An investigation on neuroprotective potential of hydro alcoholic extract of Tectona grandis leaves in albino mice

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Abstract:

Tectona grandis also called teak, is a member of the Verbenaceae family and is native to South-East Asia and India. The plant's pharmacological properties, including its anti-plasmodial, hypoglycemic, cytotoxic, analgesic, anti-inflammatory, anti-pyretic, and wound-healing properties, have all been studied. It is widely recognized, and the conventional medical system makes use of all of its components. The locomotor activity, Muscle grip strength on Rota-rod, anticonvulsant, and anti-anxiety activities of hydro-alcoholic extract were assessed in the current investigation on the areal part of Tectona grandis. This study focuses on several pharmacognostical analyses, such as anatomical, microscopic, or organoleptic features. The present study reveals that the hydro alcoholic extracts of Arial parts of Tectona grandis possess neuroprotective properties.

1. Introduction:

Medicinal herbs are an important part of our life. In the present scenario a large number of peoples in the whole world have a faith on these herbs to treat various health related issues. A large number of patients from developing as well as developed countries get their medical care from traditional healers and the herbs they cultivate. There are many marvelous herbs present in our society but we don’t know that how to use them to obtain maximum benefit. Herbal medicines are now an important part of our life. The important phytoconstituents like flavonoids, polyphenols, terpenoids etc. Present in herbs make them unique in their action [1] since ancient times the man has dependent on the plants for food and medicines. Many ayurvedic plants like Ginger, Turmeric, Basil etc. are the very important among them. The herbs are the very rich source of antioxidants and other nutritious substances. If we correctly use these medicines in a proper way then we can treat many health issues with least or no side effects.

Now a day herbal preparation has secured a very good market position all over the world. The demand of herbal products is increasing day by day due to its action on the root cause of the disease and its very less or no side effects. [2] Since ancient time the medicinal herbs are the important resource to treat various health issues. As our knowledge has been increased regarding these plants we prepared the suitable doses forms also to serve our society. Ayurveda is the root of all the health care system and it is very old tradition to treat health issues with the help of ayurvedic plants. Now Ayurvedic medicines are used in almost whole world even allopathic practitioners also rely on the medicinal plants.[3] Ayurveda is approximately five-thousand-year-old medical system that is largely used in India. It emphasizes the vata, pitta & kapha in the prevention and treatment of disease, and it incorporates herbal remedies and diet. With the statement, "A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore, or improve health." In our society among population there is a misconception that herbs can't produce adverse drug reactions which is not true, they can do so. The majority of herbal remedies still require scientific investigation, but the knowledge gathered through years of traditional use shouldn't be disregarded. The majority of the herbal medications currently in use lack sufficient data to address concerns about their safety and effectiveness, according to standard scientific methods. [4]

2. Material Methods:

2.1 Selection of Plant: On the basis of huge literature survey the plant was selected due to its biological activities.

2.1.1 Collection of plant parts: The leaves of *Tectona grandis* was collected from Sehore (M.P.), during the month of March.

2.1.2 Authentication of plant: - Identification of the *Tectona grandis* leaves was performed by Dr. Zia ul Hasan H.O.D. Department of Botany, Saifia College Bhopal and stored in the herbarium of the Institute and a specimen voucher no.512/Bot./Saf./21 was assigned.

2.1.3 Defatting of plant material: - From the coarsely powdered plant material the fats and oil removed by soxhlation process with petroleum ether. After defatting the material was undergone soxhlet extraction with suitable solvent [5]

2.1.4 Soxhlet extraction method: A 48-hour soxhlation process was used to extract 150 gm of dried powdered aerial component (leaf) of *Tectona grandis.* The extract was then filtered and dried at 200 degrees Celsius in a vacuum evaporator. The extracted material was also tested for colour, aroma, and consistency.

2.1.5 Percent yield of the extract: Following the sox halation extraction method, the crude extract was dried using a vacuum evaporator utilizing a hydro-alcoholic solvent (ethanol: water, 70:30 v/v). This was done for the aerial section of *Tectona grandis*. Twenty gm was determined to be the extract yield %.

