maze test (EPM)

In the EPM method, diazepam (DZP) increased significantly the percentage of time spends in open arm and percentage of entries in open arm (***p<0.001) as comparable with control (figure. 1: A and B). Picrotoxin (PCT) as anxiogenic agent which induced significant reduction in these parameter (***p<0.001). Mice which received fraction of various doses of SFTZ 75, 150 and 300 mg/kg i.p shown significantly increased percentage of time spend in open arm and percentage of entries in open arm (*p<0.05,**p<0.01,***p<0.001) as comparable with control (figure. 1. A and B).

Light –Dark Test (LD)

In the LD method, Mice which received fraction of various doses of SFTZ 75, 150 and 300 mg/kg i.p shown significantly increased time spent in light compartment and decreased the time spent in dark one (***p<0.001) as comparable with negative control (figure. 2. A and B). However, no changes were found in the latency time to enter in dark compartment (figure.2. C) and (figure.2. D) number of crossing (p>0.05). Diazepam (DZP) significantly increased time spent in light compartment, the latency time to enter in dark compartment and number of crossing.

Forced swimming test (FST)

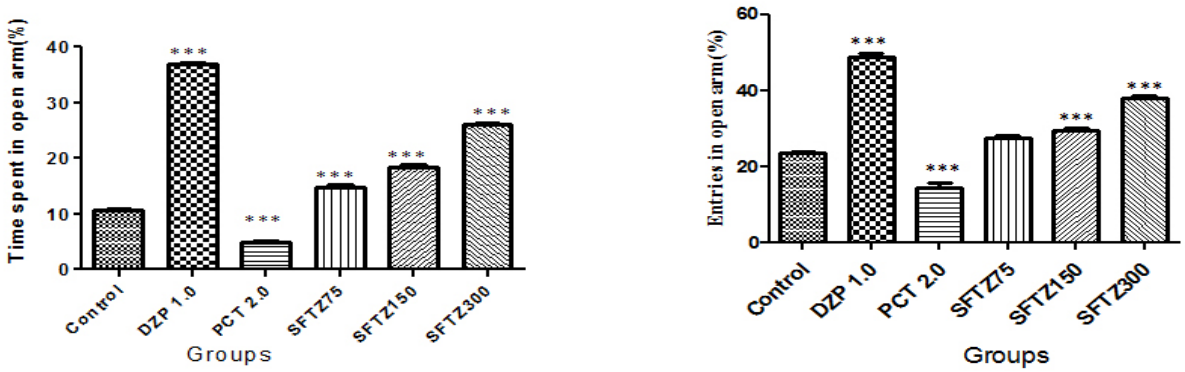
In FST study, mice received various doses of SFTZ 75, 150 and 300 shown significantly decreased the time of immobility when compared with negative control group. Fluoxetine (FLU) was used as positive control in FST model of antidepressant. It produced significantly (***p<0.001) decreased the time of immobility when compared with negative control group (figure. 3).

Tail Suspension Test (TST)

In TST study, mice received various doses of SFTZ 75, 150 and 300 mg/kg i.p shown significantly decreased the time of immobility when compared with control group. Fluoxetine (FLU) was used as positive control in FST model of antidepressant. It produced significantly (***p<0.001) decreased the time of immobility when compared with negative control group (figure. 4).

Figure 1. Effect of SFTZ-75,150 and 300 mg/kg, i.p. on percentage of time spent in open arm compartment (A), Percentage of entries in open arm (B), in Elevated plus maze test performed on mice

