IJBPAS, September, Special Issue, 2021, 10(9): 470-480
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

NEUROPROTECTIVE AND ANTI-ALZHEIMER'S EFFECTS OF PLANT- *DALBERGIA DIPHACA*

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Received 25th June 2021; Revised 28th July 2021; Accepted 29th Aug. 2021; Available online 25th Sept. 2021

https://doi.org/10.31032/IJBPAS/2021/10.9.1036

ABSTRACT

Neurodegenerative disorder can be described as an irreversible gradual loss of neuronal cell which is essential to perform the normal brain functions and the continuous loss of neuronal cell ultimately leads to brain death. Alzheimer's disease is defined as an irreversible neurological disorder which impairs the cognitive and intellectual function of human brain.

The fresh leaves of *Dalbergia diphaca* (DD) was collected from the local flora in Vellore district, Tamil Nadu India for the Neuroprotective activity of Indian medicinal plants in Alzheimer's disease. The doses (200 mg/kg and 400 mg/kg) of ethanolic extracts of ZL was used for the Neuroprotective activity of Indian medicinal plants in Alzheimer's disease. The tested doses at 200 mg/kg and 400 mg/kg of ethanolic extracts of ZL showed significant neuroprotective behavioral study. These extracts bring back the declined level of brain neurotransmitters like dopamine, glutamate and antioxidant enzymes like catalase, glutathione peroxidase and glutathione reductase. *In vitro* neuroprotective activity for ethanolic leaves extracts of *Dalbergia diphaca* (DD) was performed on SH-SY5Y cells. The copper oxide nanoparticles synthesized from the ethanolic leaves extracts of ZL showed very good neuroprotective activity.

Keywords: Neuroprotective, Alzheimer's disease, *Dalbergia diphaca*

INTRODUCTION:

Neurodegenerative disease can be described a disease with an irreversible gradual loss of neuronal cell which is essential to perform the normal brain functions and the continuous loss of neuronal cell ultimately leads to brain death. The neurodegenerative disorder includes Alzheimer's disease, Parkinson's disease, Huntington's disease and Amyotrophic lateral sclerosis (Marcello *et al*., 2010). The number of dementias affected cases mounts very higher in number in the recent days which is much more than expectedand will ascend to over and above sixty-five million peoples get affected by dementia throughout the world before the year 2030 (Korolev, 2014). Dementia is a collective term of medical manifestation characterized by the significant decline in the normal intellectual nature of human brain (Gilman, 2010). Reversible dementia and irreversible dementia are the two major types of dementia. Reversible dementia is also known as pseudo dementia which is caused by the secondary manifestation of any other primary disorders like endocrine or exocrine gland secretion disorders, metabolic disorders, malnutrition or depressions. Alzheimer's disease is defined as an irreversible neurological disorder which impairs the cognitive and intellectual function of human brain. Alzheimer's disease is characterized by a major loss of neuronal cells which disorders the normal function of human brain. At molecular level, Alzheimer's disease is illustrated by the loss of cortical neuronal cells particularly pyramidal cell which is majorly responsible for intellectual and cognitive functions (Mann, 1996; Norfray, 2004). The earlier stage of Alzheimer's disease is characterized by the synaptic dysfunction which is responsible for the transmission of neuronal circuit for normal cognitive functions (Selkoe, 2002). Alzheimer's disease originally affects the neuronal cells of temporal lobe particularly the neuronal cells of hippocampal and entorhinal cortex (Jack *et al*., 1997).

METHODOLOGY

PREPARATION OF PLANT EXTRACTS

The fresh leaves of *Dalbergia diphaca* was washed in running tap water to remove the filth and dust. The hygienic leaves materials were dried over the shadow in a room temperature for about 72 hours. The dried leaves materials were made into fine particles by using the mechanical grinder. The plant materials were extracted with petroleum ether and ethanol by Soxhlet apparatus for 4 hours and subjected to rotary evaporator to remove the excess

solvent. The concentrated petroleum ether and ethanol leaves extract of *Dalbergia diphaca* was filtered and collected for further process. The leaf powder of *Dalbergia diphaca* (100gm) was successively extracted by Soxhlet apparatus using the petroleum ether and ethanol solvents. The leaves of *Dalbergia diphaca* were concentrated in vacuum to afford 7.90gm (7.90%w/w) of dry extract of petroleum ether and 9.60gm (9.60%w/w) of dry extract of ethanol. These extracts were then subjected to preliminary phytochemical tests, in-vitro bioactivity evaluations, neuroprotective pharmacological activity.

