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PHARMACOLOGICAL EVALUATION OF ALKALOID RICH FRACTION FROM THE AERIAL PARTS OF *TURNERA SUBULATA SM*. (*TURNERACEAE*)

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ABSTRACT

The alkaloid rich fraction isolated from the aerial parts of *Turnera subulata Sm*. has been evaluated for acute toxicity and pharmacological screening for anti-anxiety anti-depressant and antistress activities. Acute toxicity study was performed according to the OECD guidelines no:423 and the pharmacological evaluations were also carried out in Swiss albino mice. Anti-anxiety activity evaluated using Elevated plus maze method, Y-maze and antidepressant activity evaluated using Forced swim test and Tail suspension test and anti-stress activity evaluated using cold Swim test and Anoxic stress tolerance test. Phytochemical tests and thin layer chromatography were also performed for the confirmation of alkaloids in the fraction. The alkaloid rich fraction did not show any sign of toxicity up to dose of 2000mg/kg body weight. The alkaloid rich fraction showed an appreciable antidepressant, anti-stress activity and is devoid of anti-anxiety activity. The significant results of both anti-depressant and anti-stress activity were attributed to the phytoconstituents, 'alkaloid' which is present in the fraction isolated from the aerial parts of the plant *Turnera subulata Sm.*

Keywords: Acute oral toxicity, *Turnera subulata Sm*, Anti-Anxiety activity, Anti-stress activity, Anti-depressant activity

INTRODUCTION

Anxiety, Depression and Stress are the prevalent and highly comorbid psychiatric disorders globally. Aboutt 5-7% of adult across the globe can be victim of it. Mostly women are affected more than men **[1]**. The genus *Turnera* has great importance as it contains a good number of species holding various pharmacological activities and among them *Turnera aphrodissiaca* is the only one which is widely used clinically for various CNS related disorders **[2]**. *Turnera subuluta Sm*. is a Tropical American taxon which is now completely naturalised in Indian Subcontinent **[3]** *Turnera subulata Sm.* seems to hold great potential in connection with other species, for in depth investigation of various biological activities, especially CNS activities. An anxiolytic flavonoid Apigenin has been isolated from the aerial parts of *Turnera aphrodisiaca* **[4]** and pharmacological evaluation of this Apigenin, reveals that Apigenin have antianxiety activity and is devoid of antidepressant, anti-stress activities **[5]**. Apigenin -8 –c-α-glucopyranoside was one of the compounds present in *Turnera subualta* as that of *Turnera aphrodissiaca*.

Like other members of the genus bioactive properties of the *Turnera subulata Sm.* is very little known and so it is necessary to investigate the therapeutic potential of this plant. The plant *Turnera subulata Sm* requires proper scientific investigation to establish the biologically active chemical constituents' profile and mode of action. These findings prompted us to a hypothesis that apart from the flavonoid content of the crude extract's other fractions of the extracts like alkaloid rich fractions may possess some CNS activities. The present study was undertaken with an objective that the plant *Turnera subulata Sm* has never been subjected to pharmacological activities, like antianxiety, antidepressant and antistress activities and also detect the particular bioactive constituents present in the plant responsible for these activities. Therefore, it was considered worthwhile to undertake pharmacological evaluation of the aerial parts of the plant *Turnera subulata Sm*.

MATERIALS AND METHODS

Plant material

Turnera subulata Sm were collected from kottakkadavu, Calicut district, Kerala, India

and authenticated by Mr. A. K. Pradeep., Assistant Professor., Department of Botany., University of Calicut. and the voucher specimen was deposited to the Department of Botany., University of Calicut for future reference. (Reference number 86997)

Preparation of alkaloid rich fraction [6]

The aerial parts were separated and subjected to drying process and homogenized into fine powder and about 2000gm of the powdered material was then subjected for the preparation of alkaloid rich fraction using Harborn method (1973). The plant material was treated with 10% acetic acid in ethanol in a beaker. Then covered it and stand for overnight. Then filtered and concentrated to one quarter of its original volume on a water bath. Added concentrated ammonia drop wise to the extract until complete precipitation. The whole solution then allowed to stand till its settlement. Collected the precipitate and dissolved in chloroform solution. The chloroform soluble portion collected by evaporation of the chloroform using a rotatory evaporator. The resulted alkaloid rich fraction has been designated as ARF (8.39 gm) and stored in an air tight container for further studies.

