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NEUROMODULATORY PROPERTIES OF ADANSONIA DIGITATA LEAF EXTRACT AND ITS FLAVONOIDS ON ANIMAL MODELS OF NEURODEGENERATION

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Abstract
Neurodegeneration is a progressive damage of structure and function of neurons involving numerous cellular pathways leading to a condition termed as neurodegenerative disease (NDD), affecting the normal functioning of motor activity and cognition. Irrespective of the various pathways and cascades involved in initiation and progression of NDD, oxidative stress, emerges as a chief runner in executing the severity of NDD. In general, oxidative stress and mitochondrial dysfunction are known to play key roles in the pathophysiology of NDD. *Adansonia digitata* plant leaves extracts play a very significant role in neuroprotection as compare to synthetic medications with fewer side effects. Oxidative damage or oxidative stress leads to the production of reactive oxygen species (ROS), and leading to the peroxidation of cell membrane leading to disrupt the integrity and function of the cell membrane and causing cell death or neurodegeneration. Our study showed that groups treated with leaves extract of *Adansonia Digitata* significantly reduced the level of MDA and other free radicle species and have anti scavenging activity. Mineral components of the plant extract from *Adansonia digitata* may potentially contribute to its pain-relieving actions in neuropathic pain in addition to its flavonoid contents. The electrolytes that are most prevalent in the *Adansonia digita*ta (Seed, Fruit, Leaf, and Bark) plant consist of Mg, Ca, Fe, Zn, and Mn. It has been reported to have a broad range of therapeutic value. Thus, plant extract of *Adansonia digitata* may be explored further for its potential in neuromodulation and neurodegenerative diseases.

Keywords- ROS-reactive oxygen species, NDD- neurodegenerated diseases.

INTRODUCTION-

1.1 Neurodegenerative Diseases-

A neurodegenerative disease is caused by the progressive loss of structure or function of neurons, as a result of which leads to neurodegenerative diseases. Such neuronal damage may ultimately involve cell death [1]. Neurodegenerative diseases include amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease, Alzheimer's disease, Huntington's disease, multiple system atrophy and various other diseases. Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies. Baobab (*Adansonia digitata* L) is a multi-purpose tree species native to Africa. Its fruit pulp has very high vitamin C content (- ten

times that of orange), and can be used in seasoning, as an appetizer and to make juices[2]. Seeds contain appreciable quantities of and protein, digestible carbohydrates and oil, whereas they have high levels of lysine amine Ca and Fe. Several plant parts have interesting anti-oxidant and anti-inflammatory properties, and baobab has been used extensively since ancient times in traditional medicine[3].Young leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice. Available data show that leaves contain (dry weight): 13-15% protein, 60-70% carbohydrate, 4-10% fat and around 11% fiber and 16% ash. Energy value varies from 1180-1900kJ/100g of which 80% is metabolizable energy: In terms of protein content and WHO standards, leaves of baobab can be rated "good in that they score well for 5 of the 8 essential amino acids [4].

baobab leaves have a high content of iron compared to numerous other wild-gathered foods, and are a rich source of calcium[5]. Comparisons between published data for the minerals iron, calcium, zine and phosphorus show wide variations in content. Iron is of especial importance because of the prevalence of iron- deficiency anemias in savannah areas [6].

The total lipid content of baobab leaves at 55 mg/g of dry weight and that they were not a significant source of linoleic acid. the level of vitamin A was about one-third the content in Amaranthus dried leaves. The absence of vitamin C but a significant content of vitamin B2. [7].

Material and method-

Experimental animals

In-vivo Protocol bearing number (LRIP/ IACEC/2023/PH-09) for carrying out neuromodulator activity of *Adansonia digitata* plant leaves was passed by IAEC of the institute. CPCSEA guidelines were followed for carrying out animal studies. Wistar rats (Rattus Norvegicus) were purchased from National Institute of Pharmaceutical Education and Research (NIPER) Mohali. Animals were kept in animal house LR group of institutes solan. Animals are kept at 12-14h light and dark photoperiod and are continuously aerated. Animals were fed with pellets (contains crude proteins, crude fat, crude fiber) obtained from the pet store. Animals were acclimatized to the laboratory conditions for about 10 days before commencement of the experiment.

Drugs and chemicals

Ethanol (BRG Biomedicals), Molisch Reagent, Benedict's Reagent, Fehling's Solution A & B (SDFCL, SD Fine Chem Limited), Mayers Reagent, Hager's Reagent (Ozone international Mumbai), Sulphuric acid, Ferric chloride, Lead acetate, Hydrochloric acid (SDFCL (SD Fine Chem Limited), Sodium hydroxide, Chloroform, Ninhydrin Solution (Fisher Scientific), Glacial acetic acid (Paskem Fine Chem Pvt. Ltd), Rivastigmine (Gift sample Dr. Reddy Pvt.Ltd), Diazepam (Gift sample Lifecare Neuro Products Pvt.Ltd),n-butanol, acetic acid, petroleum ether, ethyl acetate, glucose standard, citric acid, sodium citrate, Tris hydrochloride, sodium lauryl sulphate, thiobarbituric acid, trichloroacetic acid, sodium sulphate (sigma-Aldrich Pvt Ltd).

Collection of plant material

The dried leaves of *Adansonia digitata* were provided by Grrenforce renewable solutions Cop office A-14 first floor, Wazirpur, Industrial area, ring road, New Delhi 110052, India. These leaves were dried in shade, then pulverized into small pieces and sieved. The obtained coarse powder was stored in air tight containers until the beginning of experiment.

Preparation of the aqueous extract of leaves of *Adansonia digitata*:

The leaves of A. digitata were washed, air dried and pulverized with an electric blender. The defatted leaves powder was subjected to aqueous extraction by soaking 1000 g in 2000 mL of distilled water at room temperature and allowed to stand for 48 hour. The filtrate was carefully separated from the residue and concentrated by the use of rotary evaporator to give a paste-like residue. The residue was

stored in a sterilized container at 4 °C for subsequent use. The dose of extract used in this was based on result obtained from preliminary investigation and previous study [8].

Preparation of the methanol extract of *Adansonia digitata* leaves:
Adansonia digitata leaves were dried at 40 °C, grounded into a granulated powder and defatted with petroleum ether. The methanolic extract was obtained by extracting 4 kg of defatted leaves powder with methanol (95 %) at 50 ℃ for 72 h in Soxhlet followed by filtration and concentrated in rotatory vacuum evaporator at 50°C to its one third volume. The residual methanolic fraction was dried on water bath separately to yield extract. All the extract were stored at temperature below 10 °C and were freshly prepared with 2 % tween 80 for pharmacological experiments.