RESULTS AND DISCUSSION

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical screening of the petroleum ether and ethanol leaves extracts of *Dalbergia diphaca* (DD) by qualitative study showed the presence of phytochemical alkaloids, terpenoids, carbohydrates, proteins, phenolics, anthraquinones, flavonoids, glycosides, saponins and tannins.

PHYSICOCHEMICAL ANALYSIS OF *DALBERGIA DIPHACA*

The physico-chemical analysis like total ash, acid insoluble ash, water soluble ash, petroleum ether extractive value, ethyl alcohol extractive value and chloroform extractive value were performed and tabulated as shown in the **Table 1** *Dalbergia diphaca* (DD).

FLUORESCENCE ANALYSIS OF *DALBERGIA DIPHACA*

The fluorescence analysis for the different leaves were carried out with different chemical reagents to determine the phytochemicals present in it and the results were tabulated as shown in the **Table 2** for the leaves of *Dalbergia diphaca.*

DETERMINATION OF TOTAL FLAVONOIDS CONTENT

The total flavonoids content for the different leaves of *Dalbergia diphaca* was carried out and tabulated (Mean±SD) as shown in the **Table 3**. The ethanol extracts of leaves of *Dalbergia diphaca* (DD) have higher flavonoids content than the petroleum ether leaves extracts.

The standardization of medicinal plant is very much important to ensure the safety and quality of medicinal drugs prepared from the plant source. World Health Organization has emphasized the importance of pharmacogenetic analysis of medicinal plants which state that pharmacogenetic analysis is the first and foremost step to ensure the purity, safety and quality of medicinal plant drug materials before commencing any kind of plant materials drug tests.

Dalbergia diphaca **(DD)** have higher phenolic and flavonoids content than the petroleum ether leaves extracts.

It could be concluded that the leaves of *Dalbergia diphaca* plant is of phytopharmaceutical significance and this study helps to undertake further studies towards these plants to explore the pharmacological bioactivity profile of *Dalbergia diphaca*

IN VITRO BIOACTIVITY EVALUATIONS

IN VITRO ANTIOXIDANT ACTIVITY

The in vitro antioxidant activity for the petroleum ether and ethanol leaf extracts of *Dalbergia diphaca* was performed by DPPH (1, 1- diphenyl-2-picrylhydrazyl) scavenging activity method and the results are tabulated as shown in the **Table 4**.

The in vitro antioxidant activity is measured by the parameter called IC50 value. The IC50 value is defined as the concentration of the plant extracts required to scavenge 50% of the DPPH radical. The higher antioxidant property is evident by the lower IC50 value and the higher in IC50 value results in the lower antioxidant property (Maisuthisakul *et al*., 2007).

The ethanol extracts of leaves of *Dalbergia diphaca* (DD) has higher antioxidant activity than the petroleum ether leaves extract *Zaga latifolia* (DD).

IN VITRO ANTIDIABETIC ACTIVITY

The in vitro antidiabetic activity of *Dalbergia diphaca* was performed by alpha-amylase enzyme inhibition method and the results are tabulated as shown in the **Table 5**. The ethanol extracts of leaves of *Dalbergia diphaca* (DD) have higher dose dependent antidiabetic activity than the petroleum ether leaves extract of *Dalbergia diphaca* (DD).

IN VITRO ANTI-INFLAMMATORY ACTIVITY

The in vitro anti-inflammatory activity for the petroleum ether and ethanol leaf extracts of *Dalbergia diphaca* was performed by Human Red Blood Corpuscles membrane stabilizing method and the results are tabulated as shown in the **Table 6**.

The ethanol extracts of leaves of *Dalbergia diphaca* (DDE) has higher significant ($p<0.0001$) anti- inflammatory activity than the petroleum ether leaves extract of *Dalbergia diphaca* (DDPE).

