

Anxiolytic Activity of *Rauvolfia Tetraphylla* Leaf Extract in RodentsAaditya Singh¹, Shalini Tripathi², P.N Singh³¹Aryakul College of Pharmacy & Research Lucknow.²Rameshwaram Institute of Technology & Management Lucknow³Department of Pharmaceutics, IIT, BHU, Varanasi***Corresponding author: E-Mail: aadityasingh85120@gmail.com****ABSTRACT****Objective:** Anxiolytic activity of ethanolic extract (90%) of *Rauvolfia tetraphylla* was aimed to evaluate by this study.**Methods:** For the study of Anxiolytic activity the model used is elevated plus Maze (EPM) model, statistical analysis was done by one way ANOVA using Imipramine as standard drug.**Result:** The in vivo studies shows that ethanolic extract (90%) of *Rauvolfia tetraphylla* possess significant anxiolytic activity (p<0.05).**Conclusion:** The plant *Rauvolfia tetraphylla* possesses anxiolytic activity.**KEY WORDS:** Anxiolytic activity, *Rauvolfia tetraphylla*.**1. INTRODUCTION**

Mental diseases including anxiety are considered leading global healthcare burden. Anxiety is associated with various physical, psychological and behavioural changes mainly found in those who were suffering from chronic medical conditions such as diabetes, asthma and epilepsy. Anxiety is a problem faced by everyone but for some people it becomes frequent.

There are various drugs like benzodiazepines, beta blockers, tricyclic antidepressants, anticonvulsants, MAOIs, SSRIs, SNRIs etc. were available for treatment of anxiety disorder but they have some side effects such as ataxia, confusion, neuroleptic malignant syndrome, tardive dyskinesia, and impaired cognition. Due to these side effects traditional herbs are very useful because they are safe, effective and affordable.

There are various herbal drugs are also available for treatment of anxiety disorders but here we selected a medicinal plant *Rauvolfia tetraphylla* belongs to apocynaceae family (Kumar, 2011). It grow like a bush or a small tree or cultivated both as an ornamental or pharmaceutical plant. It has approximately 85 species in the genus which mainly grow in tropical regions. (Jyothi, 2012) *Rauvolfia tetraphylla* is a medicinal plant in folk medicines it is used to cure various diseases like hypertension, fever, malaria, gingivitis etc.

2. EXPERIMENTAL

Plant material: Fresh leaves of *Rauvolfia Tetraphylla* were collected during the month of Aug 2014 from Lucknow (U.P). The collected leaves are authenticated by a taxonomist from NBRI (CSIR Lab) Lucknow. A specimen copy (NBRI/PH/6/3) was also deposited for NBRI herbarium.

Preparation of ethanolic Extract: The leaves of plants were collected washed thoroughly in tap water and dried under shade in air. These dried leaves materials are powdered and sheaved using 80 meshes. 100 gm of dried leave powder was extracted by Soxhlet apparatus using (90%) ethanol for 48 hr. (Suresh, 2008). The extract was dried and concentrated under reduced pressure and stored in refrigerator in glass bottles for further experimentation.

Phytochemical screening: Various chemical tests for determination of primary and secondary metabolites like alkaloids, carbohydrates, caumarins, flavonoids, fixed oil, glycosides. Gums and resins, mucilage's, proteins & amino acids, saponins, steroids, tannins, triterpenoids were performed (Kavitha, 2012).

Table.1. Preliminary phytochemical screening of leaves of *Rauvolfia tetraphylla*

Constituents	Ethanol	Constituents	Ethanol	Constituents	Ethanol
Alkaloids	+++	Glycosides	+	Saponins	+
Carbohydrates	+	Gums and resins	++	Steroids	+
Caumarins	-	Mucilages	+	Tanins	+++
Flavonoids	++	Proteins and amino acid	-	Triterpenoids	-
Fixed oil	++				

Note: +++ High, ++ Moderate: + Slight: ----- Negative

Animals: Albino mice (20±2 g) of either sex were obtained from the Central Animal House, Rameshwaram Institute of Pharmacy (Reg.No- 1397/ac/10 CPCSEA). Animals were kept in ambient temperature of 25±1°C with a 12 hour light/dark cycle (lights on at 7:00 a.m) with free access to standard pellet (Hindustan lever, Mumbai) and water ad libitum by prior permission was obtained from Institutional Animal Ethics Committee to carry out the experiments. Albino mice were divided in four group (n=5) for each test.

Acute toxicity studies: The procedure for acute toxicity of ethanolic extract of leaf of Tetraphylla was followed as per the OECD guideline no.423 (Acute Toxic Class Method) (OECD, 2002). A dose of 800, 1000, 2000mg/kg body weight is administered and animals were observed for 15 days.

Drug Treatment: 20 mice were grouped into four: Group 1 is used as control receives vehicle (10% v/v PEG, 10 ml/kg, p.o). Group 2 receives the standard drug i.e diazepam (1mg/kg, i.p.). Group 3 & 4 receives the drug extract in a dose of 200 & 400 mg/kg, p.o respectively. Diazepam was administered 1hr and 30 min before test session.

