PHYTO CHEMICAL EVALUATION AND INVESTIGATION OF ANTICONVULSANT AND ANXIOLYTIC ACTIVITY OF METHANOLIC EXTRACT OF INDIGOFERA MYSORENSIS

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Abstract: Background In recent year there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications.

Objectives The objective of this study was Preliminary phytochemical studies, Screening of Anxiolytic activity and Screening of Anticonvulsant activity

Methods The pharmacological and acute toxicity studies of Methanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed upto 2000mg/kg of body weight.Preliminary phytochemical screening done by using various chemical tests. Evaluation of anxiolytic activity by Elevated Plus Maze (EPM) Test, Open Field Test and Rota rod test. Evaluation of anticonvulsant activity by PTZ Induced Convulsion, maximal electro shock model.

Results The preliminary phytochemical investigation of Methanolic extract of leaves of Indigofera mysorensis showed the presence of Carbohydrate, Alkaloids, Phytosteroids, Flavonoids, Phenolic compounds and Tannins. Results showed that plant extracts treated mice exhibited significant increase in the number of open arm entries but decreases in time spent in closed arm, which reflects plants anxiolytic property. animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Mice treated with extract showed increase in number of rearings and time spent in the center. Plant extract showed significant decrease in the locomotory score and fall of time of the mice from the rotating rod.

The extract exhibited dose dependent protection in the MES and PTZ induced convulsions. Nevertheless, in unprotected animals, the extract significantly increased seizure latency and reduced seizure duration compared with the control group in all two models at all tested doses. The effect of most of antiepileptic agents is to enhance the response to GABA by facilitating the opening of GABA-activated chloride channels. GABA receptors were involved in epilepsy and their direct activation would have an antiepileptic effect.

Conclusion Methanolic extract of Indigofera mysorensis possesses anxiolytic and anticonvulsant effects and these findings collaborate with the ethnomedicinal uses of this plant. The isolation of active chemicals from this plant might serve as lead compounds for the synthesis of drugs which could be used in the management of these nervous disorders.

Keywords: Phyto Chemical Evaluation, Anticonvulsant Activity, Anxiolytic Activity Methanolic Extract, Indigofera Mysorensis

INTRODUCTION
There is currently no cure, so symptomatic pharmacological treatment remains the mainstay of therapy for people with epilepsy. [3] By definition, antiseizure medications (ASMs) prevent or suppress the generation, propagation, and severity of epileptic seizures. The term “antiseizure medication” has replaced the old term “anticonvulsant drugs” because epilepsy therapies suppress not only convulsive but also nonconvulsive seizures.[4, 5] Furthermore, the term “antiseizure medication” more and more replaces the term “antiepileptic drug” because such drugs provide symptomatic treatment only and have not been demonstrated to alter the course of epilepsy. [1, 6] Achieving complete seizure control is the most important objective in the treatment of epilepsy. For this goal, ASMs are administered chronically to prevent
seizure recurrence in patients with spontaneous recurrent seizures (SRS). In addition, ASMs are being used to treat status epilepticus (SE) and interrupt acute symptomatic seizures in response to a variety of causes, including intoxication. However, despite the availability of numerous ASMs with different mechanisms of action (MOAs), both SRS and SE may be resistant to treatment in about 30% of all patients with epilepsy. [7–10] Interestingly, seizure freedom outcomes have not changed much since 1939, the year that phenytoin came into use, in spite of the development of numerous novel ASMs in recent decades. [9–11] Mechanisms of ASM resistance are incompletely understood.[12]

The primary use of sedative–hypnotic and anxiolytic drugs is to encourage calmness (anxiolytics or sedatives) or to produce sleep (sedative–hypnotics). All people are subjected to states of emotional tension and uneasiness. For otherwise healthy individuals, these occasions are usually mild and short that pharmacological intervention is unnecessary.[13] Anxiety almost invariably accompanies many medical and surgical conditions, and it is often a symptom of psychiatric illness. When the symptoms become intolerable or interfere with the treatment of the underlying disease, and if counseling is not sufficient, drug treatment can be considered as a means of helping patients cope with their anxiety.[14]

