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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-ANXIETY ACTIVITY OF *TINOSPORA CORDIFOLIA* (WILLD.) LEAF

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ABSTRACT
The present study was carried out to evaluate the anxiolytic potential of petroleum ether extract and ethanolic extract of Leaf of *Tinospora cordifolia* Linn. *Tinospora cordifolia*, the versatile herbal drug is the distinctive source of constituents which is having antidiabetic, immunomodulatory, antioxidant, antimicrobial, antitoxic and anticancer activity. Leaves of *Tinospora cordifolia* was collected, dried and powdered used for 7 days cold maceration process. Petroleum ether and ethanolic extract was subjected to the preliminary phytochemical screening to analyse the presence of active constituents. Both the extracts at a dose of 100mg/kg, 200mg/kg, 400mg/kg body weight of animal was evaluated for antianxiety activity in mice using 3 experimental designs; elevated plus maze, open filed, light/dark transition model. Test group animals were compared with group of animal treated with diazepam (1mg/kg) as standard drug. Petroleum ether extract at 100, 200, and 400mg/kg showed activity in dose dependent manner and at a dose of 400mg/kg it has showed significant increase; in elevated plus maze, number of entries in open arm and time spent in open arm; number of entries into light chamber and time spent in light chamber in light and dark transition model and in open field; number of square crossed, number of rearing and time spent in central square. The results supported traditional use of *Tinospora cordifolia* in nervous disorders. Study concluded that petroleum ether extract of leaves of *Tinospora cordifolia* at a dose of 400mg/kg has significant antianxiety activity.

KEYWORDS: Anti-anxiety activity, *Tinospora cordifolia* Linn, Diazepam, Elevated plus maze, Light and dark model, Open field.

INTRODUCTION
Anxiety disorder is common mental disorder with symptoms that range from mild to severe. Anxiety disorders are characterized by a feeling of fear, often chronic, in response to the presence of threatening or unfamiliar situations. Certain drugs also induce anxiety like CNS stimulant, withdrawal of alcohol, drugs and metabolic changes like hyperglycaemia, thyrotoxicosis, and hyperventilation. From the studies it was confirmed that the likely cause of anxiety is the imbalance in brain chemistry. The problems associated with the number of neurotransmitters like norepinephrine, serotonin, GABA are expressed in individuals having anxiety disorders.[1] Many of the current anxiolytic drugs exhibit undesirable side effects that contribute to poor patient compliance with the treatments.[2-3] This has been associated with an increase in the demand for medicinal plants as safer alternative therapies. Many plants have anxiolytic and/or antidepressant potential and contain diverse phytoconstituents which may be exploited for the development of new drugs to treat these disorders, particularly in cases where patients do not respond to current medications.[4-5]

Tinospora cordifolia, the versatile herbal drug is the distinctive source of constituents which is having antidiabetic, immunomodulatory, antioxidant, antimicrobial, antitoxic and anticancer activity.[6] Number of investigations and research has been done on chemical constituents of guduchi.[7] It also has potentiating activity on other substances in the form of shodhan – vidhi. "Guduchi" is the Sanskrit name which indicates that one protect from diseases.[8] In hindu mythology, hindi name of *Tinospora cordifolia*"giloya" is believed to be the heavenly elixir which prevents aging.[9]

There are many studies which report various therapeutic potential of this herb. However, no published scientific data are available on the anti- anxiety activity of leaves of *Tinospora cordifolia*. Hence the present study was undertaken.

MATERIALS AND METHODS

Preparation of the Extract

The leaves of *Tinospora cordifolia* Linn were collected from the Sattenapalli region, Guntu, A.P, India and shade dried until it was moisture free. The dried leaves were made into coarse powder by using electric grinder. *Tinospora cordifolia* leaves were extracted with petroleum ether (TCPE) and ethanol (TCEE) using soxhlet extraction process. After the extraction, the contents were filtered by using a muslin cloth. The separated content was kept on boiling water bath to remove the petroleum ether and ethanol and petroleum ether and ethanol extracts of *Tinospora cordifolia* leaves were given code name with TCPE and TCEE using soxhlet extraction process and they were stored in desiccators for experimental use.

Preliminary Qualitative Phytochemical Screening

Qualitative phytochemical examination of the petroleum ether and ethanolic extract of *Tinospora cordifolia* Linn Leaf was performed for identifying the presence of active constituents in it.[10] Standard methods were followed to perform test.[11]

Evaluation of Anti-anxiety Activity Dosing

Antianxiety potential of leaf of *Tinospora cordifolia* Linn was evaluated by 3 dose levels. One tenth of the higher dose in acute oral toxicity trails was the mid dose i.e, 1/10th of 2000 mg/kg -200mg/kg of body weight of animal. Double the 1/10th of dose will be the higher dose i.e, 400mg/kg of mice body weight. The lower dose was half of the 1/10th dose which was 100mg/kg of the mice body weight. Albino mice of approximately 20-30g were divided into five classes of 6 animals each.