Qualitative phytochemical screening
The plant extract was used for preliminary screening of active constituents such as phenols, alkaloids, steroids, resins, proteins, carbohydrates and saponins. The phytochemical analysis was performed using the standard procedures to investigate the constituents presentin the plant.

Test for saponins
2 g of the extract was taken with 20 ml of water and boiled. It was filtered and 10 ml of the resultant filtrate is taken with 5 ml of water. It was shaken until stable froth is present. The froth was taken with a small quantity of olive oil and agitated. Emulsion is formed if saponins are present[9].

Test for alkaloids (Dragendorff's reagent)
5 ml of the extract was taken along with 1.5 ml of hydrochloric acid. For a period of 20 minutes the mixture was heated and 1 ml of dragendorff's reagent was added. Theformation of an orange or reddish precipitate shows the presence of alkaloids.[10]

Test for carbohydrates (Molisch's test)
2 drops of Molisch's reagent were added to 2 ml of the extract in a test tube. The formation of reddish violet ring at the junction of two liquids indicates the presence of carbohydrates.

Test for proteins (Biuret test)
1 ml of the extract was taken in a dry test tube and in another tube 1 ml of distilled water was taken as control. 1 ml of biuret reagent was added to all test tubes and mixed well. The development of blue colour indicates the presence of proteins

Test for tannins
0.5 g of the extract was taken with 20 ml of H_2O and boiled. It was then filtered and small drops of 0.1 % $FeCl_3$ was added. It was observed for blue black or brownish green coloration.[11]

Test for flavonoids (Shinoda test)
5 ml of the extract was taken and was added to 5 ml of ethanol along with a drop of Conc. H_2SO_4. The resultant solution was taken and 0.5 g magnesium turnings were added. Formation of pink colour shows the presence of flavonoids.

Test for terpenoids (Salkowski test)
5 ml of extract was mixed with 2 ml of chloroform along with 3 ml of con. sulphuric acid. A reddish-brown color shows the presence of terpenoids.

Test for phenols ($FeCl_3$ test)
2 ml of the extract was taken with 2 ml of $FeCl_3$.The formation of a bluish green solution indicates the presence of phenols [12].

In-*vitro* anti-oxidant activity-

DPPH assay-

Principle

DPPH (1,1-Diphenyl-2-picrylhydrazyl) when mixed with a methanolic solution has purple color. When DPPH accepts a hydrogen from a donor, the solution starts to lose the purple coloration. This is a widely used study for determining the anti-oxidant capacity of various compounds. The anti-oxidant activity is determined by the increase or decrease of the concentration of 1,1-Diphenyl-2-picrylhydrazyl [13].

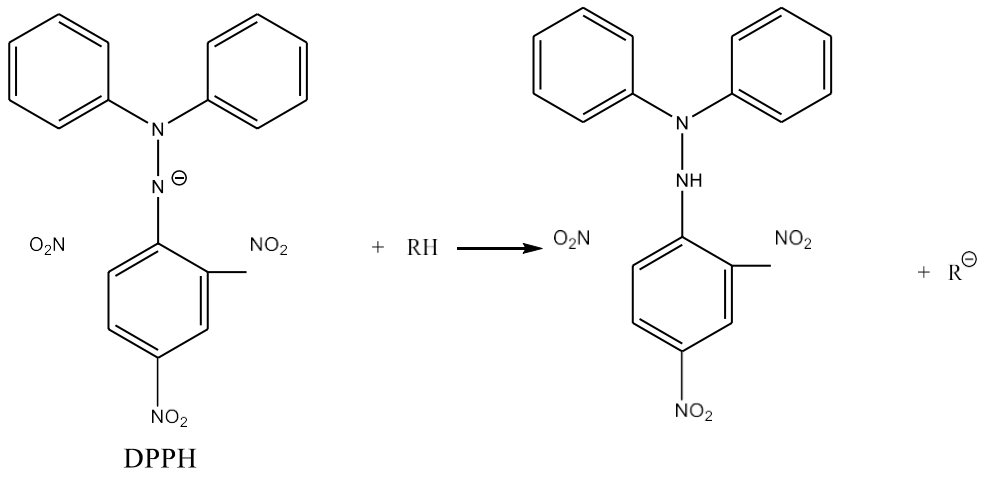


Figure 5.1: Reaction involved in DPPH assay

Reagents

DPPH - 0.1 mM (1.97 mg/50 ml methanol)
BHT - 1 mg/ml in methanol

Procedure

Anti-oxidant potential of the methanol, hexane, chloroform and ethyl acetate extracts was estimated using DPPH assay. SRE-MC was prepared with 1mg/ml in methanol and utilized as the stock solution. In the 96 well plate, the wells are made up with 200 µl SRE-MCin methanol. After addition of extract, 5 µl of the DPPH solution was added and allowed to develop for 30 minutes under darkness. Absorbance of the resultant solution was read at 517 nm [14]. The stock solution of the extracts was prepared using methanol. The varying concentration of plant extracts (e.g., 20µl, 40µl, 60µl, 80µl and 100µl) was added with DPPH (0.1mm) in methanol solution and kept at room temperature for 30 minute. The absorbance was carried out at 517nm by UV –spectrometer. The percent of DPPH scavenging activity was calculated.

% inhibition of SRE-AD and standard ascorbic acid was performed using the formula.

$$\%\ \text{of inhibition} = \frac{\text{Absorbance of test} - \text{Absorbance of control}}{\text{Absorbance of control}} \times 100$$

Selection of doses

Animal dose

Dose of the aqueous extract was chosen based on the previous work done on rodents. According to the study conducted on rodents and *Adansonia digitata* Linn. (500 mg/kg) significantly decreased the

acetylcholinesterase activity and improved cognition. The above dose 500 mg/kg is adjusted to the weight of male wistar rats and was administered orally using 1-10 µL micropipette. Standard drug used in the present study was rivastigmine, which is an acetylcholinesterase inhibitor, currently used in the market for treating patients with Alzheimer's disease. Water is used as a vehicle; both standard drug and *Adansonia digitata* were dissolved/suspended in the water. Before 2 hours of the commencement of the experiment, standard Rivastigmine and test *Adansonia digitata* Linn. extract was administered to the animals and 1 hour before the commencement of the experiment wistar rats were treated with rivastigmine 200 µM solution of rivastigmine was prepared and animals were transferred to the tank containing the rivastigmine solution before 1 hour of beginning of the experiment. Standard and test drug solutions were prepared freshly on the day of the experiment[15].