Anxiety that results from fear caused by an acute illness or a stressful event, such as loss of a loved one, is usually self-limiting and can be of relatively short duration. The current options include various kinds of psychotherapy and pharmacotherapy such as benzodiazepines, azapirones, and antidepressants and others.[15]

The recognition of anxiolytic effects of nonbenzodiazepine azapirones agents, which acts as 5-HT1A partial agonists, such as buspirone, gepirone, and ipsapirone and their therapeutic role in clinical anxiety and mood disorders has further focused attention on the 5-HT1A receptor.[16] However, the anxiolytic effects of azapirones follow a time course observed with antidepressants where therapeutic effects are delayed for 3–4 weeks, which is unlike the rapid effects observed with benzodiazepine anxiolytics.[17] Thus, there is a need for robust anxiolytic compounds that have lesser side effects than benzodiazepines and a more immediate onset of action than currently available 5-HT1A receptor acting drugs.

In recent years there has been a tremendous increase in demand for herbal drugs due to its safety, efficacy and better therapeutic results and also due to its economic pricing as compared to synthetic or allopathic drugs, which have several therapeutic complications. The selection of this plant, Indigofera mysoresensis was made on the basis of its high therapeutic value, easy availability, degree of research work which is not done. Very less pharmacological studies have been carried out on the leaves of Indigofera mysoresensis. Hence, I have decided to choose Indigofera mysoresensis on which detailed studies on Preliminary Phytochemical and Pharmacological actions on CNS is done.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION

Collection of specimen:
The species for the proposed study that is leaves of Indigofera mysoresensis has carefully collected from Chittor district, Andhra Pradesh. The plant parts are identified by Dr. Madhavashetty, Taxanomist, Dept of Botany, S.V University and Thirupathi, India and authenticated by comparing with the voucher specimen.

Shade drying:
After collection, the leaves of Indigofera mysoresensis were washed thoroughly with water to remove the dirt particles and any other foreign material adheres to leaves. Then after, the leaves were wiped off with cotton cloth and transferred to newspaper and evenly spreader on to paper.

The Indigofera mysoresensis leaves were subjected to shade drying to treat fungus until complete dryness of leaves. Then the dried leaves were powdered by mixer grinder until to get coarse powder, which was used for further detailed studies, extraction with solvent and phytochemical studies.

Extraction of Indigofera mysoresensis leaves:

Methanol extract:
About 250gm of air dried powdered material was taken in 3000ml Soxhlet apparatus and extracted with petroleum ether until green colour disappear. At the end of the day the powder was taken out and dried. After drying it was again packed and extracted by using Methanol (S.D. Fine Chemicals Ltd. Mumbai, India) as solvent, till colour disappeared. The temperature was maintained at 55°C-65°C. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield of Methanolic extract were noted.

Preliminary phytochemical screening
The Methanolic extract of Indigofera mysoresensis leaves were subjected for the different chemical tests for the identification of various active constituents.

PHARMACOLOGICAL EVALUATION
ACUTE ORAL TOXICITY STUDY

The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion.

The method uses defined doses (5, 50, 300, 2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which cause acute toxicity.

ANIMALS:
Female albino mice of 20-30 gm of body weight obtain from Animal House. Animals were kept in standard animal house condition. Prior to use, the mice were housed in polypropylene cages in group of six animals under natural light-dark cycle. They were provided with commercial food pallets and tap water ad libitum. Cleaning and sanitation work was done on alternate days. Paddy husk was provided as bedding material. All the observations were made at room temperature in a noiseless diffusely illuminated room. The cages were maintained clean and all experiments were conducted between 8 am to 3 pm.

PROCEDURE:
Twelve animals Albino mice, (25-30gm) were selected for studies. Most of the crude extracts possess LD50, value more than 2000mg/kg of the body weight of the animal used. Dose volume was administered 0.1ml/100gm body weight to the animal by oral route.