Elevated Plus Maze test

Exploratory behavior, anxiety characters and motor activity were assessed by using elevated plus maze.[12] The apparatus is about 25cm height from the ground level, consists of 2 open arm (16x5cm) and 2 closed arm (16x5cm) which are extended from common central arena.[13] Individual animals from each group of animals were placed at central platform by facing to open arm.[14-15] Group I was orally treated with 0.6%w/v CMC. Diazepam (1mg/kg)i.p treated animals served as group II. For 7 consecutive days, group III, group IV, group V was treated with TCPE at a dose of 100mg/kg, 200mg/kg, 400mg/kg AND group VI, group VII, group VIII were treated with TCEE at a dose of 100mg/kg, 200mg/kg, 400mg/kg. On 8th day, 1 hr after administration of standard, TCPE and TCEE in each groups, individually mice were placed in central platform of apparatus facing open arm. The parameters assessed for evaluation of activity during 5 min of test duration was evaluated by using ORCHID- All maze video tracking software.[16]

Light and Dark Transition Test

The light/dark apparatus is divided into two chambers (40cm × 60cm × 20cm). The light chamber is white in colour and separated from dark chamber by a wall which is connected with a small opening at bottom.[17] 20-30g weighing albino mice were divided into 5 groups, each group having 6 animals. Group I was orally treated with 0.6%w/v CMC. Diazepam (1mg/kg) i.p treated animals served as group II. For 7 consecutive days, group III, group IV and group V were treated with TCPE at a dose of 100mg/kg, 200mg/kg, 400mg/kg. Group VI, group VII, group VIII were treated with TCEE at a dose of 100mg/kg, 200mg/kg, 400mg/kg. On 8th day, 1 hr after administration of standard and TCPE and TCEE in each groups, individually mice were placed over illuminated area of chamber[18] and the parameters such as time spent in light chamber of the cage and number of entries in illuminated chamber is been noted for 5 min duration.

Open Field Model

Open field apparatus is a wooden box of dimension 40cm × 40cm × 30 cm. Apparatus consists of 16 squares of equal size and central zone is marked to separate squares from others. Lamp of 60W is suspended 100 cm above the apparatus.[19] Group I was orally treated with 0.6%w/v CMC. Diazepam (1mg/kg) i.p treated animals served as group II. For 7 consecutive days, group III, group IV, group V were treated with TCPE at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg and group VI, group VII and group VIII were treated with TCEE at a dose of 100 mg/kg, 200 mg/kg and 400 mg/kg. On 8th day, 1 hr after administration of standard, TCPE and TCEE in each groups, individually mice were placed in central zone of apparatus, 5 min of test duration evaluated parameters such as number of rearing, number of square crossed and time spent in central square.[20]

Statistical Analysis

All the data were expressed as mean ±SEM (n=6) and analyzed by one-way ANOVA followed by post hoc Schiff's test using SPSS software version 10. P value less than 0.05 was considered as statistically significant.

RESULTS

Phytochemical Analysis

The preliminary qualitative phytochemical analysis performed revealed the presence of terpenoids, saponins and proteins.

Effect of TCPE and TCEE in Elevated plus Maze Model (EPM) test

The effects of *T. cordifolia* on the time spent and the number of entries in the open arms in the EPM test are illustrated in Table 1. Administration of petroleum ether and ethanol extracts of *T. cordifolia* (100, 200, and 400 mg/kg) showed anxiolytic activity by increasing both the time spent and the number of entries in the open arms in a dose-dependent manner. The petroleum ether extract at 400 mg/kg has greatly increased the time spent (111 ± 1.17 s; $p < 0.001$) and the number of entries (16.2±1.39 s, $p < 0.001$). Mice treated with 100 mg/kg did not manifest significant improvement ($P > 0.05$) in the time spent and number of entries in the open arms. As

expected, diazepam at 1 mg/kg (positive control) significantly raised the time spent (121±3.12 s; P < 0.001 vs. control group) and number of entries (19 ±0.84; p < 0.001) in the open arms.