Experimental animals

Swiss albino mice (20-25 gm) of either sex and of approximately the same age, Procured from Central Lab Animal Fecility, Amritha Institute of Medical Sciences and Research, Kochi, Kerala, India, were used for the study. The Animals were acclimatized to our Animal house, maintained at temperature of 22± 2^0C. The animals were exposed to alternate cycle of 12 hours of darkness and light each and housed in polypropylene cages and fed with standard rodent pellet diet, and purified water ad libitum. All the experiments were performed According to CPCSEA guidelines for care of laboratory Animals with approval of Institutional Animal Ethical Committee (IAEC), Jamia Salafiya Pharmacy College Regd.no.1686/PO/Re/S/13/CPCSEA, with an approval number 803/165/JSPC/IAEC/004 Dated 04/12/2017, where the study has been conducted.

Acute toxicity study

Acute oral toxicity was performed according to OECD test guidelines- 423-Acute toxic class method **[7]**. A single dose of 2000mg/kg body weight of alkaloid rich fraction (ARF) was administered orally to the animals and observed for any sign of appearance of toxicity for 48 hours after dosing at the first 30 minutes, periodically and during the first 24 hours with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Gross behavioural changes were observed at regular intervals of time.

Experimental design

36 Animals used for each screening method and fasted for 2 hours before the experiment .The animals were divided into 6 groups each containing 6 mice and dose administration was done as follows: Group I-[vehicle –sterile water+Tween 80(5%), p.o], Group II-[Standard drugs Diazepam 2mg/kg i.p **[8]** (Antianxiety and anti-stress activity) Ethanol 2.5gm/kg i.p **[9]** (Antidepressant activity)],Group III [125mg/kg of ARF, p.o],Group IV[250 mg/kg of ARF, p.o], Group V [500 mg/kg of ARF, p.o], Group VI [1000 mg/kg of ARF, p.o]. ARF was suspended in sterile water+Tween 80(5%) and administered to the animals.

1.Evaluation of Anti-anxiety activity

a) Elevated Plus Maze Method

The elevated plus maze has four arms (30×6cm) enclosed by 20 cm height walls. Each arm converges to a central area measuring 6cm×6cm. The maze is elevated to 50 cm height from the floor. Each mouse was placed at the centre of the maze with its head facing the open arm. The duration of the experiment was five minute and the behaviour of the mouse recorded as (a) the number of entries into the open arms, and (b) average time spend by the mouse in the open arms (average time = total time spent in open arms/ number of entries in open arms) **[10]**.

b) Y- Maze method

The Y maze apparatus consists of 3 identical arms measuring 5cm×35cm×10 cm, were properly connected to an equilateral triangular centre. 2 hour fasted animals orally pretreated with extract, vehicle, drug before placing on the apparatus. Animals placed in one of the three arms and allowed to freely explore in all the three arms. The total number of visits to different arms was measure for a period of 5-minute experiment **[11]**.

2.Evaluation of anti-depressant activity

a) Forced Swim Test (FST)

Animals adapted to laboratory condition 1 hour before being exposed to FST. In this animal was individually forced to swim in a cylinder pool (10cm diameter ,13 cm height water at 22±1ºC) and the total time of immobility during a 6-minute experiment was recorded in second. Immobility time recorded when the animal showed a floating motionless state or making only the movements necessary to keep its head above the water level **[12]**.

b) Tail suspension test

Depression can be induced by suspending the mice from the edge of a table 50 cm above the floor by an adhesive tap placed approximately 1 cm from the top of the tail. Immobility time was recorded during a 6-minute period. Changes in the immobility duration studied

after administration of drug in separate groups of animals **[12]**.