After giving the dose toxic signs were observed within 3-4 hours. Body weight of the animals before and after administration, onset of toxicity and signs of toxicity like changes in the skin and fur, eyes and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems activities, motor activity and behavior pattern, sign of tremors, convulsion, salivation, diarrhea, lethargy and sleep and coma was also to be noted, if any, was observed.

The animal toxic or death was observed upto 14 days.

OBSERVATION
Acute toxicity studies and evaluation of datas are studied as per the guideline of OECD (423). No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD50 of the Methanolic extract of leaves of Indigofera mysorensis was greater than 2000mg/kg (LD50>2000mg/kg).

Hence the biological dose was fixed at three levels, 125,250 and 500mg/kg body weight for the extract

EVALUATION OF ANXIOLYTIC AND ANTICONVULSANT ACTIVITY

Animals:
Albino mice of either sex 20-30 gm of body weight obtain from Animal House. Animals were kept in standard animal house condition. Prior to use, the mice were housed in polypropylene cages in group of six animals under natural light-dark cycle. They were provided with commercial food pallets and tap water ad libitum. Cleaning and sanitation work was done on alternate days. Paddy husk was provided as bedding material. All the observations were made at room temperature in a noiseless diffusely illuminated room. The cages were maintained clean and all experiments were conducted between 8 am to 3 pm.

Drugs and Chemicals:
✓ Diazepam (Calmpose Inj. Ranbaxy, India)
✓ Pentylentetrazole (Sigma, USA)
✓ Methanol extra pure (S.D fine chemicals, Mumbai).

Experimental Design:
Animals are divided into 5 groups, each group containing 6 mice.

- Group I: Normal control mice fed with vehicle only.
- Group II: Mice treated with Diazepam 5mg/kg
- Group III: Mice treated with 125 mg/kg Methanolic extract of Indigofera mysorensis
- Group IV: Mice treated with 250 mg/kg Methanolic extract of Indigofera mysorensis
- Group V: Mice treated with 500 mg/kg Methanolic extract of Indigofera mysorensis
EVALUATION OF ANXIOLYTIC ACTIVITY

Elevated Plus Maze (EPM) Test.
The EPM test is the most frequently employed model for the assessment of the anxiolytic activity of novel substances (R.G.Liser 1987). The elevated plus maze apparatus consisted of two perpendicular open arms (50 X 10 cm) and two perpendicular enclosed arms (50 X 10 X 40 cm). The entire maze was constructed of wood and elevated 50 cm above floor. The maze was placed inside a light (25 lx) and sound attenuated room.

The animals were divided into five groups, each group comprised six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic Extract of Indigofera mysorensis at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, the rat was placed in the center platform of the maze facing the enclosed arm and was observed for 10 min. The parameters assessed were the time spent in open and enclosed arms and numbers of open and enclosed arms entries. All tests were taped by using a video camera and every precaution was taken to ensure that no external stimuli could evoke anxiety in the mice. After each test, the maze was carefully cleaned up with a wet tissue paper (70% ethanol solution) to eliminate the interference of the olfactory cues on the next rat.

Open Field Test.
The study was conducted according to method previously described by Brown et al with some modifications. The apparatus was made up of plywood measuring 72 cm X 72 cm X 36 cm. One of the walls was made of transparent Perspex glass to ensure that the mouse under investigation is visible to the observer. The floor, made of cardboard, was divided into 16 equal squares (18 cm X 18 cm) with blue marker and a central square drawn with black marker. The cardboard was covered with a transparent Plexiglas. The animals were divided into five groups; each group comprised six rats. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of Indigofera mysorensis at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, each mouse was placed individually at the corner of the arena and its behavior monitored for 5 min. The number of rearings and number of square crossed by each mouse was recorded. The apparatus was wiped between observations with 70% ethyl alcohol and allowed to dry to remove any olfactory cue.

Rota rod:
The equipment of Rotarod was used to evaluate motor coordination produced by drugs in animals. The mice were trained before the experiment to acquire the capacity to remain for 300 s on a diameter rod, rotating at 20 rpm. Two or three trials were sufficient for the animals to learn this task. Thirty mice were divided into five groups; each group comprised six rats. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of leaves of Indigofera mysorensis at doses of 125, 250, and 500 mg/kg, BW. Then, the animals were placed in the four paws on the rotating bar, which is 2.5 cm in diameter and 25 cm high from the floor. The animals were observed for a period of five minutes. The difference between the fall-off time of the mice before and after treatment was considered as an index of muscle relaxation (Farkas.S et al., 2005).