Effect of TCPE and TCEE in the light-dark box (LDB) test

The effects of *T. cordifolia* on the time spent in the light and the dark compartments in the LDB test are illustrated in Fig. 2. When compared to the control, TCPE at 400 and 200 mg/kg significantly (P < 0.001) increased the time spent in the light box (192.8±3.9 and 123.51.±4.52 s, respectively) and significantly (P < 0.001) decreased the time spent in the dark box (107.2±4.68 and 176.49±8.56 s, respectively). The group of animals treated with 100 mg/kg did not manifest a significant increase/decrease (P > 0.05) in the time spent in the light/dark box. In the standard drug diazepam-treated group (1 mg/kg), the times spent in the light and dark box were 176.49±8.56 and 89.46±1.65 s, respectively (P < 0.001 vs. control group). The values for the time spent in the light/dark box observed for MECR (400 mg/kg) were comparable to those obtained after administration of the standard drug diazepam.

Effect of TCPE and TCEE in the Open Field Test

a) Number of Square Crossed

Significant increase (p<0.001) in number of square crossed in open field was observed in the group of animals treated with diazepam (1mg/kg) as standard drug. At the doses of 100mg/kg, 200mg/kg and 400mg/kg of TCPE and TCEE showed number of square crossed in dose dependent manner and 400mg/kg showed significant increase (p<0.001) in number of square crossed in open field comparing to other dose.

b) Number of Rearing

There was a significant increase (p<0.001) in number of rearing showed by group of animals treated with diazepam (1mg/kg) as standard drug. The TCPE and TCEE at a dose of 100mg/ kg, 200mg/kg, 400mg/kg has increased the number of rearing by animals in dose dependent manner. The TCPE at 400 mg/kg exhibited significant (p<0.001) activity in number of rearing.

c) Time Spent in the Central Square

Group of animals treated with diazepam (1mg/kg) as standard drug showed significant increase (p<0.001) in time spent in central square of open field. The TCPE and TCEE at a doses of 100mg/kg, 200mg/kg and 400mg/kg has showed activity in dose dependent manner and high dose (400mgkg) of TCPE exhibit significant increase (p<0.001) in time spent in central square of open field.

Table 1: Effect of TCPE and TCEE on number of entries and Time spent in open arm in elevated plus maze.

Sl.No	Groups	Number of entries into open arm Mean±SEM	Time spent in open arm Mean±SEM (s)
1.	Control	1 ±0.58	6.1±2.41
2.	Standard	19 ±0.84 [b]	121±3.12 [b]
3.	TCPE (100 mg/kg)	4.83 ±0.401 [a]	47.4±2.74 [a]
4.	TCPE (200 mg/kg)	9.6±0.55 [b]	65.3±7.4 [b]
5.	TCPE (400 mg/kg)	16.2±1.39 [b]	111±1.17 [b]
6.	TCEE (100 mg/kg)	3.83 ±0.401 [a]	41.4±3.68 [a]
7.	TCEE (200 mg/kg)	7.6±0.55 [b]	59.3±3.57 [b]
8.	TCEE (400 mg/kg)	12.2±1.39 [b]	93.6 ±1.49 [b]

All the values are expressed as mean±SEM (n=6). a=p<0.05 when compared with control, b=p<0.001 when compared with standard. P <0.05 was considered as statistically significant.

Table 2: Effect of TCPE and TCEE on number of entries and time spent in light chamber in light /dark transition model.

Sl.No	Groups	Number of entries into light chamber Mean±SEM	Time spent in light chamber Mean±SEM (s)	Time spent in dark chamber Mean±SEM (s)
1.	Control	2±0.516	14.53±2.92b	285.47±2.92
2.	Standard	21.5±2.09[b]	210.54±4.51 [b]	89.46±1.65 [b]
3.	TCPE (100 mg/kg)	5.1±0.65[a]	71.1±4.24 [a]	228.9±5.29 [a]
4.	TCPE (200 mg/kg)	11.47±0.44[b]	123.51.±4.5[b]	176.49±8.56 [b]
5.	TCPE (400 mg/kg)	18.29±1.064 [b]	192.8±3.9 [b]	107.2±4.68 [b]
6.	TCEE (100 mg/kg)	4.1±0.65 [a]	58.1±5.23 [a]	241.9±3.29 [a]
7.	TCEE (200 mg/kg)	9.52±0.44 [b]	99.19±4.29 [b]	200.81±4.56 [b]
8.	TCEE (400 mg/kg)	15.19 ±1.64 [b]	175.8±3.91 [b]	124.2±3.83 [b]

All the values are expressed as mean±SEM (n=6). a=p<0.05 when compared with control, b=p<0.001 when compared with standard. P <0.05 was considered as statistically significant.