Elevated Plus Maze (EPM): The EPM (Techno Mumbai M.S) was made of wood and consisted of two opposed open arms (50x10 cm) and two opposed enclosed arms of same size with 40cm high walls. The arms were connected with a central square 10x10 cm, giving the apparatus a plus sign the maze was kept in dimly lit (lit intensity of 20 lux), sound proof room and elevated 50 cm above the floor (OECD, 2002). Each naive rat/mice was placed at the center of the maze facing an enclosed arm and allowed to explore the maze 5 min. The standard spatio-temporal measurement, such as the number of entries and the time spent in open and enclosed arms were recorded. The exploratory behaviour in the open arm was expressed as the percentage of entries in to (% open arm entries) and the time spent (% open arm time). An arm entry was defined when all four paws of the rat were in the arm.

Statistical Analysis: Statistical analysis was done using Sigma Stat Statistical Software 2.0 (Jandel Scientific Corporation, USA). All observation was expressed as mean± SD. The data was analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test (two tailed). $P < 0.05$ was considered as statistical significance.

3. RESULTS AND DISCUSSION

In Elevated Plus Maze (EPM) test, all dose levels of *R. tetraphylla* extract exhibited a significant ($P < 0.05$) increase in the open arm time spent, open arm entries percentage entries and percentage time spent. Total entries of *R. Tetraphylla* and diazepam treated groups were not altered significantly compared to vehicle treated animals.

Table.2. Effect of Ethanol Extract of Rauvolfia tetraphylla in Elevated plus maze Test on mice

Treatment	Dose (mg/kg)	No of Entries (N)		Time spent (sec)	
		Enclosed	Open arm	Enclosed	Open arm
Vehicle (5)		21.80±0.69	2.40±0.19	218.00±0.82	18.00±0.26
R.tetraphylla (5)	200	12.80±0.32***	4.20±0.22***	186.00±1.81***	58.00±0.82***
R.tetraphylla (5)	400	11.00±0.26***	5.00±0.26***	176.00±1.09***	62.00±0.63***
Diazepam (5)	1	7.60±0.19***	6.80±0.32***	162.00±0.98***	72.00±0.59***

N denotes for number, Values in parenthesis indicate number of animal used. * indicates a significant difference compared to control group at $P < 0.05$.

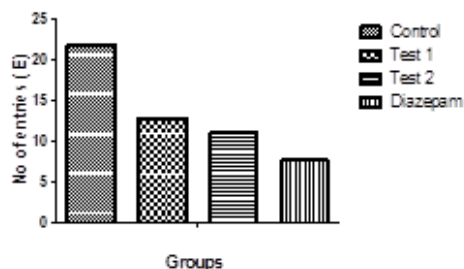


Figure.1. Effect of ethanolic extract of *R.tetraphylla* (200, 400 mg/kg) and standard Diazepam (1 mg/kg) on percentage of no entries in enclosed arm in elevated plus maze

$p^* < 0.05$, $p^{**} < 0.01$ when compared to control

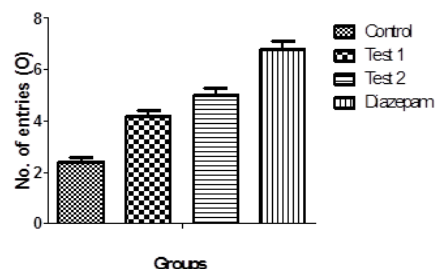


Figure.2. Effect of ethanolic extract of *R.tetraphylla* (200, 400 mg/kg) and standard Diazepam (1 mg/kg) on percentage of no entries in open arm in elevated plus maze

$p^* < 0.05$, $p^{**} < 0.01$ when compared to control

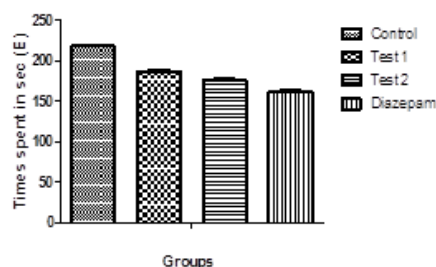


Figure.3. Effect of ethanolic extract of *R.tetraphylla* (200, 400 mg/kg) and standard Diazepam (1 mg/kg) on percentage time spent by mice in enclosed arm in elevated plus maze

$p^* < 0.05$, $p^{**} < 0.01$ when compared to control

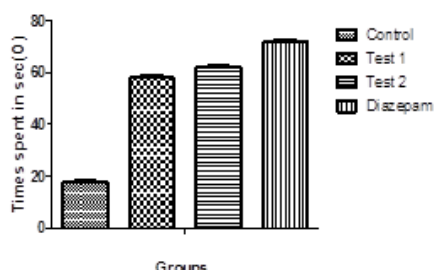


Figure.4. Effect of ethanolic extract of *R.tetraphylla* (200, 400 mg/kg) and standard Diazepam (1 mg/kg) on percentage time spent by mice in open arm in elevated plus maze

$p^* < 0.05$, $p^{**} < 0.01$ when compared to control

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5. CONCLUSION

The conclusion based on our present study is that the ethanolic extract of *Rauvolfia tetraphylla* has shown significant anxiolytic activity in a dose of 200mg/kg and 400 mg/kg body weight.

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