2.2 Phytochemical analysis

2.2.1 Estimation of Total polyphenol content (TPC)

The Folin Ciocalteau reagent-based test was used to determine the extract's total polyphenol content. Methanol served as the blank, and standards ranged from 5 to 50 µg/ml methanolic Gallic acid solutions.

With a UV-Vis spectrophotometer, the generated color's absorbance was measured at 765 nm. Every determination was made in triplicate for both the plant extract and Gallic acid. The three determinations' average is used to represent the data. A Gallic acid standard curve was created using these values. [6] The calibration line was used to determine the phenolic concentration (µg/ml) based on the measured absorbance of the plant extract. Gallic acid equivalent (mg of) was used to calculate and express the extract's polyphenol content.

2.2.2 Estimation of total flavonoids content (TFC)

Aluminum chloride technique was used to estimate total flavonoid concentration. Ten milliliters of methanol were used to dissolve the 10 mg of Quercetin, and different aliquots containing 5, 10, 15, 20, and 25 µg/ml were made. After dissolving the 10 mg of dried extract in 10 ml of methanol, the mixture was filtered. For the flavonoid evaluation, 3 ml of this solution was employed. Furthermore, 3 ml of extract or normal was mixed with 1 ml of 2% methanolic solution of aluminum chloride, and the mixture was left to remain at room temperature for 15 minutes. The absorbance was then measured at 420 nm. [8]

2.3 Pharmacological activity:

According to literatures the *Tectona grandis* plant has a large number of biological activities.

2.3.1 Animals

In the experiments, Swiss albino mice weighing 25–35 gm are employed. The animals were divided into treatment groups and placed at random. To get over diurnal and circadian fluctuations, all of the trials were run from 9:30 to 16:30. Animals were kept in a room with a relative humidity of 65±5% and a temperature of 25±2.0C. There was a 12 hour light-to-day cycle. Every animal was kept in polypropylene cages with bedding made of paddy husk, free access to water, and animal food pellet as its food source. [9]

The institutional animal ethics committee at the Radharaman Institute of Pharmaceutical Sciences, Bhopal (M.P.) reviewed all of the experimental methods and procedures utilized in this work, and they were all in compliance with the IAEC's guidelines (proposal number IAEC/Rips/2021/03).

2.3.2 Acute oral toxicity study. The acute toxic class technique (OECD-423) recommendations were followed in the conduct of the acute oral toxicity investigation. Tectona plant extract (5, 50, 300, and 2000 mg/kg) was given orally to six groups of mice (n = 6), and the animals were observed for behavioral changes and mortality during the seven-day period. [10]

2.3.3 Effect of plant extract on locomotor activity of mice on Actophotometer. Swiss albino mice weighing 20–30 g were collected and placed into groups of six animals each. One group was designated as the control group, and the other as the standard group. The remaining two groups received differing dosages of plant extract (100 and 200 mg/kg.p.o.).After turning on the rotarod and verifying that every photocell was operational for precise recording, each mouse was given a five-minute period in the activity cage. Every animal's basal activity score was recorded. After giving each mouse a 2 mg/kg injection of diazepam, they were placed in activity cases for 5 minutes after 30 minutes. [11] Take note of the score and how the activity changed before and after the diazepam therapy. For varying quantities of hydro alcoholic extracts (100, 200 mg/kg p.o.), repeat the above process. It was estimated as a percentage change in motor activity.

2.3.4 Effect of plant extract on muscle strength of mice on Rota-rod apparatus: Swiss albino mice weighing between 30 and 35 g were collected and put into 4 groups, each with 6 mice. One group was designated as the control group, and the other as the standard group. The remaining two groups received varying dosages of the plant extract (100 and 200 mg/kg p.o.). The rotation speed of the rota-road was set to 22–25 rpm when it was turned on. [12] The rodents were arranged individually on a rotating rod, and the moment the mouse fell off the rod was recorded.

The animal in the second group received an injection of the medication (diazepam, 2 mg/kg i.p.), and after 30 minutes, the aforementioned parameter was noted. The test group then underwent the identical process. [13] The fall-off times for the entire animal were compared.