3)Evaluation of anti-stress activit

a) Cold swimming test

In this animal were placed in an open tank (50 cm height × 80 cm diameter) filled with cold water (6±2ºC) and allowed to swim for 10 minutes. The water temperature monitored regularly trough out the experiment. If the animals showing drowning behaviour (ceased swimming or barely floating) removed from water and swimming latency time was recorded. After each session animals dried with towel and placed under a warmer and then returned to their home **[10]**.

b) Anoxic stress tolerance test: Mice were subjected to anoxic stress by keeping them in a confined air tight 250 ml glass jar. Time taken for the mice to exhibit the first clonic convulsion taken as the end point, the animals removed from the vessel for recovery and resuscitated if needed **[13]**.

Antinxity activity

Antidepressant activity

Antistress activity

Statistical Analysis

The results were expressed as mean ± standard error of mean (S.E.M). The test doses were compared with standard and control by analysis of variance (ANOVA) followed by Studentized Tukey's test **[14]** using Graph Pad Prism 7.04

RESULTS AND DISCUSSION

The qualitative phytochemical tests and TLC profiling of the prepared alkaloid rich fraction confirmed the presence of alkaloids.

S. No.	CHEMICAL TEST	OBSERVATION
1	Mayer's test	+
2	Wagner's test	+
3	Hager's test	+
4	Dragendroff's test	+

In acute toxicity studies, the fixed doses of 2000 mg/kg, as per OECD guidelines no:423 were performed in female mice. The sign elicited by the mice such as respiration, salivation and sence of touch were normal. The other gross behavioural changes were also normal.

S. No.	GROUPS	NUMBER OF ANIMALS/GROUP	DOSE IN mg/kg	NUMBER OF DEATH OF ANIMALS
1	I	3	2000mg/kg	No death
2	II	3	2000mg/kg	No death

S. No.	OBERVATION	EFFECTS								
	GROSS ACTIVITY	Upto3 hrs	3½ hrs	4 hrs	4½ hrs	5 hrs	5½ hrs	6 hrs	12 hrs	24 hrs
1	Tremor	-	-	-	-	-	-	-	-	-
2	Writhing	-	-	-	-	-	-	-	-	-
3	Respiration	+	+	+	+	+	+	+	+	+
4	Convulsion	-	-	-	-	-	-	-	-	-
5	Sense of touch and sound	+	+	+	+	+	+	+	+	+
6	Salivation	+	+	+	+	+	+	+	+	+
7	Diarrhea	-	-	-	-	-	-	-	-	-
8	Skin color changes	-	-	-	-	-	-	-	-	-
9	Body weigt	-	-	-	-	-	-	-	-	-
10	Mortality	-	-	-	-	-	-	-	-	-

Anti anxity activity by Elevated plus maze method

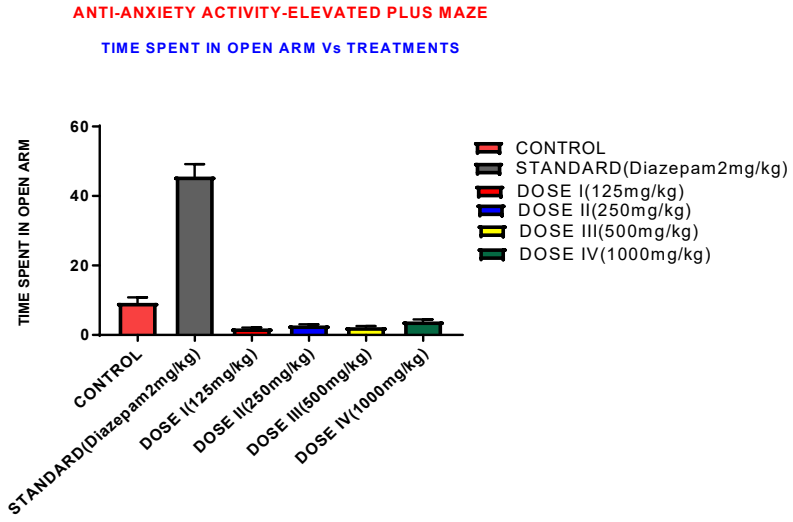
In the case of pharmacological evaluation for anti-anxiety activity in mice using Elevated plus maze apparattus and Y-Maze at various dose levels -125, 250, 500,1000 mg/kg, the number of open arm entries by the mice of all the tested doses were very less compared to standard as well as control. Animals spend more time in the closed arm and avoid the open arm entries probably to avoid falling off. Avoidance of the open arms clearly demonstrates the fear response. It suggests that absence of anxiolytic activity. An

increase in the number of visits in the three arms of the Y-maze observed in the all the treated dose of the alkaloid rich fraction compared to control animals, suggests that absence of anxiolytic activity.