EVALUATION OF ANTICONVULSANT ACTIVITY

Pentylenetetrazole Induced Convulsions:
Pentylenetetrazole (PTZ) induced convulsions test was performed to evaluate anticonvulsant property of drugs (Ahmadiani.A et al.,).Thirty male mice were divided into five groups, each group comprising six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of Indigofera mysorensis at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, convulsions were induced by the intraperitoneal administration of 60 mg/kg BW of PTZ. Following the administration of PTZ, mice were placed in separate transparent plexiglass cages (25 x 15 x 10 cm) and were observed for the occurrence of seizures over a 30 min time period. Latency of convulsions (the time prior to the onset of tonic convulsions), duration of tonic convulsions, and mortality protection (percentage of deaths in 24 h) were recorded (R.S. Fischer, 1989).

Maximal Electro Shock (MES) Induced Convulsions:
The animals were divided into five groups, each group comprising six mice. Different groups were treated with distilled water (10 mL/kg), diazepam (5 mg/kg), and Methanolic extract of Indigofera mysorensis at doses of 125, 250, and 500 mg/kg, BW. Thirty minutes later, convulsions were induced in all the groups of animals using electro convulsimeter. A 60 Hz alternating current of 150 mA for 2 s was delivered through the ear electrodes (Balamurukan.G.et al.,). The animal was observed for the occurrence of tonic hind limb extension.

Data analysis
Results of the experiments and observations were expressed as mean ± standard deviation (SD). The significance of differences between groups was determined using one-way analysis of variance (ANOVA) followed by at least one of
the following post hoc tests: Dunnett’s multiple comparison tests $P < 0.05$ where level of significance was considered for each test. The data is presented as mean ± S.D.

**RESULTS AND DISCUSSION**

Based on literature review, the leaves of *Indigofera mysorensis* was collected, authenticated and the project was carried out. The result of the present study show that the Methanol extract of *Indigofera mysorensis* leaves shows significant anticonvulsant and anxiolytic activities.

**PRILIMINARY PHYTOCHEMICAL STUDIES**

<table>
<thead>
<tr>
<th>Table No. 1: Percentage Yield of <em>Indigofera mysorensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of extract</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Methanol</td>
</tr>
</tbody>
</table>

The extract obtained were subjected to qualitative Phytochemical test to find out the active constituents.

<table>
<thead>
<tr>
<th>Table No. 2: Qualitative Phytochemical analysis of the extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEST FOR PHYTOCONSTITUENTS</td>
</tr>
<tr>
<td>Saponins</td>
</tr>
<tr>
<td>Alkaloids</td>
</tr>
<tr>
<td>Glycosides</td>
</tr>
<tr>
<td>Tannins and phenolic compounds</td>
</tr>
<tr>
<td>Carbohydrate</td>
</tr>
<tr>
<td>Terpenoids</td>
</tr>
<tr>
<td>Flavonoids</td>
</tr>
<tr>
<td>Steroids</td>
</tr>
</tbody>
</table>

(+) - Present      (-) - Absent
DISCUSSION:
The preliminary Phytochemical studies were done in the Methanolic extract of Indigofera mysorensis leaves, the result suggest that presence of Alkaloids, Carbohydrate, Terpenoids, flavonoids, Steroids, phenolic compounds and tannins.

PHARMACOLOGICAL STUDIES
ACUTE ORAL TOXICITY STUDIES
The acute oral toxicity of the Methanolic extract of Indigofera mysorensis was carried out as per OECD 423-guidelines (Acute toxic class method). Acute toxicity studies revealed that LD50>2000mg/kg for the extract. Hence, the biological dose was fixed at 125, 250mg and 500mg/kg body weight.