Table 3: Effect of TCPE and TCEE on number of rearing, Number of square crossed and Time spent in central square in open field.

Sl.No	Groups	Number of square crossed in open field Mean±SEM	Number of rearing in open field Mean±SEM	Time spent in central square Mean±SEM (s)
1.	Control	78±6.93	7.1±1.07	15.5±6.10
2.	Standard	190.3±12.8 [b]	31.6±3.31 [b]	268±29.1 [b]
3.	TCPE (100 mg/kg)	101±10.63[a]	10.1±1.35 [a]	82.7±4.13 [a]
4.	TCPE (200 mg/kg)	137±9.6 [b]	18.4±1.16 [b]	156.87±2.63 [b]
5.	TCPE (400 mg/kg)	179.4±8.43 [b]	27±1.69 [b]	248.28±5.71 [b]
6.	TCEE (100 mg/kg)	96.14±10.6[a]	8.1±1.17 [a]	64.7±3.1 [a]
7.	TCEE (200 mg/kg)	129.87±9.6[b]	15.4±1.13 [b]	115±2.63 [b]
8.	TCEE (400 mg/kg)	159.42±8.43 [b]	24±1.78 [b]	216.3±4.88 [b]

All the values are expressed as mean±SEM (n=6). a=p<0.05 when compared with control, b=p<0.001 when compared with standard. P <0.05 was considered as statistically significant.

DISCUSSION

The results from the present study showed that petroleum ether extract of leaves of *Tinospora cordifolia*(TCPE) possessed significant anti-anxiety activity in dose dependent manner. Investigation on *Tinospora cordifolia*stem, leaf, whole plant part confirmed the vast medicinal properties.[21] The experimental model elevated plus maze is commonly used for the screening of anxiolytic drugs.[22] In many other experimental models the elevated plus maze exhibits more acceptance than Y-maze, radial maze. Anxiolytic drugs were successively identified by using this model.[23] Chemical/extract having anti-anxiety activity shows increase in number of entries into open arm and time spent in open arm. The anxiolytic property is exerted through $GABA_A$ receptor complex and evaluated by using diazepam (1mg/kg) as standard drug.[24] Study was conducted by 7 days consecutive oral treatment of petroleum ether and ethanolic extract at doses of 100, 200, 400mg/kg and on 8th day of dosing, after 1 hr animals are placed in elevated plus maze showed anxiolytic property by increase in number of open arm entry and time spent in open arm. Activity is confirmed by comparing parameters with control group.

In light/dark transition model, animal spent more time in dark chamber than in light chamber due to fear for facing new environmental condition. The parameters such as number of entries into light chamber and time spent in light chamber are assessed for the evaluation of antianxiety property.[25] The petroleum ether extract of leaf of *Tinospora cordifolia* (TCPE) at a dose of 100, 200, 400mg/kg was given orally to mice of respective groups for 7 days. After 1 hr, of oral administration on 8th day activity was evaluated by keeping animal on model. The antianxiety activity exhibited by increase in number of entries and time spent in light chamber as compared to the control treated group.

In open field, animal shows emotional disturbance, fear and anxiety. Animals having anxiety spent more time towards outer zone, corners/ peripheral arenas of the open field.[26] In open field, parameters like number of square crossed, rearing rate (standing on hind limb) and time spent in central Square are assessed for evaluating activity.[27] Oral treatment of 7 consecutive day with TCPE and on 8th day, after 1 hr of dosing, respective groups of animals showed increase in square crossed, number of rearing and time spent in central square as compared to the normal control group.

In summary, from the results of study it was confirmed that the petroleum ether extract of leaf of *Tinospora cordifolia*(TCPE) treated animal produced anxiolytic effect in dose dependent manner and at high dose 400mg/kg showed a significant anti-anxiety activity, when compared with normal control group.

CONCLUSION

Study was carried out to evaluate the antianxiety activity of TCPE and TCEE. Presence of terpenoids, saponins and proteins are established by phytochemical analysis. The antianxiety potential was evaluated by elevated plus maze, light/dark transition test and open field test. It was found that petroleum ether extract of *Tinospora cordifolia* (TCPE) Shows anxiolytic property in dose dependent manner. At a dose of 400mg/kg, TCPE has showed significant increase in parameters compared with control treated group of animals; in open arm entries and time spent in open arm of elevated plus maze, number of entries into the light chamber and time spent in light chamber. In light/dark transition model; number of square crossed, rearing rate and time spent in Central Square in open field. From the results, it is confirmed that petroleum ether extract possess significant antianxiety activity and further studies can be carried out to find out the exact mechanism to confirm its anxiolytic activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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