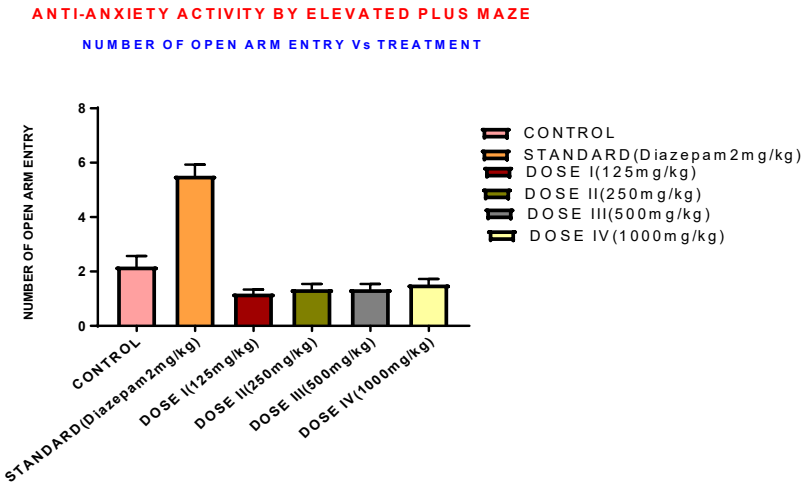
Table 1

S. No.	TREATMENT	DOSE mg/kg	MEAN[a] NUMBER OF ENTRIES±S.E.M.	MEAN[a]TIME[b](S)± S.E.M
1	Control	Vehicle	1.5±0.2236	2.833±0.5426
2	Diazepam	2.0	5.5±.4282	45.52±3.656
3	Dose I	125	1.67±0.1667	6.833±2.75
4	Dose II	250	1.5±0.2236	7.833±3.081
5	Dose III	500	1.333±0.2108	4±1.033
6	Dose IV	1000	1.67±0.1667	5.833±1.276

n= 6; values are expressed as mean ± standard error mean; ANOVA. followed by student's Tukey's test .P<0.05 considered as significant ns= not significant