EVALUATION OF ANXIOLYTIC ACTIVITY
Elevated plus maze:
Administration of diazepam (5mg/kg) significantly increases number of open arm entries, time spent in open arms and the number of rearings in open arm. They showed a reduction in the time spent in closed arm. Plant extracts treated mice exhibited significant increase in the number of open arm entries. The number of arm entries, but decreases in time spent in closed arm as shown in the table no .3.

Open field test:
There was significant anxiolytic activity observed with diazepam, plant extracts when compared to control. In the open field test, plant extract showed significant increase in number of rearings, number of squares crossed and number of assisted rearings during 5 min intervals of test as compared with control as show in table 4.

Rota rod:
Table 5.shows the effects of Methanolic extract of leaves of Indigofera mysorensis in the Rotarod test, a method used for evaluating motor coordination and presence of any muscle gripping effect. It revealed that there was significantly increased grip force and fall time after administration of Methanolic extract of Indigofera mysorensis (125, 250, and 500 mg/kg) when compared to control. All the plant extract treated animals retained on the rotating rod for more than 276.35 ± 7.58 s at 500 mg/kg as shown in Table 6.5 indicate the Methanolic extract of Indigofera mysorensis to be devoid of neurotoxicity.
Table No. 3: Effect of Methanolic extract of Indigofera mysorensis on Elevated plus maze in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Time spent in open arm (s)</th>
<th>Entries in open arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline</td>
<td>10ml/Kg</td>
<td>40.25±4.41</td>
<td>3.98±0.52</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam</td>
<td>5mg/kg</td>
<td>239.59±3.52**</td>
<td>12.64±0.47**</td>
</tr>
<tr>
<td>III</td>
<td>Plant extract</td>
<td>125mg/kg</td>
<td>100.83±3.97</td>
<td>6.48±0.39</td>
</tr>
<tr>
<td>IV</td>
<td>Plant extract</td>
<td>250mg/kg</td>
<td>173.81±4.32*</td>
<td>6.53±0.42*</td>
</tr>
<tr>
<td>V</td>
<td>Plant extract</td>
<td>500mg/kg</td>
<td>213.92±4.80**</td>
<td>10.32±0.21**</td>
</tr>
</tbody>
</table>

The data represent the mean ±S.D (n=6) *p<0.01, **p<0.001 significantly different compared to normal control and diazepam.

Fig. 2. Effect of Methanolic extract of *Indigofera mysorensis* on Open arm entries in EPM Test

Table No.4 : Effect of Methanolic extract of Indigofera mysorensis on Open field test in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Number of square crossed</th>
<th>Number of rearing of</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline</td>
<td>10ml/kg</td>
<td>40.3±2.1</td>
<td>10.1±1.4</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam</td>
<td>5mg/kg</td>
<td>29.5±3.6**</td>
<td>8.2±1.8**</td>
</tr>
<tr>
<td>III</td>
<td>Plant extract</td>
<td>125mg/kg</td>
<td>22.9±2.4</td>
<td>5.4±2.3</td>
</tr>
<tr>
<td>IV</td>
<td>Plant extract</td>
<td>250mg/kg</td>
<td>24.7±3.2*</td>
<td>7.3±3.5*</td>
</tr>
</tbody>
</table>
V | Plant extract | 500mg/kg | 26.4±2.8** | 9.3±1.2**

The data represent the mean ±S.D (n=6) *p<0.01, **p<0.001 significantly different compared to normal control and diazepam.

Table No. 4: Effect of Methanolic extract of Indigofera mysorensis leaves on Rota rod performance

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>Experimental mean time(10min) (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10ml/kg</td>
<td>180.21±9.1</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam</td>
<td>5mg/kg</td>
<td>157.61±6.9**</td>
</tr>
<tr>
<td>III</td>
<td>Plant extract</td>
<td>125mg/kg</td>
<td>140.82±5.7</td>
</tr>
<tr>
<td>IV</td>
<td>Plant extract</td>
<td>250mg/kg</td>
<td>145.41±3.6*</td>
</tr>
<tr>
<td>V</td>
<td>Plant extract</td>
<td>500mg/kg</td>
<td>152.13±5.8**</td>
</tr>
</tbody>
</table>

The data represent the mean ±S.D (n=6) *p<0.01, **p<0.001 significantly different compared to normal control and diazepam.