2.3.5 Effect of plant extract on learning & memory checked on elevated plus- maze.
Swiss albino mice weighing between 25 and 35 grams were collected and put into 4 groups, each with 6 mice. One set of people was labeled as control, and another as standard. Two groups' worth of rests was labeled with varying plant extract dosages (100 and 200 mg/kg p.o.). Each animal was positioned individually in the middle of the plus maze with their head towards the open arm, and for five minutes, their subsequent actions were observed. [14] Mice prefer an enclosed arm to an exposed one. The quantity of entries in both enclosed and open arms (where one arm entrance is the result of four paws entering the arm). Each animal's average time in each arm is calculated as follows: average time = total time in the arm / number of entries. The animals in the third and fourth groups received injections of a standard medication (Diazepam 2 mg/kg.i.p.) and two different doses of plant extract (100 and 200 mg.kg. p.o.) after 30 minutes. The settings listed above were followed. The animal preferences for open and enclosed arms were compared. [15] The amount of time on average and the quantity of entries in the open arm for every group.

2.3.6 Effect of plant extract on MES induced convulsion in rat.
Swiss Albino rats of approximately 150 g rats were used in the trials. The animals were sorted into five groups and marked. Every group has six creatures in it. The first group was assigned as control, while the second and third groups received normal medication treatment (45 mg/kg of phenobarbitone and 120 mg/kg of phenytoin, intra-peritoneal). [16] Remaining two groups were assigned two distinct plant extract dosages (100 and 100 mg/kg.p.o., respectively). The animal was held with great care. The cornea was covered with corneal electrodes, and for 0.2 seconds, a 150-mA current was applied, several convulsion phases, such as (a) tonic flexion (b) tonic extensor (c) clonic (d) stupor (e) after applying electric current, recovery or death was observed
It was recorded how long the animal spent in each stage of the convulsions in seconds. All of the animal group's go through the same process once more. [17] The animals in each group were given the conventional medication and plant extract. The same current was used for a comparable amount of time after 30 minutes, and the amount of time spent in each stage was recorded. For each group, the duration of the tonic extensor phase of MES-convulsions was reduced or eliminated.

3. Result:

Table 1: Qualitative analysis of *Tectona grandis* hydro alcoholic extract for different phytochemicals [18]

S.NO.	TEST	INFERENCE
1	**Alkaloid**	
	Wagner's test	+ ve
	Dragendorff's test	+ ve
	Mayer's test	+ ve
2	**Glycoside**	
	Keller Killiani test	+ ve
	Conc. sulphuric acid test	+ ve
	Molish’s test	+ ve
3	**Steroid**	
	Solkowski Test	+ ve
	Libermann Burchard’s Test	+ ve
4	**Carbohydrates**	
	Molisch Test	+ ve
	Benedict's Test	+ ve
5	**Phenolic compounds**	
	Ferric chloride test	+ ve
	Lead acetate test	+ ve
	Iodine test	+ ve

Table: -2 Estimation of total phenolic content (TPC)

Concentration µg/ml	Absorbance
0	0
5	0.132±0.002
10	0.311±0.004
15	0.439±0.001
20	0.578±0.001
25	0.663±0.004

Table: -3 Estimation of total flavonoid content (TFC)

Concentration µg/ml	Absorbance
0	0
5	0.326±0.001
10	0.437±0.002
15	0.619±0.001
20	0.875±0.002
25	1.035±0.001

Table: -4 Effect of plant extract on locomotor activity of mice on Actophotometer.

Groups	Dose (mg/kg)	Locomotion Score (M±SEM) (Min.)		% Change in locomotor activity
		Basal	After 30 min. drug administration	
Vehicle control	5 ml/kg/p.o.	195.5±10.500	-	-
Disease control	Diazepam 2 mg/kg/i.p.	133.5±1.500	5.5±1.500***	95.88
Test drug low dose	Extract100 mg/kg/p.o.,Diazepam 2mg/kg ip	455.5±262.50	161±101.00**	64.65
Test drug high dose	200 mg/kg/p.o. Diazepam 2mg/kg ip	535±55.000	153.5±28.500*	71.30

Values are expressed as mean ±S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc test)

Table: -5 Effect of plant extract on muscle grip strength of mice on Rota-rod apparatus.