IN VITRO ANTIMICROBIAL ACTIVITY

The in vitro antimicrobial activity for the petroleum ether and ethanol leaf extracts of *Dalbergia diphaca* (DD) was performed by agar well diffusion method and the results of in vitro antimicrobial activity for the petroleum ether and ethanol leaf extracts of *Dalbergia diphaca* (DD) was tabulated as shown in the **Table 7**.

PHARMACOLOGICAL ACTIVITY

ACUTE TOXICITY STUDIES

The acute toxicity study for the ethanol leaves extracts of *Dalbergia diphaca* (DD) was studied and tabulated as shown in the **Table 8**. The ethanol leaves extract of *Dalbergia diphaca* (DD) had not shown up any mortality or any kind of toxic symptoms on mice even at the dosage of 2000 mg/kg body weight through oral route of administration. The guidelines for the acute toxicity studies as per the OECD-423 guidelines suggests that the LD_{50} dosage of above 2000 mg/kg termed as unclassified drugs and ethanol leaves extracts of *Dalbergia diphaca* (DD) were viewed as a secured and non-toxic drug for the other pharmacological studies (Muralidharan *et al*., 2010). Since the dosage of extracts found to be safe and non-toxic up to 2000 mg/kg, the one-tenth (200 mg/kg) and one-fifth (400mg/kg) dosage of ethanol leaves extracts of *Dalbergia diphaca* (DD) were chosen for the neuroprotective activity study.

OPEN FIELD TEST

The open field test for the ethanol leaves extracts of *Dalbergia diphaca* (DD) were studied and the results are tabulated as shown in the **Table 9**. There is a significant increase in the locomotor activity of ethanol leaves extracts of *Dalbergia diphaca* (DD) when compared with the locomotor activity of toxic negative control group as shown in the **Fig. 1**.

ELEVATED PLUS MAZE TEST

The elevated plus maze test for the ethanol leaves extracts of *Dalbergia diphaca* (DD) were studied and the results are tabulated as shown in the **Table 10**. There is a significant increase in the transfer latency of ethanol leaves extracts of when compared with the transfer latency of toxic negative control group as shown in the **Figure 2**.

ESTIMATION OF ANTIOXIDANT & ACETYLCHOLINESTERASE ENZYME

The antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and brain enzyme acetyl cholinesterase (AChE) were estimated for the animals treated with ethanol leaves extracts of *Dalbergia diphaca* (DD) and the results are tabulated as shownin the **Table 11**.

There is a significant improvement in restoring the decreased level of antioxidant enzymes and the brain enzyme acetyl cholinesterase by the ethanol leaves extracts of *Dalbergia diphaca* (DD) when compared with the other treated groups and toxic negative control group as shown in the **Fig. 3-7**.

The level of antioxidant enzymes and the brain enzyme acetyl cholinesterase restored by ethanol leaves extracts of *Dalbergia diphaca* (DD).

Table 1: Physicochemical analysis of leaves of DD

WHO parameters	Leaves value (%w/w)
Total ash	4.6
Acid insoluble ash	1.23
Water soluble ash	1.65
Petroleum ether extractive value	4.41
Alcohol extractive value	7.27
Chloroform extractive value	1.54

Table 2: Fluorescence analysis of leaf powder of *Dalbergia diphaca*

S. No.	Particulars of treatment	Under ordinary light	Under UV light
1	Powder as such	Green	Dark green
2	Powder and Sulphuric acid (1:1)	Yellowish green	Pale green
3	Powder and Nitric acid (1:1)	Greenish yellow	Dark green
4	Powder + NH3	Light green	Dark green
5	Powder + I2	Yellowish green	Green
6	Powder + 5% Ferric chloride	Greenish black	Dark green
7	Powder+ CH3COOH	Greenish yellow	Dark green

Table 3: Total flavonoid content of DD

Extracted samples	DD
Ethanol	139.54±0.18
Petroleum ether	74.20±0.86

Table 4: *In Vitro* Antioxidant Activity of DD

Extract samples	*Dalbergia diphaca* (ZL) IC50 ± SD (µg/ml)
Ethanol extracts	88.12±6.2
Petroleum ether extracts	116.34±9.2