![Fig. 4. Effect of Methanolic extract of Indigofera mysorensis on Rota rod performance](image-url)
EVALUATION OF ANTICONVULSANT ACTIVITY

PTZ Induced Convulsion:
Pentylenetetrazole produced tonic seizures in the entire animals used. A dose of 125 mg/kg of Methanolic extract of leaves of Indigofera mysorensis protected 33.33% of the animals against seizures and did not affect the onset (latency) of seizures to any significant extent. Methanolic extract of leaves of Indigofera mysorensis at the dose of 250 and 500 mg/kg protected 50.0% and 100% of the mice against seizures and increased the latency of the seizures.

Maximal Electro Shock Model:
Maximal electroshock produced hind limb tonic extension (HLTE) in all the animals. The vehicle treated mice showed tonic hind limb extension for duration of 12.88 ± 0.35 s. Administration of Methanolic extraction of leaves of Indigofera mysorensis (125–500 mg/kg) showed a dose dependent increase in the delay of the onset time of seizures induced by maximal electroshock induced convulsion and also decreased duration of tonic hind limb extension.

Table No. 5: Effect of Methanolic extract of leaves of Indigofera mysorensis on PTZ induced convulsions in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Latency of Tonic convulsion (s)</th>
<th>Duration of Tonic convulsions (s)</th>
<th>Mortality (% death)</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>100.20±3.34</td>
<td>446.10±5.19</td>
<td>6/6(100)</td>
<td>0.0</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam (5mg/kg)</td>
<td>478.34±6.07**</td>
<td>126.69±1.93**</td>
<td>0/6(0.0)</td>
<td>100</td>
</tr>
<tr>
<td>III</td>
<td>Plant extract (125mg/kg)</td>
<td>141.43±1.98</td>
<td>216.29±1.23</td>
<td>4/6(66.66)</td>
<td>33.33</td>
</tr>
<tr>
<td>IV</td>
<td>Plant extract (250mg/kg)</td>
<td>298.16±4.45*</td>
<td>189.19±1.72*</td>
<td>3/6(50.00)</td>
<td>50.0</td>
</tr>
<tr>
<td>V</td>
<td>Plant extract (500mg/kg)</td>
<td>416.42±6.14**</td>
<td>137.11±2.61**</td>
<td>0/6(0.0)</td>
<td>100</td>
</tr>
</tbody>
</table>

The data represents the mean S.D ± (n=6) *p<0.1, **p<0.001 significantly different compared to normal control and diazepam.
### Table No.6: Effect of Methanolic extract of *Indigofera mysorensis* on Tonic seizures induced by MES method in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Seizure onset time (s)</th>
<th>Duration of Tonic Hind Limb Extension (Sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>8.38±1.88</td>
<td>12.88±0.35</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam (5mg/kg)</td>
<td>59.88±1.35**</td>
<td>2.63±1.72**</td>
</tr>
<tr>
<td>III</td>
<td>Plant extract (125mg/kg)</td>
<td>28.81±1.10</td>
<td>8.28±1.19</td>
</tr>
<tr>
<td>IV</td>
<td>Plant extract (250 mg/kg)</td>
<td>32.43±1.44*</td>
<td>7.44±1.01*</td>
</tr>
<tr>
<td>V</td>
<td>Plant extract (500mg/kg)</td>
<td>48.84±1.25**</td>
<td>3.21±1.25**</td>
</tr>
</tbody>
</table>

The data represent the mean ±S.D (n=6) *p<0.05, **p<0.001 significantly different compared to normal control and diazepam.
SUMMARY AND CONCLUSION

The leaves of Indigofera mysorensis has been examined to gain an insight of its Phytochemical and pharmacological behaviors.

The preliminary phytochemical investigation of Methanolic extract of leaves of Indigofera mysorensis showed the presence of Carbohydrate, Alkaloids, Phytosteroids, Flavonoids, Phenolic compounds and Tannins.