Acute toxicity study

Adansonia digitata was employed as a neuromodulator due to its flavonoids, minerals and anti-oxidant role. Previous study showed that *Adansonia digitata* at 10-15 mg/kg dose in rats was well effective as anti-epileptic; which states the neuroprotective role of *Adansonia digitata.* It is essential to perform the acute toxicity studies according to OECD 203 guidelines in order to decide lethal concentration (LC50) of *Adansonia digitata* as a test compound in adult male wistar rats.[16]

The male wistar rats were exposed to test compounds for a period of 96 hours. Changes in the behavior movements like sniffing, crawling movement and survival was observed for every 24 hours until 96 hours. Mortalities noted at 24, 48, 72 and 96 hours and the concentration killing 50 % of the wistar rats was determined.

Experimental protocol

Grouping of animals

S.No.	GROUPS(n=6)	TREATMENT	Number of Animals(n=4)
1	CONTROL GROUP	NORMAL SALINE (0.9% Nacl)	4
2	TEST GROUP (I)	*Adansonia* chloroform extract (250mg/kg)	4
3	TEST GROUP (II)	*Adansonia* aq. extract (500mg/kg)	4
4	STANDARD GROUP-(I)	Rivastigmine (0.75µg, i.p)	4
5	STANDARD GROUP-(II)	Diazepam (2 mg (ip)	4

Male Wistar rats, bodyweight between 250 g and 300g of 11 – 14 weeks old rats were used.

The animals were divided into 5 groups, with 4 animals in each group. The animals would be on a normal diet of food and drinking water, The first group act as a control group and would be given normal saline orally, and the second group act as a test group (1, 2) and administered with different doses of plant extract. And the last group is the standard group administered rivastigmine (0.75µg, i.p) and Diazepam (2 mg (i p). Then the animals were subjected to chronic unpredictable mild stress for 28 days, for the first 7 days animals were only subjected to the stressors, and then after that the stressors the animals were administered the drugs as per their divided groups for 21 days. And from day 29 to 31 different behavioural evaluations were conducted, 29 day the T-maze test is conducted, and on 30 day the Forced swim test is conducted, and on 31 days, the open field test is conducted. then after that, the statistical data is collected and the animals were sacrificed.

Laboratory model employed

The various behavioral laboratory tests were performed in male wistar rats as follows-

Table 5.1: Parameters for the behavioral assessments

S. No.	Behavioral assessment	Parameters
1	Passive avoidance test	Learning and memory
2	Elevated plus-maze test	Spatial memory
3	Force swim test	Motor activity and stress

6. RESULTS AND DISCUSSION

The present project is done to explore the potential of herbal drugs for the treatmentof CNS disorders with a view to perform phytochemical investigation and assess Neurobehavioral and Neurochemical screening. The study also involves investigation of medicinal active ingredient associated with the extracts followed by characterization and other evaluation parameters employing *Adansonia digitata.* The various observation and results obtained from evaluations are discussed in this chapter.

6.1 PHYTOCHEMICAL PROFILING OF CRUDE EXTRACTS

6.1.1 Physical characteristics of extracts

Extraction of plant materials was done with 90 % methanol. % yields of extracts as wellas their characteristics are reported in Table-6.1.

Physical characteristics of methanolic extracts

S.No.	Extracts	Consistency	Colour ofextract	Odour ofextract	Yield(in gms)	% Yield(w/w)
1.	90% methanolic extract of *Adansonia digitata*	Greasy	Yellowishbrown	Characteristic	92.3	13.14
2.	Aqueous extract of *Adansonia digitat*a leaves	Non greasy	Dark brown	characteristic	90.9	9.02

6.2 Qualitative chemical tests

Results obtained from qualitative chemical tests of all the medicinal plants are tabulated in Table.

Table-6.2: Qualitative chemical tests of methanolic extracts of *Adansonia digitata*

S.no	Test for active constituents	Ethanolic extract
1	Alkaloids	+ve
2	Glycosides	+ve
3	Sterols	+ve
4	Flavonoids	+ve
5	Tannins	+ve
6	Phenolic compounds	+ve
7	Carbohydrate	++ve
8	Proteins	-ve
9	Resins	-ve
10	Fats & oils	+ve
11.	Trace elements	+ve