Groups	Dose (mg/kg,)	Fall off time in Sec. (M±SEM)		% Change in fall off time
		Basal reaction time (M±SEM)	After 30 min.of drug administration (M±SEM)	
Vehicle control	5 ml/kg/p.o.	1125 ±135.00	-	-
Disease control	Diazepam 2 mg/kg/i.p.	717.5±155.50	75±25.000***	89.19
Test drug low dose	Extract100 mg/kg/p.o. Diazepam 2mg/kg ip	212.5±57.500	145±55.000**	31.76
Test drug high dose	200 mg/kg/p.o. Diazepam 2mg/kg ip	144±4.000	172±3.000*	50

Values are expressed as mean± S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Turkey' s post hoc test).

Table: -6 Effect of plant extract on parameter of learning & memory on elevated plus-maze in mice.

Groups	Dose (mg/kg,)	% preference in open arm	Total No. of entries (M±SEM)	% open arm entries
Vehicle treated	5 ml/kg/p.o.	44.31	22±5.000	27.84
Disease control	Scopolamine 1mg/kg	18.64	4±1.00	19.32
Standard group	Donepezil 5mg/kg po	38.12	20±1.32	25.74
Test drug low dose	100 mg/kg/p.o.	42.13	13.32±1.95 ns	29.71
Test drug high dose	200 mg/kg/p.o.	51.23	15.55±1.69 *	39.37

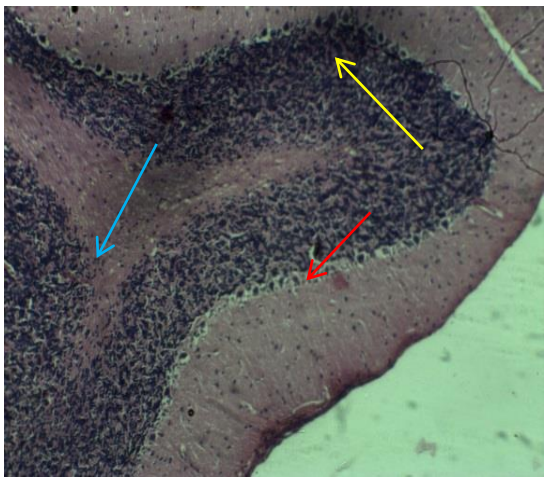
Values are expressed as mean ±S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc test).

Table: -7 Effect of *Tectona grandis* extract on MES induced convulsion on rat.

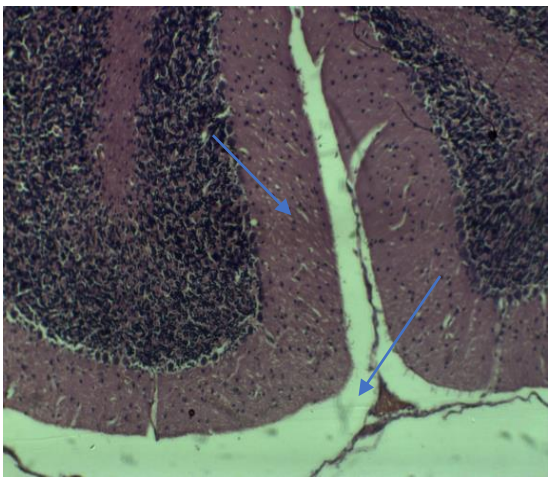
Group	Dose mg/kg	Flexon phase in sec. (M±SEM)	Extensor phase in sec. (M±SEM)	Clonus phase in sec. (M±SEM)	Stuper phase in sec. (M±SEM)	Recovery/ Death
Vehicle control	5 ml/kg/p.o.	10.5±1.500 ns	12.5±1.500 ns	22.5±1.500 ns	345±135.00 ns	Recovery
Phenytoin	120mg/kg/i.p.	Absent	Absent	12.5±0.5000 **	170±10.000**	Recovery
Test drug low dose	100 mg/kg/p.o.	12.5±0.5000 ns	7.5±0.5000 ns	27±2.000 ns	187.5±17.500 ns	Recovery
Test drug high dose	200 mg/kg/p.o.	14.5±0.5000 ns	4.5±0.5000. **	17±3.000*	215±5.000 **	Recovery