Values are expressed in mean ± SD for the four determinations

Table 5: *In Vitro* Antidiabetic Activity of DD

Samples	Concentration (µg/ml)	% Inhibition	IC50 (µg/ml)
Acarbose (Standard)	100	34.86 ± 0.3536	339.85 ± 5.9
	200	50.11 ± 0.4805	
	400	60.19 ± 0.3944	
	800	68.33 ± 0.2544	
	1000	74.98 ± 0.4847	
ZLPE	100	25.63 ± 0.3674	687.95 ± 4.97
	200	35.80 ± 0.2691	
	400	39.67 ± 0.3465	
	800	57.94 ± 0.4925	
	1000	63.62 ± 0.4920	
ZLE	100	20.84 ± 0.3864	595.84 ± 4.58
	200	31.95 ± 0.2497	
	400	35.69 ± 0.3847	
	800	52.48 ± 0.4836	
	1000	58.53 ± 0.4658	

Values are expressed in mean ± SEM for the three determinations

Table 6: *In Vitro* Anti-inflammatory Activity of DD

Treatment	Absorbance	% Inhibition
Control	0.67 ± 0.43	_
ZLPE	0.48 ± 0.27^{a}	33.97
ZLE	0.32 ± 0.19^{aaa}	57.08
Diclofenac potassium	0.16 ± 0.07^{aaa}	77.41

Values are expressed in mean ± SEM for triplicate experiments. All the data wereassessed by student't' test using ^{aaa}P<0.0001, ^{aa}P<0.001, ^{a}P<0.05 values to indicate significant levels compared to control group for the all different extracts at concentration of 1000 mcg/ml.

Table 7: *In Vitro* Antimicrobial Activity of DD

Organism	Zone of inhibition (mm)		
	Petroleum ether extract	Ethanolic extract	Ampicillin
	Concentration 10mg/ml	Concentration 10mg/ml	Concentration 1mg/ml
	Dose: 0.2ml	Dose: 0.2ml	Dose: 0.2ml
Escherichia coli ATCC 25922	14	17	20
Staphylococcus aureus ATCC 29213	13	16	23
Klebsiela pneumonia ATCC 27738	15	18	22
Pseudomonas aeruginosa ATCC 27853	16	19	21

Table 8: Individual mortality data of DD in acute toxicity study

Maximum dose level	Sex	Number of animals died during day of dosing (hr)					Number of animals died during period after dosing (Days)								Deaths
		1/2	1	2	3	4	1	2	3	4	5	6	7	8-14	
ZL 2000mg/kg	M/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3
	F/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3

Table 9: Effect of DD and on Locomotor activity

Groups	Treatment	Locomotor activity (Counts/5min)
I	Control 0.1 ml of Normal saline	384.94 ± 3.44
II	Negative control β-amyloid (25-35) peptide (10µL)	189.78 ± 4.39^{**a}
III	β-amyloid (25-35) peptide (10µL)+ ZL 200mg/kg b.wt., p.o	267.94 ± 5.50^{**b}
IV	β-amyloid (25-35) peptide (10µL)+ ZL 400mg/kg b.wt., p.o	329.44 ± 3.71^{**b}
V	β-amyloid (25-35) peptide (10µL)+ DD 200mg/kg b.wt., p.o	274.28 ± 3.42^{**b}
VI	β-amyloid (25-35) peptide (10µL)+ DD 400mg/kg b.wt., p.o	349.44 ± 4.64^{**b}
VII	β-amyloid (25-35) peptide (10µL)+ Donepezil 1.5mg/kg b.wt.,i.p	365.44 ± 2.58^{**b}