Graph 1



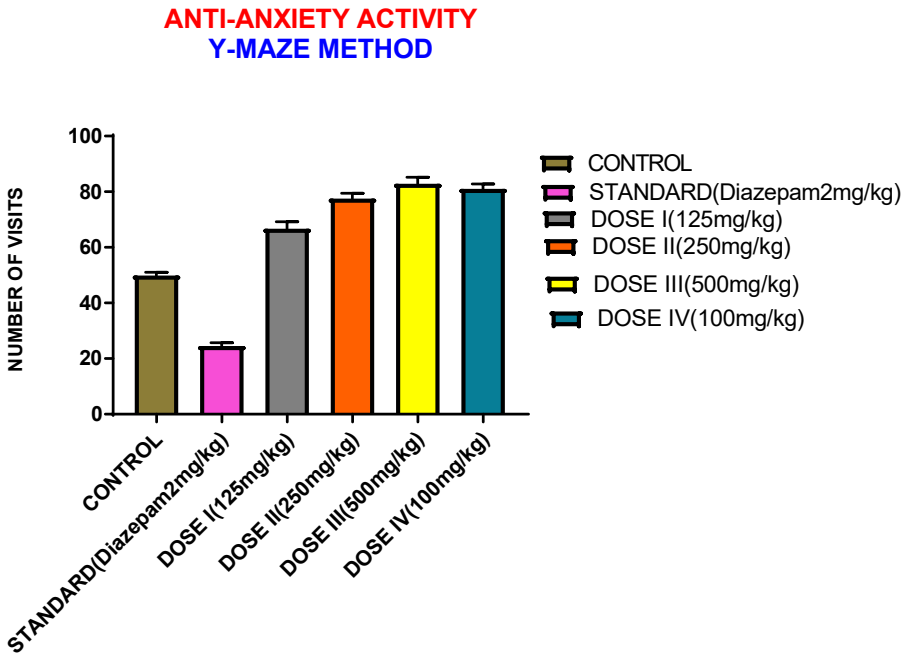
Graph 2

Anti anxity activity by Y-maze method

Table:2

S. No.	TREATMENT	DOSE mg/kg	MEAN [a]TOTAL NUMBER OF VISITS±S.E.M
1	CONTROL	VEHICLE	49.83±1.195
2	DIAZEPAM	2.0	24.5±1.176
3	DOSE I	125	66.67±2.525
4	DOSE II	250	77.5±1.979
5	DOSE III	500	82.83±2.386
6	DOSE IV	1000	81±1.751

n= 6; values are expressed as mean ± standard error mean; ANOVA. followed by student's Tukey's test.P<0.05 considered as significant ns= not significant



Graph 3

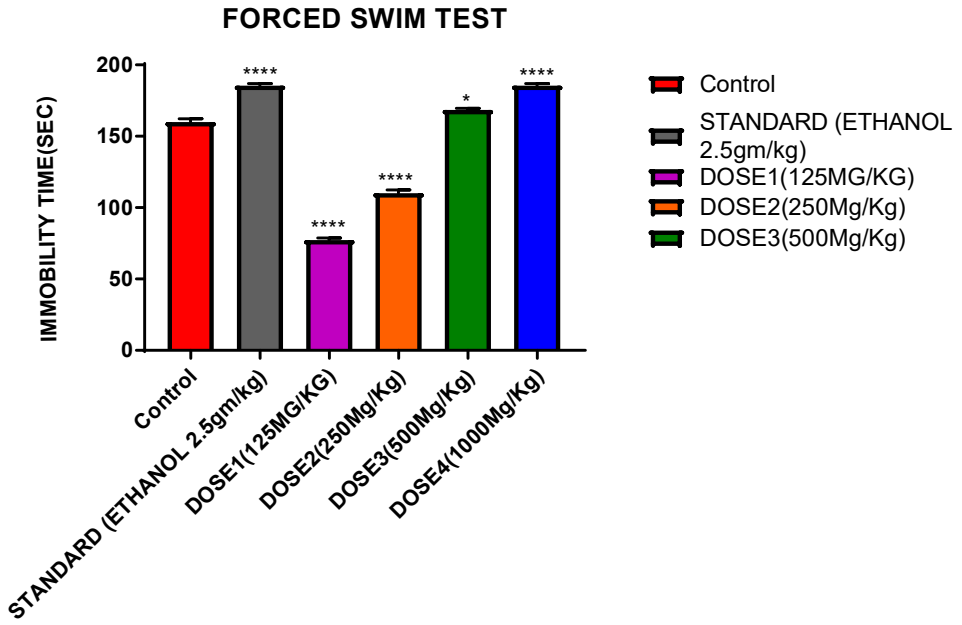
Antidepressant activity

In evaluation for anti-depressant activity in mice using Forced swim test and Tail suspension test at various dose levels -125, 250, 500,1000 mg/kg, both method Test dose 1 (125mg/kg) and Test dose 2 (250 mg/kg) Showed significant reduction in the immobility time thus produced significant antidepressant activity compared to control groups as well as standard group(ethanol 95%-2.5gm/kg). An increase in immobility time showed in higher doses (500mg/kg and 1000mg/kg) compared to control groups as well as standard groups.

Table 3

S. No.	TREATMENT	DOSE	MEAN[a]TIME OF IMMOBILTY(Min)±S.E.M.
1	Control	Vehicle	159.8±2.442
2	Ethanol	2.5gm/kg	185.3±1.542
3	Dose I	125mg/kg	77.17±1.579
4	Dose II	250mg/kg	110±2.338
5	Dose III	500mg/kg	168.3±1.229
6	Dose IV	1000mg/kg	185.3±1.542

n-6; Values are expressed as mean ±Standard error mean;**p<0.000001,*p=0.02 Vs Control;ANOVA followed by Studentized Tukey's test.**