Values are expressed as mean ±S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc

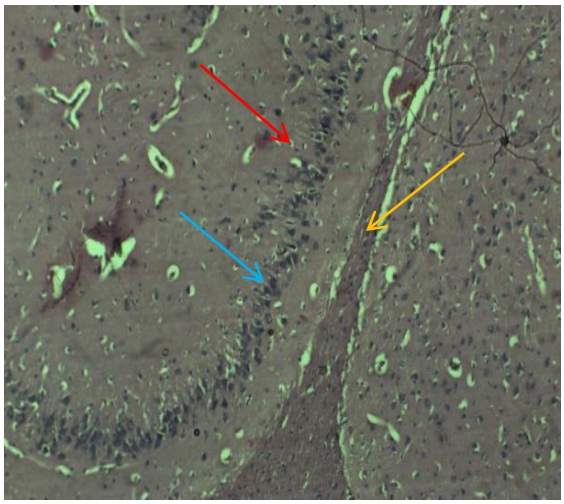
3. Histopathology of brain of animal's treated with electric shock & Phenytoin:



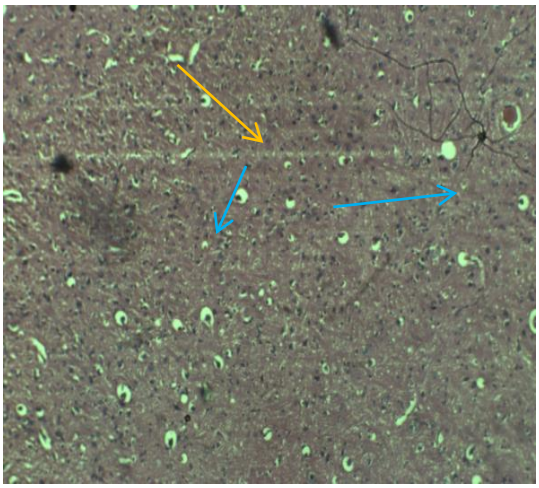
Normal Control



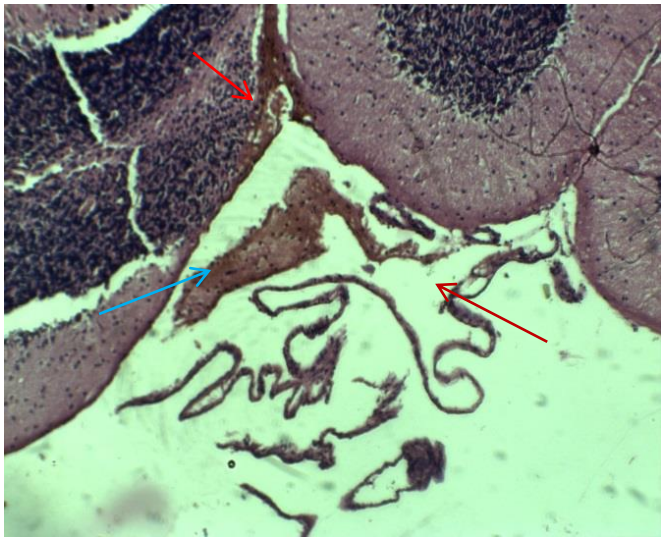
Phenytoin treated



Test group low dose



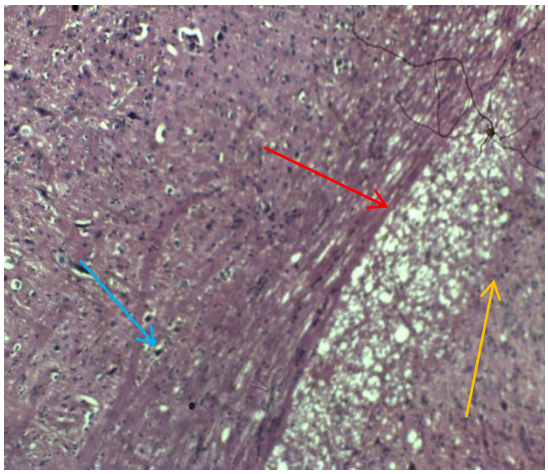
Test group high dose



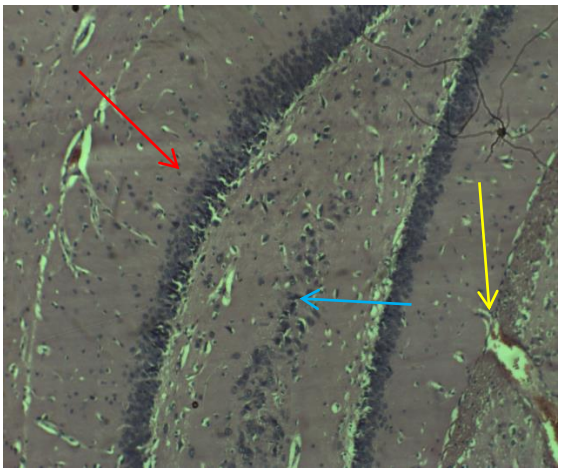
Negative control (Treated with electric shock)

If we observe the different slides of the brain of animals treated with electric shock, phenytoin and test drug we see that in