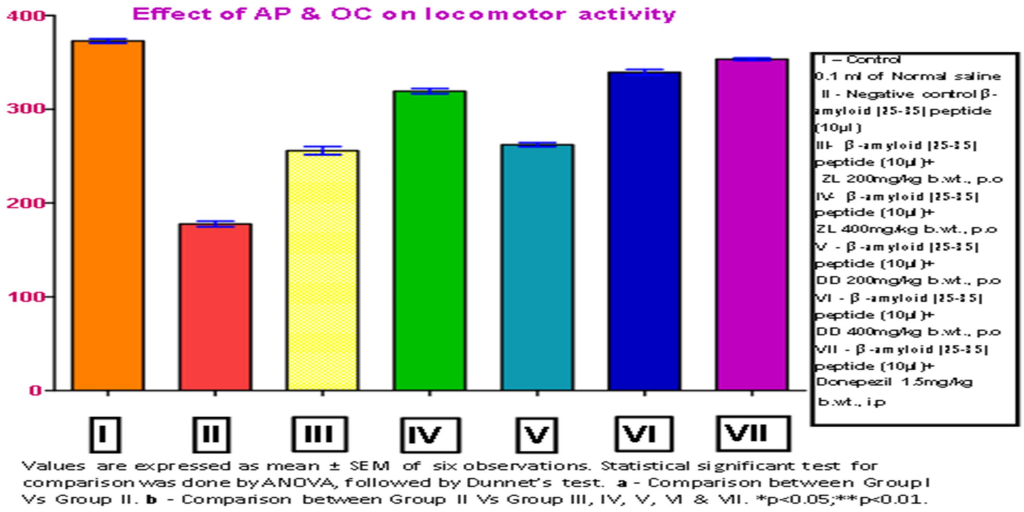

Fig 1: Effect of DD on Locomotor Activity

Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test. a - Comparison between Group I Vs Group II. b - Comparison between Group II Vs Group III, IV, V, VI& VII. *p<0.05; p<0.01**

Table 10: Effect of DD on Transfer Latency

Groups	Treatment	Transfer latency (TL)
I	Control 0.1 ml of Normal saline	93.44 ± 2.41
II	Negative control β-amyloid (25-35) peptide (10µL)	29.28 ± 2.36^{**a}
III	β-amyloid (25-35) peptide (10µL)+ ZL 200mg/kg b.wt., p.o	49.94 ± 2.90^{**b}
IV	β-amyloid (25-35) peptide (10µL)+ ZL 400mg/kg b.wt., p.o	63.49 ± 2.39^{**b}
V	β-amyloid (25-35) peptide (10µL)+ DD 200mg/kg b.wt., p.o	58.49 ± 2.27^{**b}
VI	β-amyloid (25-35) peptide (10µL)+ DD 400mg/kg b.wt., p.o	78.51 ± 0.97^{**b}
VII	β-amyloid (25-35) peptide (10µL)+ Donepezil 1.5mg/kg b.wt.,i.p	83.28 ± 0.82^{**b}

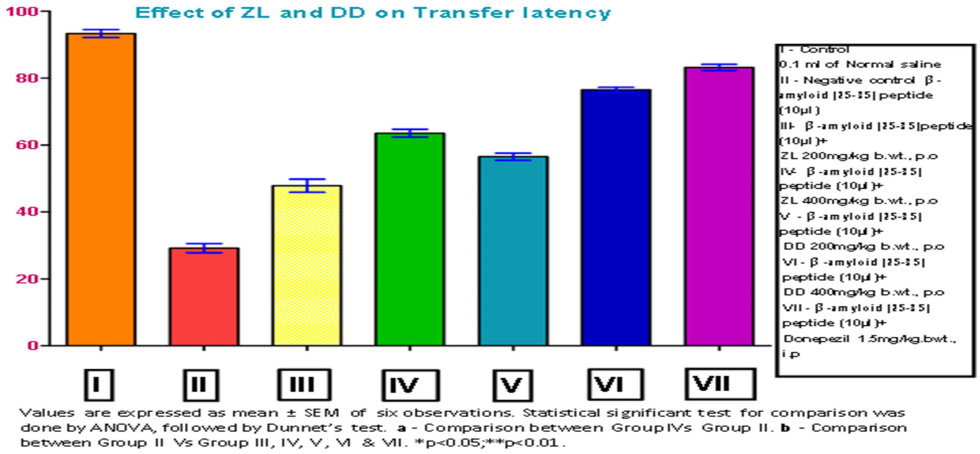

Fig 2: Effect of DD on Transfer Latency

Table 11: Effect of DD on Antioxidant & Acetylcholinesterase Enzymes

Groups	Antioxidant enzymes				AchE µmol/min/mg Protein
	SOD U/min/mg Protein	Catalase U/mg Protein	Glutathione peroxidase U/min/mg Protein	Glutathione reductase U/min/mg Protein	
I	7.73±0.25	2.30±0.05	34.73±0.57	36.73±0.63	14.57±0.43
II	2.62±0.13^{**a}	0.83±0.03^{**a}	20.47±0.63^{**a}	20.37±0.67^{**a}	21.27±0.73^{**a}
III	3.73±0.03^{**b}	1.34±0.04^{**b}	23.83±0.67^{**b}	24.63±0.53^{**b}	20.72±0.67^{**b}
IV	5.28±0.07^{**b}	1.93±0.04^{**b}	27.07±0.58^{**b}	27.32±0.35^{**b}	18.47±0.43^{**b}
V	5.12±0.05^{**b}	1.78±0.03^{**b}	25.72±0.73^{**b}	25.73±0.79^{**b}	19.85±0.45^{**b}
VI	6.71±0.06^{**b}	2.26±0.07^{**b}	28.57±0.57^{**b}	30.61±0.47^{**b}	16.26±0.41^{**b}
VII	7.37±0.05^{**b}	2.47±0.05^{**b}	32.62±0.53^{**b}	33.19±0.37^{**b}	14.73±0.35^{**b}

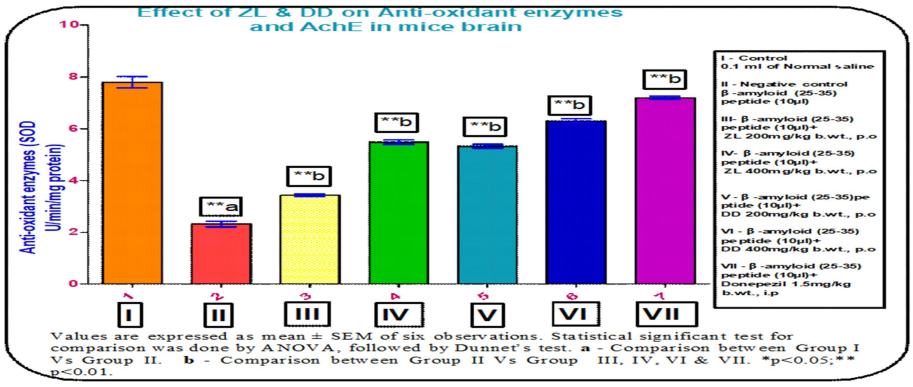

Fig 3: Effect of DD on anti-oxidant enzymes (SOD U/min/mg Protein)

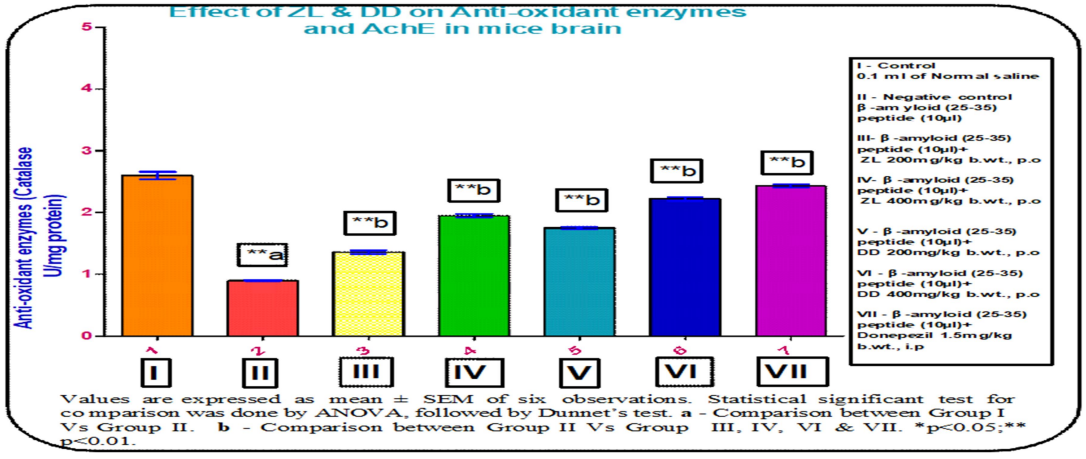

Fig 4: Effect of DD on anti-oxidant enzymes (Catalase U/mg Protein)

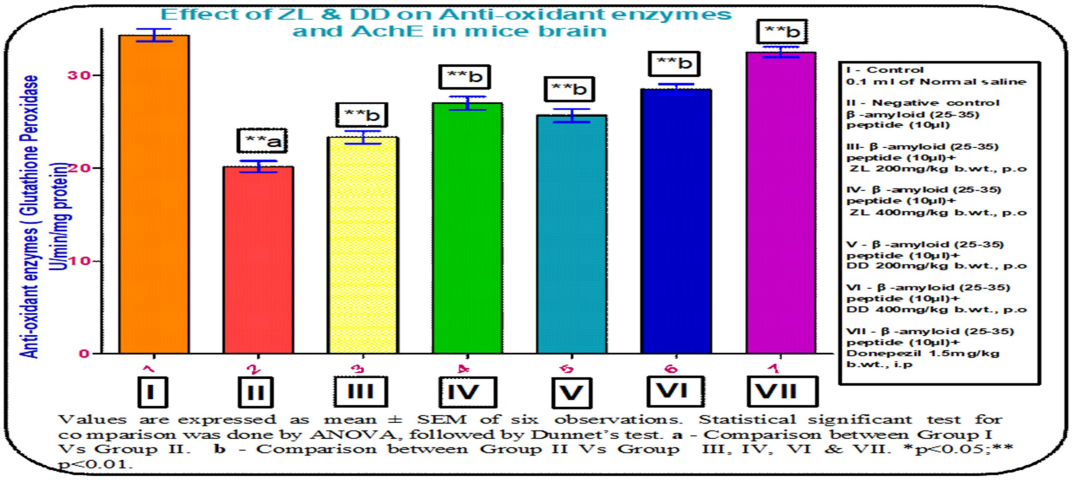

Fig 5: Effect of DD on anti-oxidant enzymes (Glutathione peroxidase U/min/mg Protein)

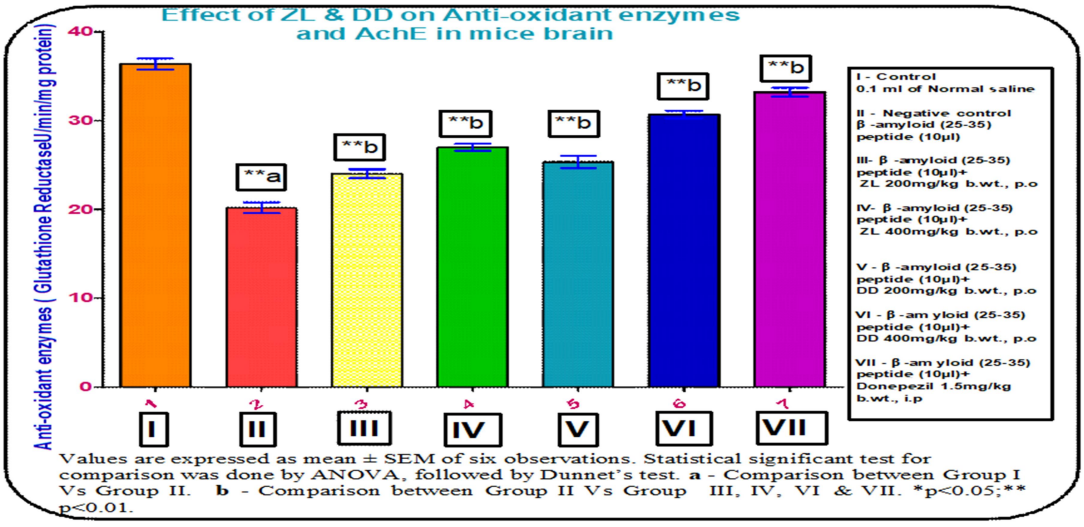

Fig 6: Effect of DD anti-oxidant enzymes (Glutathione reductase U/min/mg Protein)