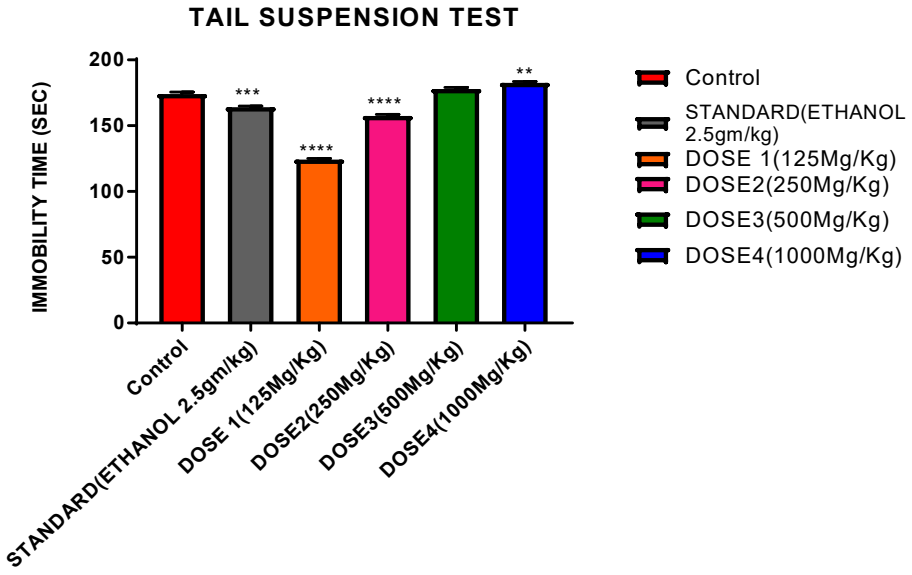
Graph 4

Tail suspension method

Table 4

Sl.No	TREATMENT	DOSE	MEAN[a]TIME OF IMMOBILTY(Min)±S.E.M
1	Control	Vehicle	173.8±1.778
2	Ethanol	2.5gm/kg	163.8±1.014
3	Dose I	125mg/kg	124±1.065
4	Dose II	250mg/kg	157.2±1.352
5	Dose III	500mg/kg	177.7±1.333
6	Dose IV	1000mg/kg	182.3±1.202

n-6; Values are expressed as mean ± Standard error.mean;**p<0.000001, ***p=0.0001, **p=0.001 Vs Control ; ANOVA followed by Studentized Tukey's test**



Graph 5

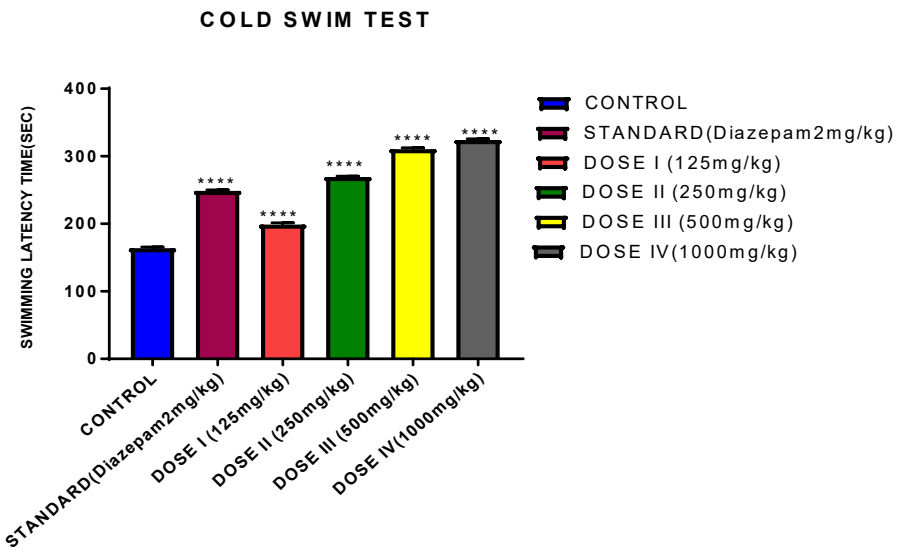
Anti stress activity

The alkaloid rich fraction from the aerial parts of *Turnera subulata* when subjected to pharmacological evaluation for anti-stress activity in mice using Cold swim test and Anoxic stress toleance test at various dose levels -125, 250, 500,1000 mg/kg. In the cold swim test mice treated with the alkaloid rich fraction from the aerial parts of the plant *Turnera subulata* showed significant improvement in the swimming time and in case of anoxic stress test significant increase in the latency of anoxic convulsion.