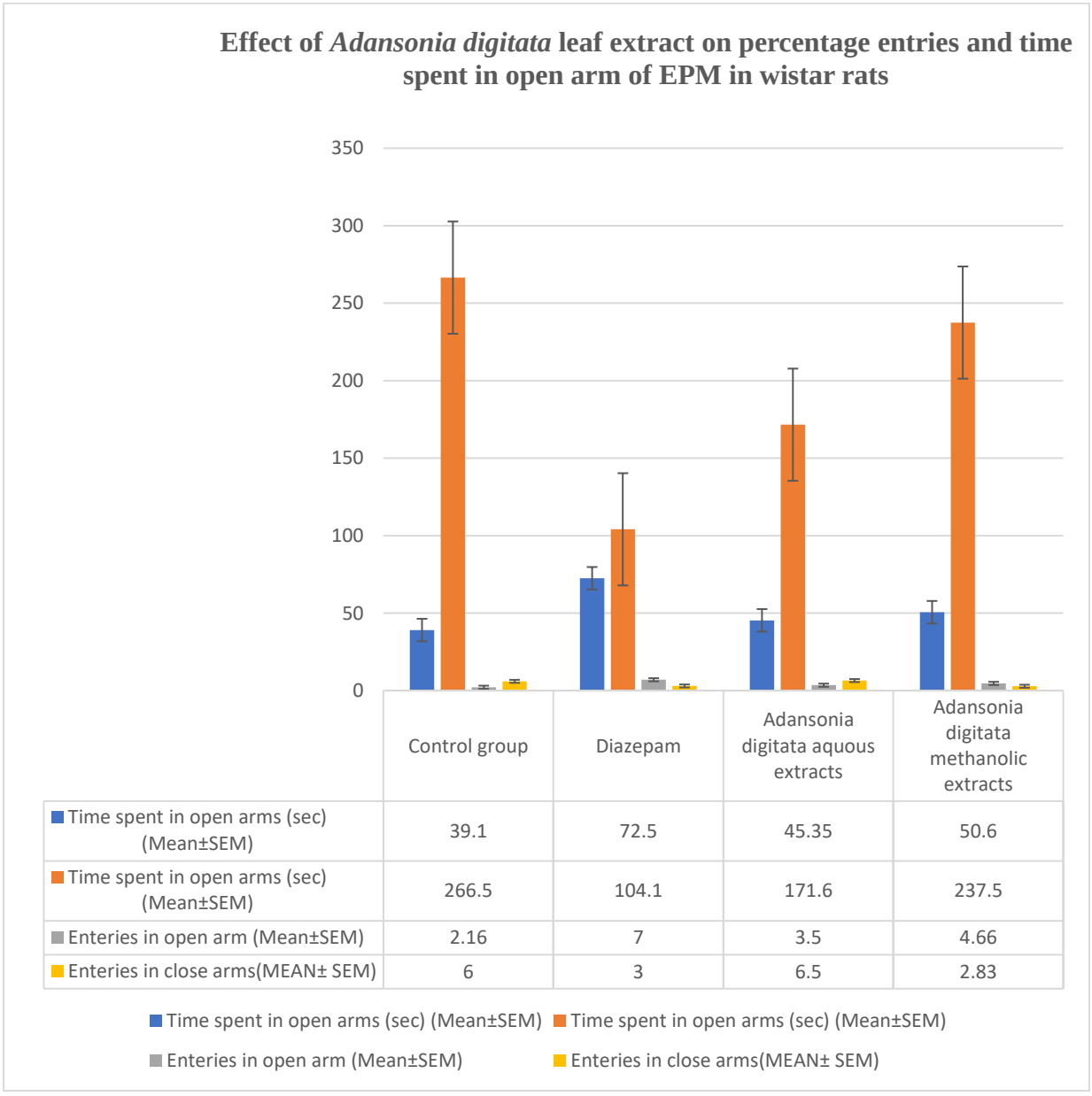
+ve: Present; **-ve:** Absent

Pharmacological studies

Elevated plus maize study- From the experiment it was observed that rats taken aqueous and methanolic leaf extract at dose of 250 and 500 mg/kg body weight, stayed more time in open arm of Elevated plus Maze apparatus in comparison to standard and negative control group. Moreover, they were also stayed less time in closed arm of elevated plus maze apparatus in comparison to standard and negative control group.

Table showing time spent or number of entries taken by rats in open or close arms.

S.NO	Treatments	Dose (KG^{-1})	Time spent in open arms (sec) (Mean±SEM)	Time spent in open arms (sec) (Mean±SEM)	Enteries in open arm (Mean±SEM)	Enteries in close arms (MEAN± SEM)
1	Control group 0.9% normal Nacl solution	Normal diet	39.1 ± 2.58	266.5 ± 9.27	2.16 ± 0.3	6 ± 1.0
2	Diazepam	2 mg (ip)	72.5 ± 0.76	104.1 ± 12.75	7 ± 0.25	3 ± 0.36
3	*Adansonia digitata* aqueous extracts	250 mg/kg	45.35 ± 4.4	171.6 ± 5.88*	3.5 ± 0.56	6.5± 0.56
4	*Adansonia digitata* methanolic extracts	500 mg/kg	50.6 ± 0.88	237.5 ± 6.41*	4.66 ± 0.21	2.83±0.47



All the data were mentioned as mean ± SEM. Statistical analysis was done by using one-way ANOVA followed by Tukey's multiple comparison tests. Graph Pad Prism 5 statistical software was employed. $p < 0.05$ was denoted as remarkable/ significant data.

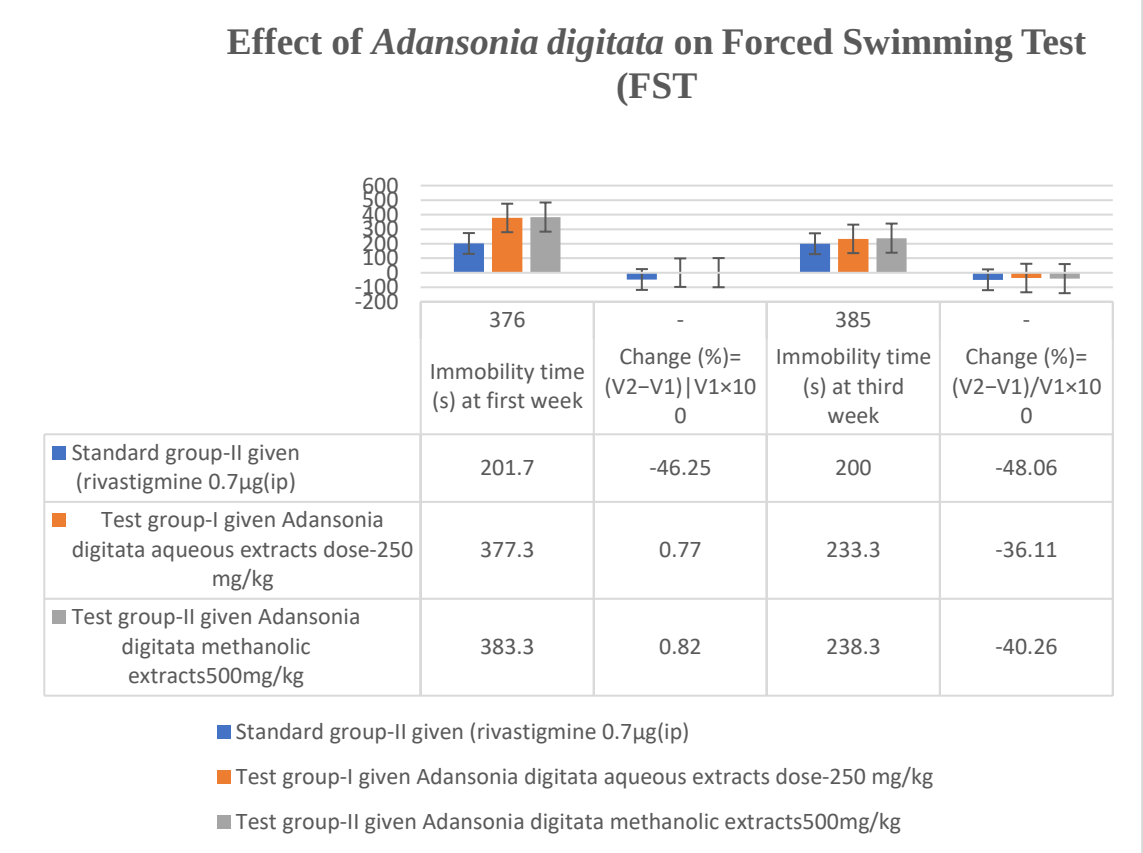
Forced Swimming Test (FST)-

According to FST, the results obtained from the first week of the day was shown a significant ($p<0.05$) decrease in the duration of immobility with respect to standard drug rivastigmine and the results obtained after administration of the *Adansonia digitata* leaves extract showed that the immobility time of rats also decreases with respect to dose dependently and rats were more active in employed models. The motor activity was also more and antianxiety effect was also stronger. However, for all different doses administered, there were also significantly differences in response to control, standard or test drugs and led to reduction in immobility time.