The pharmacological and acute toxicity studies of Methanolic extract was performed by following, OECD-423 guidelines (Acute toxic class method). No mortality or acute toxicity was observed up to 2000mg/kg of body weight.

Medicinal plants have served as sources of readily accessible, inexpensive, and effective medication since the earliest times known to man. Several ethnomedicinal plants have been found to possess neurobehavioral profile and serve as
alternative to modern medicine. Biological evaluation and scientific validation of the ethnomedicinal plants are the need of the hour. The present study was proposed to assess anxiolytic, and anticonvulsant effects of methanolic extract of leaves of an ethnomedicinal plant, Indigofera mysorensis.

Anxiety disorders are due to involvement of GABAergic, serotonergic, involvement. The adrenergic and dopaminergic system have also been shown to play a role in anxiety. BZA have been extensively, used for the last 40 years to treat several forms of anxiety, but due to their unwanted side effects, alternative treatment strategies with favorable side effect profiles. Medicinal plants are a good source to find new remedies for these disorders. Despite the wide spread traditional use of Indigofera mysorensis for treating various disorders there are no reports of scientific evaluation of its anxiolytic and anticonvulsant activity. The present work demonstrates that the Indigofera mysorensis leaf extract had anxiolytic activity in mice by Elevated Plus Maze, Rotarod and Open field models.

Elevated Plus Maze is used to evaluate psychomotor performance and emotional aspects of rodents. Results showed that plant extracts treated mice exhibited significant increase in the number of open arm entries but decreases in time spent in closed arm, which reflects plants anxiolytic property.

The open field test is used to evaluate the animal emotional state. The open field model examines anxiety related behavior characterized by the normal aversion of the animal to an open area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Mice treated with extract showed increase in number of rearings and time spent in the center.

Rota rod test, the difference in the fall of time from the rotating rod between the vehicle and extract treated groups were taken as an index of muscle relaxation. Plant extract showed significant decrease in the locomotory score and fall of time of the mice from the rotating rod.

The results of the present laboratory animal study indicate that Methanolic extract of Indigofera mysorensis leaf extract possesses anticonvulsant activity. The present study demonstrated the anticonvulsant effects of the methanolic extract of Indigofera mysorensis in both chemically and electrically induced seizures in mice. The extract exhibited dose dependent protection in the MES and PTZ induced convulsions. Nevertheless, in unprotected animals, the extract significantly increased seizure latency and reduced seizure duration compared with the control group in all two models at all tested doses. The effect of most of antiepileptic agents is to enhance the response to GABA by facilitating the opening of GABA-activated chloride channels. GABA receptors were involved in epilepsy and their direct activation would have an antiepileptic effect.

The anticonvulsant, anxiolytic, and sedative effects of benzodiazepines like diazepam are mostly attributed to enhance the action of gamma-aminobutyric acid (GABA) (Yemita O.K. et al., 2005). Actually, benzodiazepines bind to the gamma subunit of the GABA<sub>A</sub> receptor, due to which a structural modification of the receptor results in an increase in GABA<sub>A</sub> receptor activity. Benzodiazepines do not substitute for GABA, which bind at the alpha subunit, but increase the frequency of channel opening events, which leads to an increase in chloride ion conductance and inhibition of the action potential (Muhammed.N et al., and Garcia 2006). According to some researchers, the anxiolytic action of benzodiazepines may be due to the direct activation of glycine synapses in the brain (Muhammed.N et al., and P.Brambilla, 2013). This may explain the mechanism of action of the tested extract as well, because it is clear from the results that the effect of the extract was similar to diazepam.

Previous phytochemicals reported in the literature, various Flavonoids, glycosides, Alkaloids and triterpenoids, isolated from Indigofera mysorensis would be the effective constituents for their anxiolytic and anticonvulsant effect. In conclusion, Methanolic extract of Indigofera mysorensis possesses anxiolytic and anticonvulsant effects and these findings collaborate with the ethnomedicinal uses of this plant. The isolation of active chemicals from this plant might serve as lead compounds for the synthesis of drugs which could be used in the management of these nervous disorders.

REFERENCES: