

## EVALUATION OF NOOTROPIC ACTIVITY OF INDIGOFERA MYSORENSIS ROTTLE EX DC IN ALBINO RATS

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### ABSTRACT

Learning and memory are considered as psychological process. Learning is the ability to acquire new information and skills through experience whereas Memory is a process by which information acquired through learning is stored and retrieved. It is special faculty of brain where it retains the events developed during process of learning. Cognition means acquiring knowledge or information or processing of information like thinking, perception, motivation, memory, language and skilled movements, orientation, problem solving, reason, mathematical ability, ability to retain and recall events. The present investigation is to screen the methanolic extract of Indigofera mysorensis for its Nootropic activity in scopolamine induced rats using Cook's Pole Climbing Apparatus. Increase in CAR (Conditioned Avoidance Response) and less time taken by the animal as jump response to avoid shock shows that the extract has Nootropic Activity.

### 1. INTRODUCTION

Different parameters like age, external factors like alcohol and drugs leads to impairment of memory.

**COGNITIVE DISORDER** - It is a disruption or impairment at higher level functioning of brain. This disorder is due to Delirium, Dementia or Amnesia.

#### DEMENTIA

It is a mental disorder involving multiple cognitive deficits, memory impairment or it may be due to Aphasia (deterioration of language function), Apraxia (inability to execute motor functions), Agnosia (inability to recognize or name objects), and disturbance in executing functions<sup>1</sup>.

#### COMMON TYPES OF DEMENTIA INCLUDE

1. **Alzheimer's disease** – AD is an irreversible type of disease that is characterized by progressive deterioration of certain parts in brain which are essential for learning and memory.
2. **Vascular dementia** - It is the second most common cause of dementia. It is more common in men than in women which are influenced by age. This dementia is mostly caused by ischemic stroke or hemorrhagic cerebrovascular lesions. Symptoms may appear suddenly and remain stable.
3. **Pick's disease** - It is a frontotemporal dementia. The nerve cells are damaged hence get weaken and eventually die in frontal and temporal lobes. Symptoms of Pick's disease include sudden change in personality due to effect on frontotemporal lobes of brain
4. **Parkinson's disease** - It is a progressive neurological disease affecting the movements and muscle control. It destroys brain nerve cells responsible for muscle control. Symptoms include tremors, rigid posture, balance problems, difficulty in walking.
5. **Huntington's disease** - It is an inherited degenerative brain disease that affects brain and body. The main symptom of Hunting's disease is 'fidgety' movements (chorea), involuntary dance like movements followed by attention deficit and depression. Other symptoms include personality changes, impaired judgment, disturbance in speech and psychiatric problems.

### 2. NEUROTRANSMITTERS IN LEARNING AND MEMORY

There are many types of chemicals that act as neurotransmitters in human body. Acetyl choline is the main transmitter responsible for learning and memory<sup>2</sup>. Reduce in acetyl choline causes loss of cognitive function which is seen in AD. Nicotine, a tertiary amine and nicotinic agents improve working memory function. The hippocampus and the amygdale are found to be responsible for memory. Apart from this decrease in histamine, decrease in vasopressin also affects memory<sup>3</sup>. Also Glutamate<sup>4</sup>, Serotonin, Dopamine<sup>5</sup> are associated. Therefore drugs which increase these chemicals are given to the patients to increase cognitive function<sup>6</sup>. On the other hand memory can be increased or maintained providing with drugs. Drugs which are used to increase the cognitive function of the brain are recognized as NOOTROPICS which are also known as smart drugs. Piracetam was the first Nootropic drug discovered. Other Nootropics include Aniracetam, Nefiracetam, Pramiracetam, Fosracetam, Nebracetam and Oxiracetam. Investigations are lasting on in order to find out more significant and potent drug.

Indigofera mysorensis Rottl. (Fabaceae) is a robust herb, grows up to 80 cm height. It is widely distributed on hills of Deccan in Chinglaput and Mysore and also in the Western Ghats of dry Nilgiri slope in India. Popularly known as

'Bapanga' in the local language (Telugu), *Indigofera mysorensis* is used for its antidiabetic activity in rural India. The objective of this study was to investigate the hypoglycemic and

ethanolic extract of *Indigofera mysorensis* was found to contain different chemical constituents like phenolic compounds, tannins, phytosterols, alcohols, fixed oils, glycosides, flavonoids, saponins and coumarins which are confirmed by phytochemical screening<sup>7</sup>.

### 3. MATERIALS AND METHODS

**PIRACETAM - Mechanism of Action** - Piracetam improves the function of neurotransmitter Acetyl choline via muscarinic cholinergic receptors. Piracetam increases the cell membrane permeability. It increases oxygen supply to the brain and increases the adenylate kinase activity in rats<sup>8</sup>.

#### Plant collection and authentication

The whole plant of *Indigofera mysorensis* collected in the month of February, 2024 from chittur dist. The plant materials were identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference.

#### Preparation of plant extract

The freshly collected whole plant of this plant was chopped and dried. The dried material was powder. The powdered plant material (250 g) was extracted by hot continuous soxhlet extraction method and the plant material was extracted with Ethanol in a soxhlet apparatus.

It is a process of continuous extraction method in which the solvent can be circulated through the extractor for several time. The vapours from the solvents are taken to the condenser and the condensed liquid is returned to the extract for continuous extraction. The apparatus consists of body extractor (thimble) attached with side siphon tube fixed in lower and attached with distillation flask and the mouth of the extractor is fixed to the condenser by the standard joints<sup>9</sup>.

Animals – Male and Female Albino rats weighing from 150gms - 200gms were used. Study was approved by Institutional Animal Ethics Committee with a proposal no.1488/PO/Re/S/11/CPCSEA/06/2023. Animals were acclimatized to the laboratory conditions prior to the experiment, given with normal laboratory diet and water ad libitum.

### EXPERIMENTAL PROCEDURE

#### 1. Acclimatization to Cook's Pole Climbing Apparatus<sup>10</sup>

- Cook's pole climbing apparatus is one of the majorly used instruments involved in evaluating neurological activity of the animal.
- In order to find out the cognitive functioning, the animal is subjected to conditioned avoidance response. It is a learning concept where the animal is trained to climb up the pole in order to escape from the shock.
- For that each rat is allowed to get acclimatized for 2min to the cook's pole climbing apparatus.
- Then the animals are exposed to a buzzer noise.
- After 5sec of buzzer the animals are subjected to mild shock.
- The animals now try to escape from shock by climbing the pole.
- As soon as rat climbs the pole, buzzer and shock were switched off.
- At least 10 trials at 1 min gap for 10 days are performed.
- The rats avoiding the shock in all 10 trials are considered to develop CAR and they are used for further process.

#### GROUPING OF ANIMALS

The animals which developed CAR are divided into six groups under which each group contains five animals. Animals are then induced with scopolamine (antimuscarinic agent which inhibits the cholinergic transmission) 0.5mg/kg, through intraperitoneal route to produce amnesia, where the memory is partially lost. Group1 are given with normal saline, Group 2 are left untreated, Group 3 are treated with the standard (PIRACETAM) and Group four, five and six are treated with low (125mg/kg), intermediate (250mg/kg) and high doses (500mg/kg) of *Indigofera mysorensis* respectively. Now the animals are again placed in the apparatus, buzzer is given with shock 10 sec later. The animals which have high CAR jumped onto the pole immediately after the buzzer before shock. In addition to this the other two parameters unconditional response and no response are calculated. Unconditional response is the action of the animal to climb up the pole after giving the shock. Animals which show no action even after giving shock are considered to show no response. Trials are performed one hour later and at 24 hours after inducing scopolamine. Sessions are continued for 8 days and on 9<sup>th</sup> day the mean values of the CAR are calculated<sup>11</sup>.