Table Effect of *Adansonia digitata* leaves extracts on immobility time of rats.

Animal Group (Dose in mg/kg)	Immobility time (s) at first week	Change (%) = (V2−V1) |V1×100	Immobility time (s) at third week	Change (%) = (V2−V1)/V1×100
Control group given 0.9% normal Nacl solution	376	-	385	-
Standard group-II given (rivastigmine 0.7µg(ip)	201.7**	-46.25	200	-48.06
Test group-I given *Adansonia digitata* aqueous extracts dose-250 mg/kg	377.3**	0.77	233.3*	-36.11
Test group-II given *Adansonia digitata* methanolic extracts500mg/kg	383.3	0.82	238.3**	-40.26

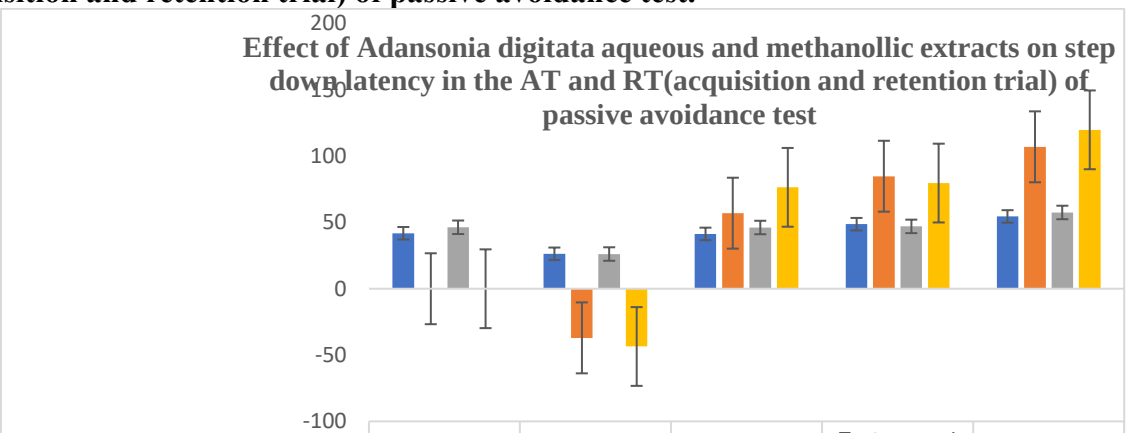
All the data were mentioned as mean ± SEM. Statistical analysis was done by using one-way ANOVA followed by Tukey's multiple comparison tests. Graph Pad Prism 5 statistical software was employed. $p < 0.05$ was denoted as remarkable/ significant data.



Passive avoidance test-

In this test, the diazepam treated group, the step-down latency (s) in passive avoidance test decreased significantly to the extent of 37.05 and 43.54 % respectively in the AT and RT (acquisition and retention trial) as compared to vehicle control animals. Thus, suggesting markedly impairment of memory. In contrast, treatment with *Adansonia digitata* aqueous and methanolic extracts at 250 and 500 mg/kg dose increased the step-down latency significantly in both the acquisition and retention trial as compared to diazepam treated group animals. The significant effect of reference standard drug rivastigmine on step down latency in passive avoidance test was to the extent of 56.97 and 76.45% respectively in the AT and RT trial. On statistical comparison we found that effect of *Adansonia digitata* aqueous and methanolic extract in both AT and RT trial was statistically more significant. The significant doses of two extracts were statistically similar indicating dose ceiling effect.

Table Effect of *Adansonia digitata* leaves extracts on step down latency in the AT and RT (acquisition and retention trial) of passive avoidance test.



	Control group given 0.9% normal Nacl solution	Standard group-I given Diazepam (2 mg (ip)	Standard group-II given (rivastigmine 0.7µg(ip)	Test group-I given Adansonia digitata aqueous extracts dose-250 mg/kg	Test group-II given Adansonia digitata methanolic extracts 500mg/kg
Step down latency (s) in the AT	41.83	26.33	41.33	48.66	54.5
Change (%) = (V2−V1) |V1×100	0	-37.05	56.97	84.81	106.99
Step down latency (s) in the RT	46.33	26.16	46.161	47	57.5
Change (%) = (V2−V1)/V1×100	0	-43.54	76.45	79.66	119.8

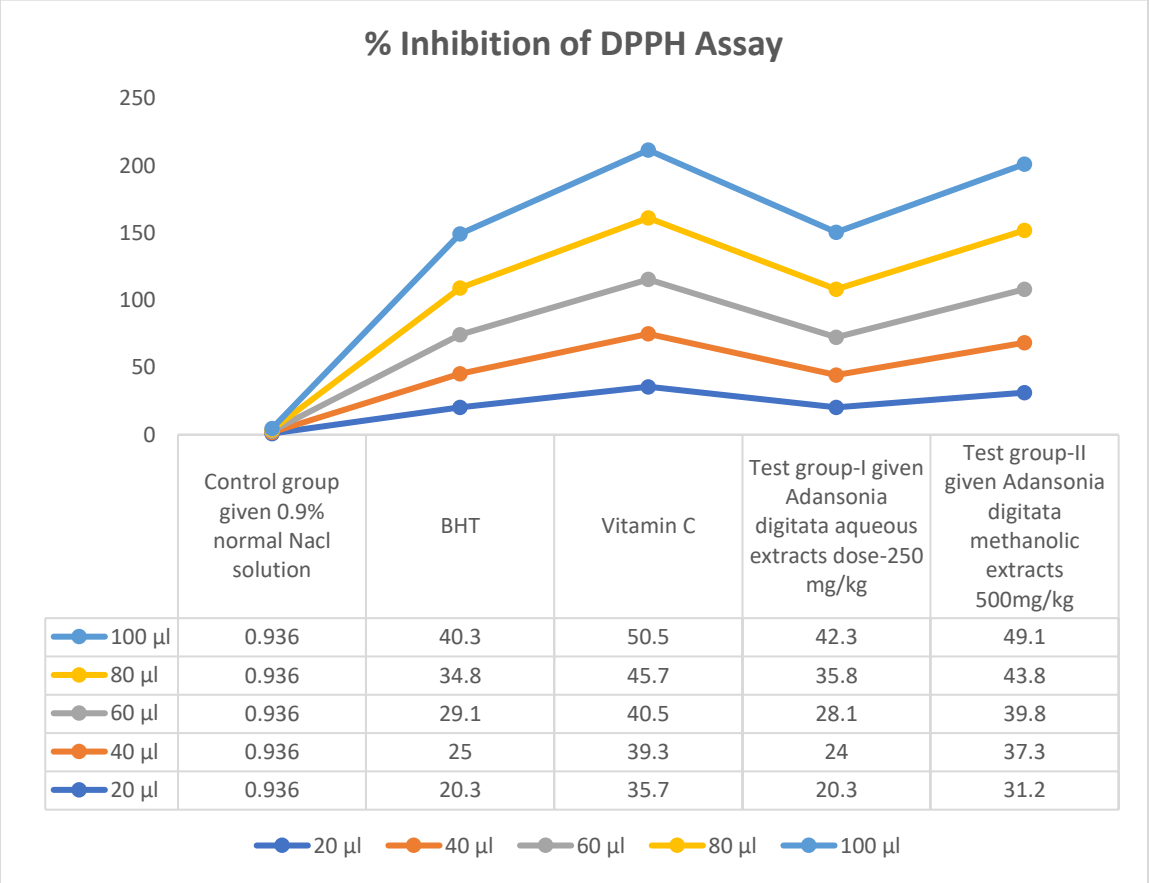
Values are expressed as mean ± SEM at n=4.The numerical results are evaluated by application of One-way ANOVA followed by Tukey's multiple comparison tests. Graph Pad Prism 5 statistical software was employed. $p < 0.05$ was denoted as remarkable/ significant data.