normal control group the neurons appear normal with no any hippocampal oedema, whereas in disease control group the group which has been treated with electric shock there is neuronal fragmentation and damage of neurons has appeared. There is neuroinflammation and reduced hippocampal volume has been observed.[19] The test group shows an improvement in the neuronal health and brain tissues.

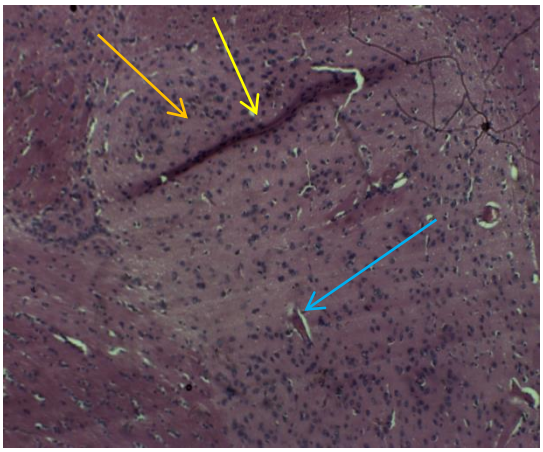
Histopathology of brain of animal's treated with Scopolamine:



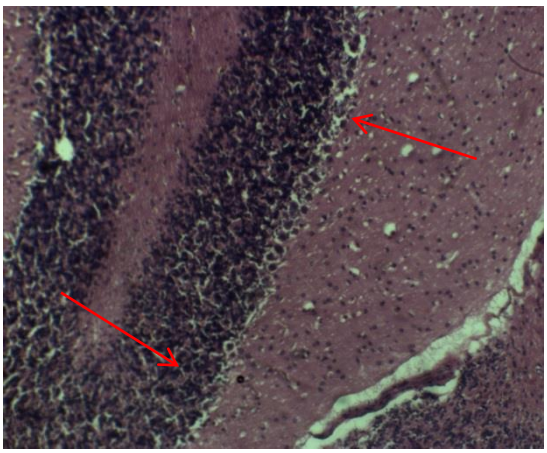
Normal control



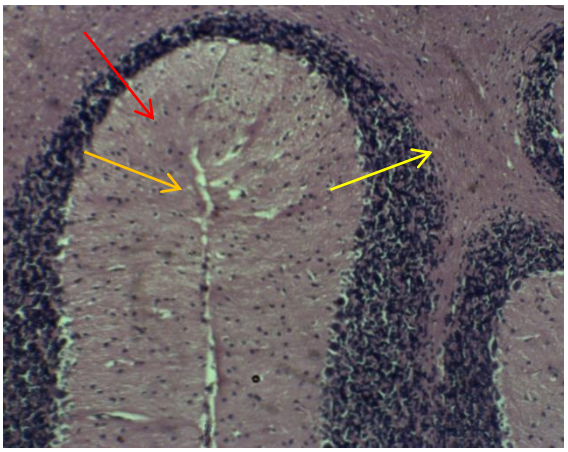
Negative control(scopolamine treated)