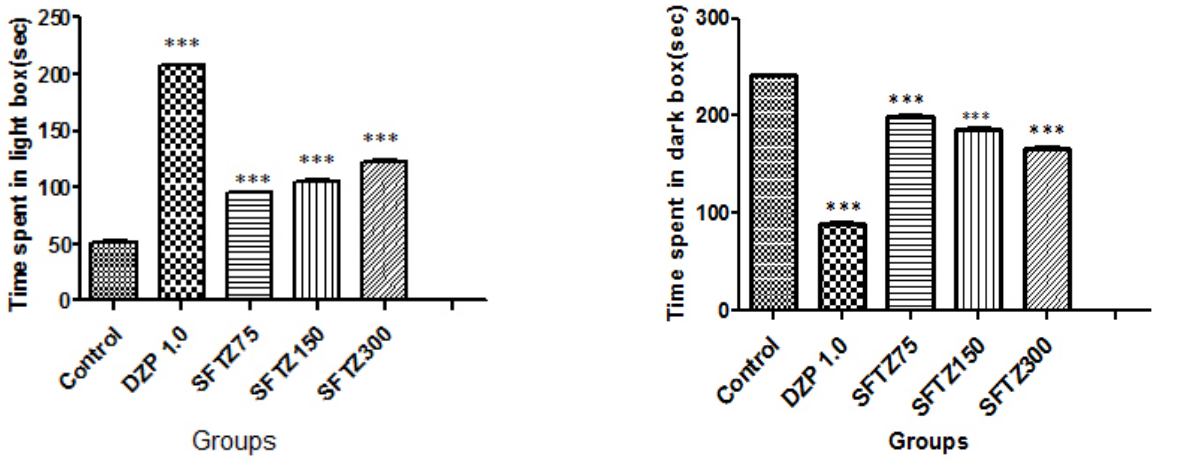
(A) (B)



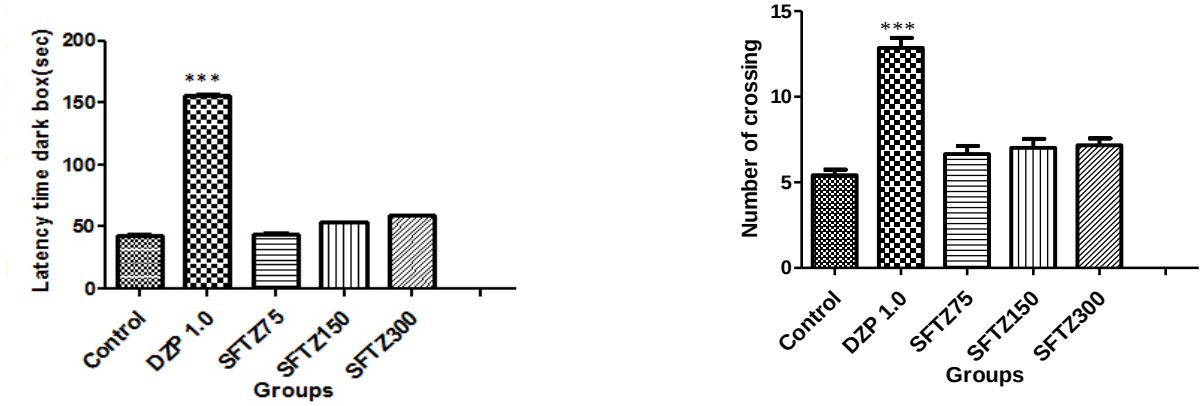
Data represents mean ± S.E.M., n=6. One–way ANOVA followed by Dunnett's test (*p<0.05, **p<0.01, ***p<0.001)

Figure 2. Effect of SFTZ-75,150 and 300 mg/kg, i.p on time spent in light compartment (A), time spent in the dark compartment(B), latency time enter in dark compartment (C) and number of crossing (D)during light-dark test performed on mice

A B

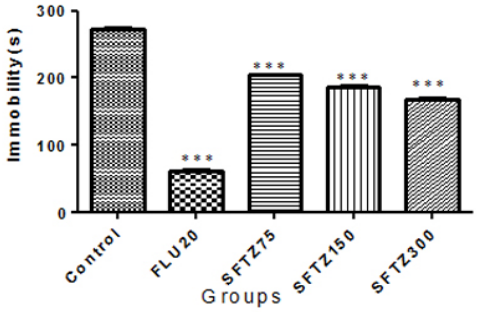


C D



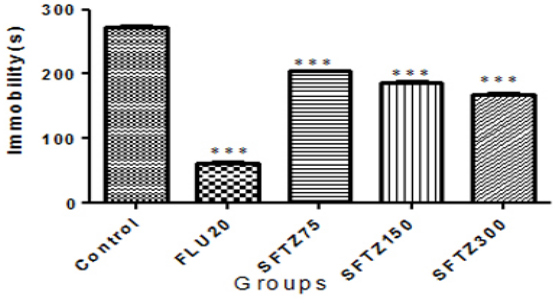
*Data represents mean ± S.E.M., n=6. One –way ANOVA followed by Dunnet's test (*p<0.05, **p<0.01, ***p<0.001)*