Antioxidant activity by DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay-

Free radical scavenging activity of various plant extracts is measured by the DPPH assay method. DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radical, which is purple in colour and its observance is measured at 517nm. Antioxidant present in the plant extract reacts with DPPH and it was reduced to produce unstable DPPH-H, which is hydrogen donor and it's decolorized to form yellow colour. Increase decolorization indicates higher reducing the ability of plant extract. The varying concentration of plant extracts (20µl, 40µl, 60µl, 80µl and 100µl) was added with DPPH

(0.1mm) in methanol solution and kept at room temperature for 30 minute. The absorbance was carried out at 517nm by UV –spectrometer. The percent of DPPH scavenging activity was calculated according to the following equation. % of inhibition= Absorbance (control) – Absorbance (sample) / Absorbance (control) × 100

S.no	Concentration	% Inhibition of DPPH Assay				
		Control	BHT	Vitamin C	Test group-I given *Adansonia digitata* aqueous extracts dose-250 mg/kg	Test group-II given *Adansonia digitata* methanolic extracts 500mg/kg
1	20 µl	0.936	20.3	35.7	20.3	20.2
2	40 µl	0.936	25	39.3	24	21.3
3	60 µl	0.936	29.1	40.5	28.1	36.8
4	80 µl	0.936	34.8	45.7	35.8	37.8
5	100 µl	0.936	40.3	50.5	42.3	49.1



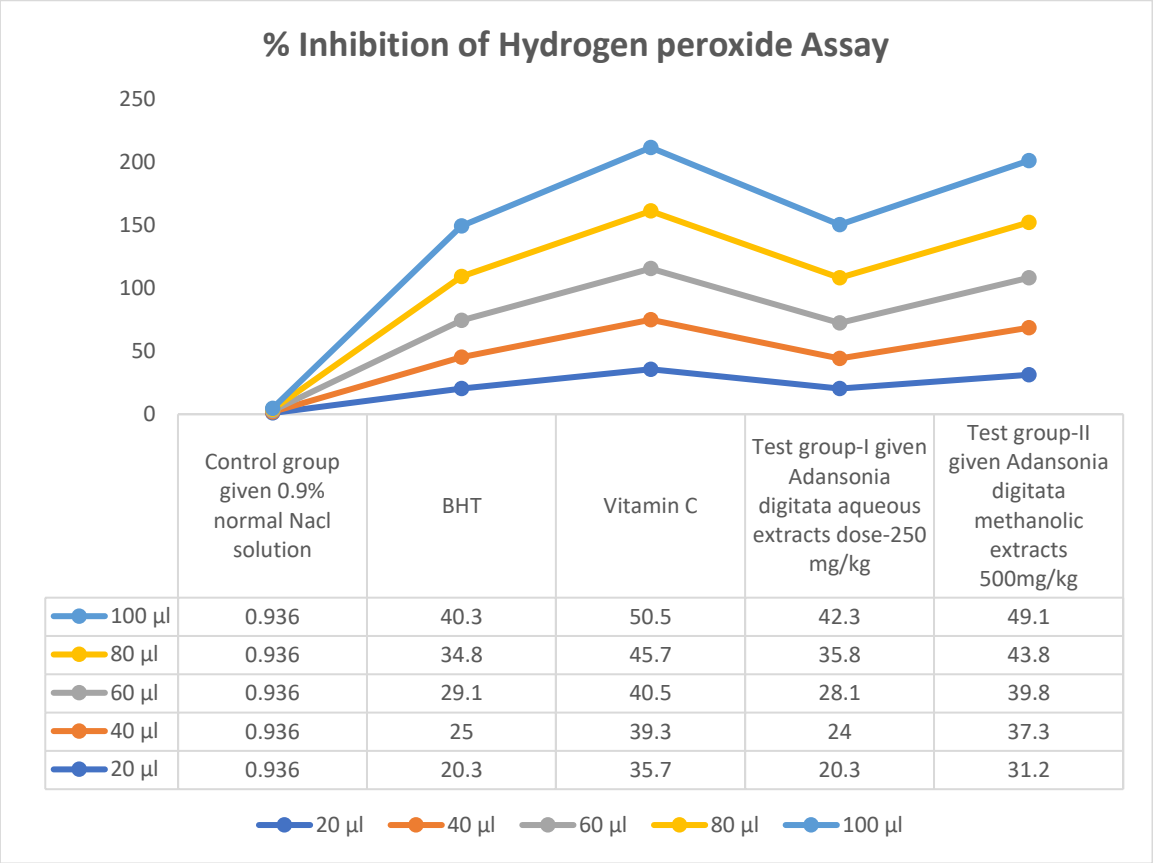
After performing DPPH assay, A graph is plotted after compilation with results the Adansonia digitata methanolic extracts showed significantly increased in scavenging free radicle activity with respect to other drugs. The numerical results are evaluated by application of One-way ANOVA with one-way ANOVA followed by Tukey's multiple comparison tests. Graph Pad Prism 5 statistical software was employed. $p < 0.05$ was denoted as remarkable/ significant data.