Test group first



Test group second



Standard group

When we look at the various brain slides of animals that were given scopolamine, donepezil, and a test drug, we can see that the neurons in the normal control group appear normal and do not show any signs of hippocampal edema, while the group that received scopolamine treatment shows signs of neuronal degeneration and damage. There is neuroinflammation and reduced hippocampal volume has been observed. The test group shows an improvement in the neuronal health and brain tissues & hippocampal regeneration with reduced oedema.[20]

4. Discussion:

Tectona grandis hydro-alcoholic extract reveals the presence of phenolic, tannin, and steroid components. Carbohydrates, glycosides, and alkaloids. Table 1 presents the findings. The hydro alcoholic extract percentage was 20 gm. Several experimental models relevant to the central nervous system, including as rotarod and locomotor activity, were used in this investigation. MES-induced convulsion and elevated plus maze are used to look into the potential central effect of *Tectona grandis.* The traditional approaches for evaluating CNS activity include details on the psychomotor performance depressing properties of anxiolytic and myorelaxant substances. The use of herbal items or natural therapies to treat depression and anxiety has garnered significant public interest. It has recently been discovered that a number of plants have anxiolytic effects in various animal models of anxiety. There have also been suggestions that a number of conventional herbal remedies contain anxiolytic properties. It was discovered that the plant had a high concentration of flavonoids and steroidal compounds, which are phytoconstituents that have a variety of pharmacological effects. [21] A decrease in locomotor activity is indicative of sedative action, as it is seen as an indicator of alertness. We can see in the present study that *Tectona grandis* extract improves the muscular function and test group shows the increased time of fall on rota rod apparatus. It effectively increases the locomotor activity in all tested doses. Because of a rise in GABA concentration in the brain, a decrease in locomotion indicates a depressive effect on GABArgic transmission. [22] The CNS depressive effect of *Tectona grandis* causes mice to lose muscle or coordination, which causes them to lose their grip strength and slip off the rota-rod. The plant extract reduces the mice's fall off time off the rotating rod. When animals are placed on an elevated plus maze (EPM), their fear of heights causes them to become uneasy, depending on how long they had been exposed to one. Fear makes the animal that is being introduced to the novel atmosphere want to avoid it and wants to stay in a close arm of elevated plus maze.

A reduction in motor activity and a tendency to stay in safer areas are the ultimate signs of fear and anxiety in animals. The elevated plus maze illustrates the animal's anxiolytic expenditure in the open arms. Diazepam enhanced the duration and number of open arm entries. One of the most extensively used and proven methods for determining the presence of anti- anxiety drugs operating at the GABA benzodiazepine chloride channel complex is the plus maze model. The present study's findings are in line with the findings of many earlier investigations, which demonstrated that diazepam and other benzodiazepines reduce anxiety in a range of anti-anxiety screening techniques, such as elevated plus-maze tests. We discovered that the plant extract was processed. [23] One of the most prevalent and dangerous brain disorders is epilepsy.

Medications like phenytoin that blocks voltage-dependent Na+ channels. Table 6 lists the effects of plant extract on rats' MES-induced convulsions, increased frequency of open arm admissions and the length of time spent in the EPM. [24] In a dose-dependent way, the tonus and extension phase were reduced. The duration of the clonus phase in the treatment group was not remarkably different from the control group across the board when compared to the plant extract group. The animals were treated and returned to their vehicles, phenytoin and all plant extract dosages.

5. Conclusion:

According to pharmacological research, the plant Tectona grandis has a central nervous system depressing effect. *Tectona grandis* hydro-alcoholic extract acts as a hypnotic and reduces anxiety, which implies it acts as an anxiolytic agent because of hypnosis. *Tectona grandis* also has an anti-anxiety, muscle-relaxing, and locomotor effect on mice. With minimal toxicity and good absorption, *Tectona grandis* is pharmacologically safe. This study provides *Tectona grandis's* complete pharmacological details in addition to a few phytochemicals. The primary emphasis is on the plant's pharmacological potential, which aids researchers in learning more about this important plant. Aside from this, there aren't many opportunities to look into the untapped potential of plants depending on their applications. To verify the conventional claims and investigate the possibility of developing a medicine, the active ingredient must be isolated and should be taken into consideration for additional in-vitro or in-vivo research.

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