Figure 3. Effect of SFTZ-75,150 and 300 mg/kg, i.p on time of immobility in force swimming test performed on mice



*Data represents mean ± S.E.M., n=6. One –way ANOVA followed by Dunnett's test (*p<0.05, **p<0.01, ***p<0.001).*

Figure 4. Effect of SFTZ-75,150 and 300 mg/kg, (i.p) on time of immobility in tail suspension test performed on mice



*Data represents mean ± S.E.M, n=6.One-way ANOVA followed by Dunnett's test(*p<0.05,**p<0.01,***p<0.001*

DISCUSSION

Nowadays stress of any kind results in progressive deterioration in brain functions. Abnormal functioning of brain leads to imbalance of various neurotransmitters like GABA, 5-hydroxytrptamine (serotonin) and various amino acids and their metabolite in pathophysiology of depression and anxiety states. Therefore, varied number of neurotransmitter plays an important role in underlying mechanism of disease as well as mechanism of action of anxiolytic and antidepressant drugs (Palucha A *et al*., 2002). In present study, EPM model shows significant anxiolytic activity, it is well established test for evaluating anxiolytic drugs in rodents.In EPM model mice shown less aversion in open arm of maze and prolong the time spent in open arm. This model is useful for modeling anxiety, and it has been developed for predicting the potency of clinically used compound for treating this disease (Herrera-Ruiz M *et al*., 2006). Another model, light-dark test is suitable model for evaluation of putative anxiolytic activity of drugs like diazepam. The time spent in the light compartment (illuminated) is the most consistent parameter for anxiolytic activity, while crossing is a considered as a sign of exploratory behaviour (Lepicard EM *et al*., 2000). In the present study, both the studied model of anxiety has shown dose dependent significant activity on acute administration of various doses of SFTZ 75,150 and 300 mg/kg i.p in mice comparable with diazepam. Neuroscience study, suggest that dysfunction of GABA nergic function may lead to anxiogenic effect (Brambilla P *et al*., 2003).

Clinically benzodiazepines facilitates action of GABA on $GABA_A$ receptor, hence diazepam shows reproducible results in EPM and LD model of anxiety (Pellow S *et al*., 1985). Based on mention mechanism of DZP, thus the various doses of SFTZ 75,150 and 300 mg/kg i.p is supposed to be able to produce anxiolytic action may be through $GABA_A$ receptor facilitation. In antidepressant study, force swimming test (FST) and tail suspension test (TST) illustrate a stimulus commonly used for inducing stress in animal studies. This aversive feature was able to induce a decrease in extracellular level of 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the amygdale and lateral septum of brain (Kirby LG *et al*., 1995; Price ML *et al*., 2002).

In present study, the SFTZ fraction of various doses have shown significant dose dependent antidepressant activity in behavioural model of depression like force swimming test (FST) and tail suspension test (TST) comparable with the immobility produced by negative standard. Fluoxetine was used as positive control in present study, it produced antidepressant action through 5-HT activation and it leads to decreased immobility time in both model of depression. All antidepressant drugs shown significant activity in the test. It is well reported that in the test, the augmentation of alpha noradrenergic transmission, played important role in accessibility of the force swimming test (Bourin M *et al*., 1996). Though, it has also been reported that the test is responsive to drug acting primarily on 5-HT function, it has been demonstrated that this neurotransmitter is closely associated with stress phenomenon. The probable mechanism of antidepressant action of SFTZ may be through noradrenergic and serotonergicpathways (Borsini F *et al*., 1988).In conclusion our data suggest that SFTZ may be good candidate for significant anxiolytic and antidepressant activity.

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