### 4. STATISTICAL ANALYSIS

Values are expressed as Mean  $\pm$  SD and results are analyzed by one way ANOVA followed by Dunnet's multiple comparison tests to find out the significance. \*\*\*p < 0.001, \*\*p < 0.01 and \*p < 0.05

## 5. RESULTS

### PHYTOCHEMICAL CONSTITUENTS

Table No.1

PHYTO CHEMICAL CONSTITUENTS	METHANOLIC EXTRACT
Carbohydrates	-ve
starch	-ve
Proteins and amino acids	-ve
Phenolic compounds and tannins	+ve
Phytosterols	+ve
Alkaloids	+ve
Fixed oils	+ve
Glycosides	+ve
Flavonoids	+ve
Saponins	+ve
Coumarins	+ve

(-) indicates absence (+) indicates presence

Table No. 2 - CAR responses after 4hrs of drugs inducing

TREATMENT	MEAN $\pm$ SD		
	CAR	UR	NR
Control	25.30 $\pm$ 0.30	0.16 $\pm$ 0.33	0.00 $\pm$ 0.00
Negative control	4.2 $\pm$ 2.23	9.30 $\pm$ 2.02	17.60 $\pm$ 4.52
Standard	18.0 $\pm$ 3.2**	8.0 $\pm$ 4.0*	3.6 $\pm$ 0.2*
T 1	22.00 $\pm$ 2.10*	3.88 $\pm$ 1.00	0.50 $\pm$ 0.20
T 2	24.00 $\pm$ 1.26**	4.86 $\pm$ 2.00*	1.26 $\pm$ 0.1*
T 3	27 $\pm$ 5***	4 $\pm$ 2*	3 $\pm$ 1*

Values expressed as Mean  $\pm$  SD, (n = 6), statistical analysis is done by ANOVA followed by Dunnet's test to find out significance \*\*\*p < 0.001, \*\*p < 0.001, \*p < 0.05, compared with negative control

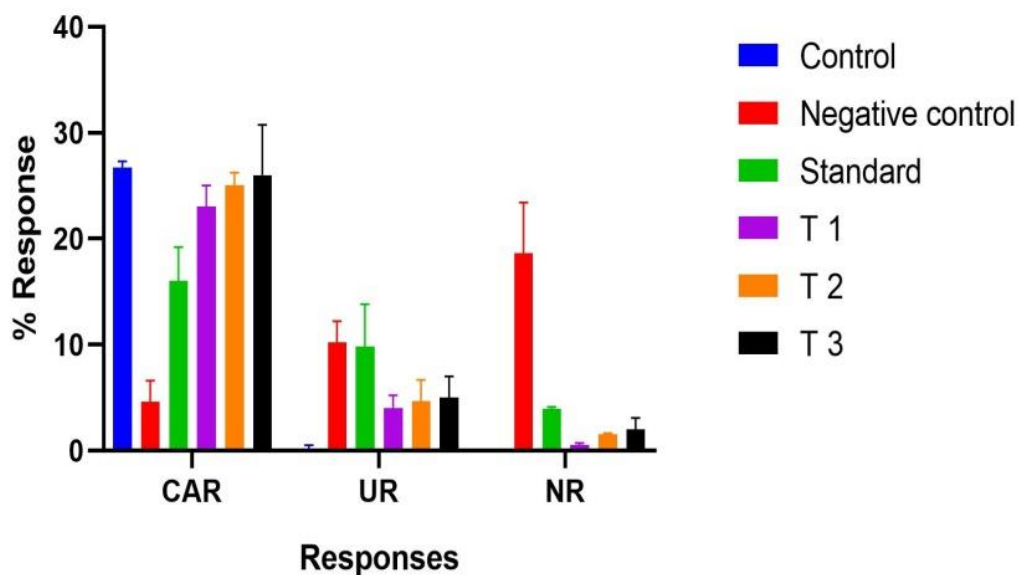
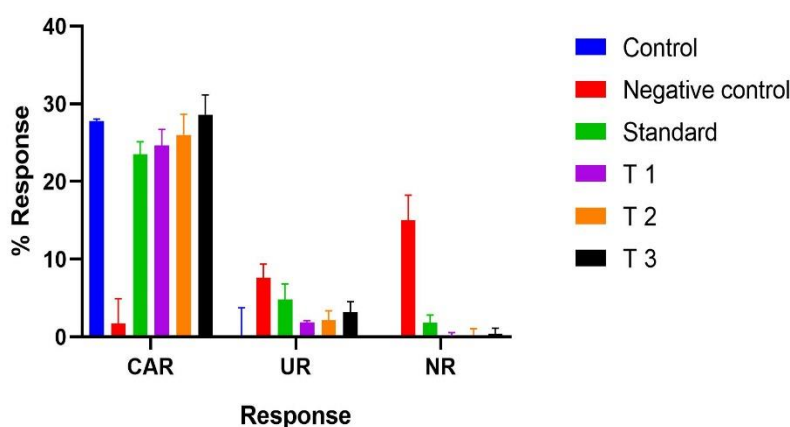