Hydrogen peroxide scavenging assay

Hydrogen peroxide is an oxidizing agent, but it's weak in nature and can deactivate several enzymes by oxidation of thiol (-SH) groups. Hydrogen peroxide may cross over the cell membrane and in the cell, hydrogen peroxide may immediately react with Fe2+, and probably Cu2+ to produce hydroxyl radical, which may initiate several toxic effects Inside the cell. IC50 values were 0.86,28.1,55.5,43.1,53.8µg/ml for normal 0.9%Nacl, diazepam, rivastigmine, methanol extract respectively.

S.no	Concentration	% Inhibition of Hydrogen peroxide Assay				
		Control group given 0.9% normal Nacl solution	BHT	Vitamin C	Test group-I given *Adansonia digitata* aqueous extracts dose-250 mg/kg	Test group-II given *Adansonia digitata* methanolic extracts 500mg/kg
1	20 µl	0.86	18.3	51.7	37.3	50.2
2	40 µl	0.86	22.5	52.3	39	51.3
3	60 µl	0.86	28.1	55.5	43.1	53.8
4	80 µl	0.86	31.8	59.7	47.8	58.8
5	100 µl	0.86	34.3	63.5	55.3	61.1

After performing hydrogen peroxide assay, A graph is plotted after compilation with results the Adansonia digitata methanolic extracts showed significantly increased in scavenging free radicle activity with respect to other drugs.

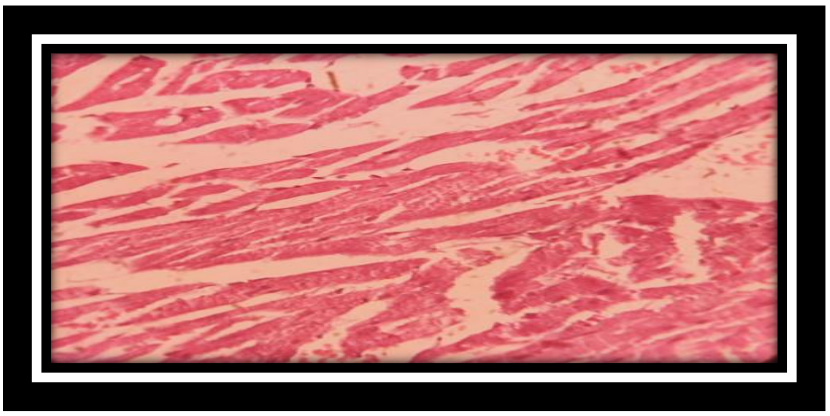


The numerical results are evaluated by application of One-way ANOVA with one-way ANOVA followed by Tukey's multiple comparison tests. Graph Pad Prism 5 statistical software was employed. $p < 0.05$ was denoted as remarkable/ significant data.

Histopathology-

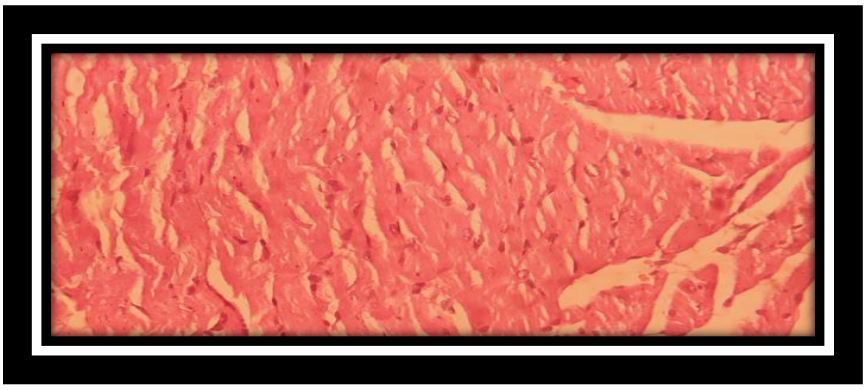
For histopathology study the brain was collected and placed in the fixative immediately. Then they were placed in the 10% neutral buffered formalin in the non-breakable plastic containers, with lid. And then the samples were sent to lab for histopathology. Following were the reports as mentioned below:

(a) Control group (given 0.9% normal Nacl solution)-: Brain frontal cortex in the control group, a normal neuron (arrow) and neuroglia are seen. There are normal neuronal cell and no neural damage. normal pyramidal layer in the control group. The brain tissues of control animals showed normal intact cellular arrangements.



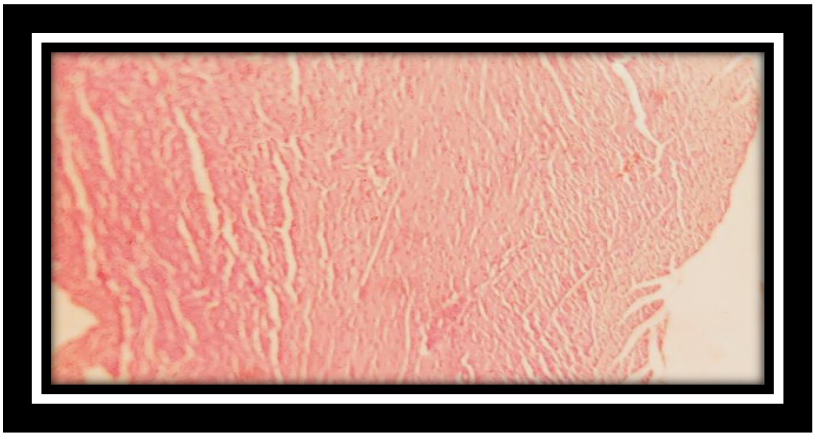
Histopathology slide of brain of Control group

(b) Standard group-1 given Diazepam (2 mg (ip) - Brain activity slows down and the transmission of electrical signals also slow down. There are changes in brain frontal cortex a neuron that is an indication for neuronal necrosis and a few normal neurons are seen. Brain of diazepam treated group showed degeneration in some neuronal cell of hippocampus and cortex.



Histopathology slide of brain of Standard group-1

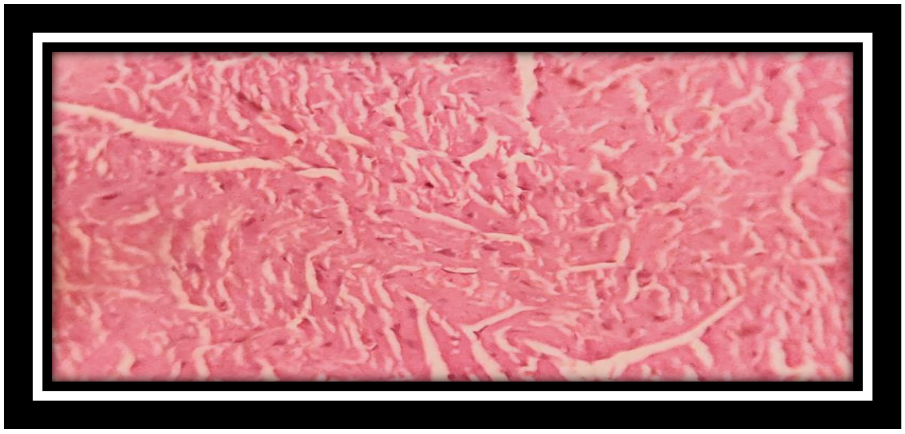
(c) Standard group-2 given (rivastigmine 0.7µg(ip) - Group showed marked perineural and mild perineuronal and perivascular edema, substantial improvement and neurogenesis. few shrunken and degenerated neurons. no significant pathology and displayed the reduced cellular alterations.