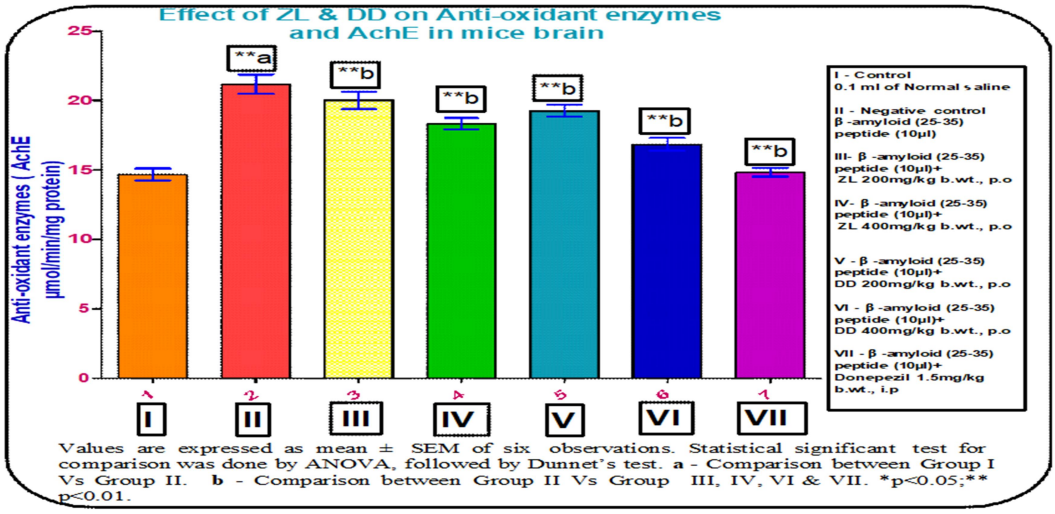

Fig 7: Effect DD on Acetylcholinesterase enzyme

SUMMARY AND CONCLUSION

The petroleum ether and ethanol leaf extracts of *Dalbergia diphaca* (DD) was studied for different *in-vitro* bioactivity evaluations anti-diabetic activity, anti-inflammatory activity, antimicrobial activity and antioxidant activity because the pathological pathway aspects of Alzheimer's disease is a very much complex which requires multiple functional drugs like anti-diabetic, anti-inflammatory, antimicrobial and antioxidant drugs for the treatment of Alzheimer's disease. The ethanol extracts of leaves of *Dalbergia diphaca* (DD) have higher anti-diabetic activity, anti-inflammatory activity, antimicrobial activity and antioxidant activity than the petroleum ether leaves extract of *Dalbergia diphaca* (DD)). The ethanol leaves extract of *Dalbergia diphaca* (DD) had not shown up any mortality or any kind of toxic symptoms on mice even at the dosage of 2000 mg/kg through oral route of administration. Hence, one-tenth (200 mg/kg) and one-fifth (400 mg/kg) dosage were chosen for the neuroprotective activity study. The neuroprotective effect of ethanol extracts of leaves of *Dalbergia diphaca* (DD) on Alzheimer's disease model caused by the β-Amyloid peptide was proved by the *in vivo* methods through behavioral studies like open field test, elevated plus maze test, water maze task and learned helplessness test. The tested doses at 200 mg/kg and 400 mg/kg of ethanolic extracts of DD showed significant neuroprotective behavioral study. These extracts bring back the declined level of brain neurotransmitters like dopamine, glutamate and antioxidant enzymes like catalase, glutathione peroxidase and glutathione reductase. Preincubation of ethanol leaves extracts of *Dalbergia diphaca* (DD) with different concentration

on human SH-SY5Y neuroblastoma cell lines produced significant neuroprotective activity against the neurotoxicity induced by 6-hydroxydopamine. The *in vitro* neuroprotective study of the ethanol leaves extracts of DD have significant neuroprotective activity against the 6-hydroxydopamine on human SH-SY5Y neuroblastoma cell line.

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