FIG.NO. 1 – Response shown by the treatments after 4hrs of drug intake

**Table No. 3 - CAR responses after 24hrs of drugs inducing**

TREATMENT	MEAN $\pm$ SD		
	CAR	UR	NR
Control	26.80 $\pm$ 0.26	0.18 $\pm$ 3.56	0.00 $\pm$ 0.00
Negative control	1.6 $\pm$ 3.2	6.5 $\pm$ 1.8	14.0 $\pm$ 3.3
Standard	24.5 $\pm$ 1.6	4.8 $\pm$ 2.0	1.8 $\pm$ 1.0
T 1	23.60 $\pm$ 2.10	1.80 $\pm$ 0.20	0.11 $\pm$ 0.45
T 2	25.0 $\pm$ 2.6	2.1 $\pm$ 1.3	0.2 $\pm$ 0.8
T 3	27.6 $\pm$ 2.5	3.2 $\pm$ 1.3	0.3 $\pm$ 0.7

Values expressed as Mean  $\pm$  SD, (n = 6), statistical analysis is done by ANOVA followed by Dunnet's test to find out significance \*\*\*p < 0.001, \*\*p < 0.001, \*p < 0.05, compared with negative control



**FIG.NO. 2 – Response shown by the treatments after 24hrs of drug intake**

## 6. DISCUSSION

Learning and memory are considered as psychological process, where learning is ability to acquire new information and memory is a process by which the learnt information is stored and Cognition means processing of information. For information to be passed, stored and memorized several neurotransmitters are involved like Glutamate, Acetyl choline, serotonin, Dopamine. Depletion in these neurochemicals, excessive stimulation of these chemicals or damage to the nerve cells leads to CNS disorders. Cognitive impairment occurs through many disorders like delirium, dementia or amnesia or less oxygen supply to brain.

Different causes of dementia include Alzheimer's disease, vascular dementia, Pick's disease, Parkinson's disease and Hunting's disease. Formation of plaques due to amyloid protein or tangles of neurons leads to Alzheimer's disease. Pick's bodies or Lewy bodies are other reasons for dementia. Mental illness, aging, alcohol consumption are some other common reasons. Alteration in neurochemicals or increase in oxygen supply to brain leads to cognitive enhancement. Flavonoids, alkaloids, triterpinoids, saponins, phenols, monoterpene, xanthenes and isothiocyanate are the chemical constituents responsible for this activity.

To find out the Nootropic activity different screening methods can be opted like Elevated plus maze<sup>12</sup>, One way Shuttle box, Spatial learning in water maze, Metabolic influence<sup>13</sup>. Amongst which activity with Cook's Pole Climbing Apparatus is selected. Animals are trained with Cook's pole climbing apparatus. Scopolamine, antimuscarinic agent used as an inducing agent blocks the action of Acetylcholine, thereby decreasing cognitive function. The standard Nootropic drug PIRACETAM is used to compare the results.

Ethanollic Extract of Indigofera mysorensis when given to the animals induced with scopolamine there is a significant increase in conditioned avoidance response (CAR). In table 2 there is a significant increase in CAR at 4hrs, more than the standard. Effect of the extract is more at high dose, also at 24hrs. Chemical constituents responsible for Nootropic activity are Monoterpenes, Isothiocyanates, Triterpenoids, Saponins, Alkaloids, Phenols, Xanthenes and flavonoids, where Triterpenoids and saponins are mainly involved in increasing learning and memory<sup>14</sup>.

Therefore saponins present in the extract of Indigofera mysorensis may be responsible for the activity which can supported by previous investigations.

## 7. CONCLUSION

Results demonstrated that the activity of brain can be improved using Methanolic extract of *Indigofera mysorens* which is confirmed by activity of the animals. Animals which are given with the extract have shown significant increase in CAR. High dose of the extract gave better results when compared to the low and intermediate doses. Presence of Saponins can be a reason for Nootropic activity which is supported by previous investigations and based the statistical analysis it can be concluded that *Indigofera mysorens* has Nootropic activity.

## 8. REFERENCES

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