Histopathology slide of brain of Standard group-2

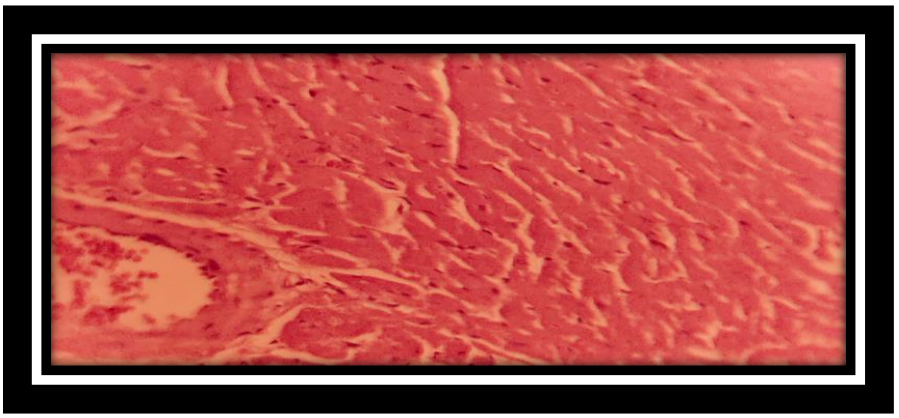
(d) Test group-1 given *Adansonia digitata* aqueous extracts dose-250 mg/kg -Brain frontal cortex that is an indication for neuronal necrosis is seen. The extract group showed mild perineuronal and perivascular edema, have few shrunken and degenerated neurons. Due to flavonoid compound, in

Adansonia digitata shows neurogenesis due to reduced glutathione level and peroxidase level. Slide shows few necrotic neuronal cells.



Histopathology slide of brain of Test group-1

(e) Test group-2 given *Adansonia digitata* methanolic extracts dose 500 mg/kg - shows that hippocampal sections have a standard pyramidal layer, very mild shrunken and mild degenerative changes of neurons as well as showed significant improvement and neurons accompanied by normal glial cells. It also showed the anti-inflammatory effect of the Extract due to the antioxidants and immunomodulatory properties of its constituents.



Histopathology slide of brain of Test group-2

Summary-

- The present study suggests that *Adansonia digitata L.* leaves extract plays a very important role in the treatment of memory impairment and used for to prevents oxidative stress due to its antioxidant properties. Various studies have shown that *Adansonia digitata* contains several bioactive constituents with strong antioxidant capabilities such as flavonoids, saponins, minerals and amino acids etc. Increased oxidative stress causes memory impairment and several diseases like Alzheimer, Parkinson, Amyotrophic lateral sclerosis etc. Several studies had explored the medicinal potentials of plants in attenuating environmental neurotoxicity and in the management of neurological disorders.
- The methanolic and aqueous leaf extract of *Adansonia digitata* leaves of different dose 250mg\kg and 500mg/kg exhibited potential neuromodulated activity in male Wister rats. Higher the dose of *Adansonia digitata* more effective was enhancing effect of neuromodulation property of plant. After performing the behavioural study like elevated plus maze, force swim test, inhibitory avoidance test on male wistar rats, the methanolic and aqueous leaf extract of *Adansonia digitata* leaves showed significantly increase in exploratory activity or neurotransmission activity.

- Anticholinesterase drug rivastigmine and diazepam was taken standard drug and activity was compared with respect to *Adansonia digitata* leaves aqueous and methanolic extracts and has shown markedly enhanced the neurotransmission or Neuromodulatory activity. The beneficial effect of *Adansonia digitata* on memory improvement is believed to be mediated through its action as a potent antioxidant that results from its direct scavenging activity of free radicals, thereby preventing oxidative stress. As a result, its antioxidants assays or parameters were also performed or graphs were also plotted.
- After comparing the results with control group or standard groups (I and II) with respect to aqueous and methanolic extract of *Adansonia digitata* showed markedly increased activity.

Conclusion-

- Antioxidant activity of plants prevents the free radicle formation and protects the brain from chemical induced damage or enhancing learning and memory performance.
- oxidative damage or oxidative stress leads to the production of reactive oxygen species (ROS), and leading to the peroxidation of cell membrane leading to disrupt the integrity and function of the cell membrane and causing cell death and neurodegeneration.
- Our study showed that groups treated with *Adansonia Digitata* significantly reduced the level of MDA, but increased the activities of SOD, CAT and GPx in the brain. Thus, antioxidant property of plants leaves is only due to presence of some bioactive compounds such as flavonoids, phenols, vitamins and minerals component of plants results in scavenging the free radical's formation and prevent the lipid peroxidation or play the neuroprotective role. Glutamate is the most abundant excitatory neurotransmitter in the CNS involved in neuronal transmission, development, differentiation and plasticity of neurons.
- However, excess accumulation of glutamate leads to abnormal depolarization of neurons, resulting in excitotoxicity and neuronal cell death but *Adansonia digitata L*. modulates glutamate concentration or activity by increasing cellular uptake to maintain low extracellular glutamate. Excitability of neuron firing increases with spontaneous or excessive firing especially with metabolic or ischemia diseases or inability of axon to conduct action potential due to structural or metabolic damages to axon and axon starts conducting action potential slowly or at low rate of firing due to narrowing or deformation of axon structure. *Adansonia digitata L*. also modulates neurons by allowing the astrocytes cells to function effectively.
- Mineral components of the plant extract from *A. digitata* may potentially contribute to its pain-relieving actions in neuropathic pain in addition to its flavonoid contents. The electrolytes that are most prevalent in the A. digitata (Seed, Fruit, Leaf, and Bark) plant consist of Mg, Ca, Fe, Zn, and Mn.
- *Adansonia digitata* have broad range of therapeutic value. Thus, plant extract of *Adansonia digitata* should be explored further for its potential in neuromodulation and neurodegenerative diseases.

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