Table 6

S .No.	TREATMENT	DOSE mg/kg	MEAN[a]SWIMMING LATENCY TIME(Min)±S.E.M
1	Control	Vehicle	163.3±2.404
2	Diazepam	2.0	248.2±2.088
3	Dose I	125	198.3±3.18
4	Dose II	250	268.7±1.944
5	Dose III	500	309.8±2.75
6	Dose IV	1000	323.5±2.487

n-6;Values are expressed as mean ±Standard error mean;**p<0.01 Vs Control;ANOVA followed by Studentized Tukey's test.**

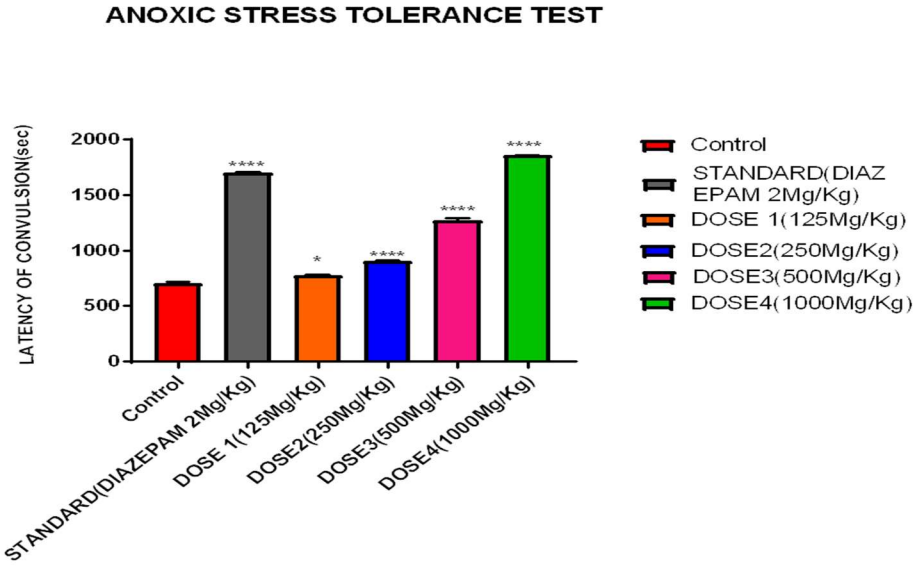


Graph 6

Table 7

S. No.	TREATMENT	DOSE mg/kg	MEAN[a]DURATION OF ANOXIC STRESS TOLERANCE(SEC)±S.E.M
1	Control	Vehicle	7O5.8±15.05
2	Diazepam	2.0	1703±3.887
3	Dose I	125	779±5.125
4	Dose II	250	908±2.176
5	Dose III	500	1272±20.33
6	Dose IV	1000	1858±3.631

n-6; Values are expressed as mean ±Standard error mean;*p=0.04,**p<0.01 Vs Control;ANOVA followed by Studentized Tukey's test**



Graph 7

CONCLUSION

The anti- anxiety activity of alkaloid rich fraction from the aerial parts of *Turnera subulata Sm* by elevated plus maze method and Y-maze method clearly suggests that the alkaloids were devoid of anxiolytic activity. Thus it is confirmed that the anxiolytic activity of the *Turnera subulata Sm* is due to the presence of the flavonoid 'Apigenin'. It can be inferred that the alkaloid rich fraction from the aerial parts *of Turnera subulata Sm* possess anti stress and antidepressant activity and devoid of antianxiety activity. Therefore, this alkaloid rich fraction could be considered for the treatment of stress and depressant related neuropsychiatric disorders by conducting further pharmacological studies and find out the compounds responsible for these bioactivity in the animal model as well as to confirm the mechanism of antistress and antidepressant action.

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