ISSN 0975-2366
DOI: https://doi.org/10.31838/ijpr/2020.12.04.687

Research Article

Neuroprotective And Anti-Alzheimer's Effects Of Plant-Zaga Latifolia

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Received: 12.10.20, Revised: 09.11.20, Accepted: 05.12.20

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 Zaga latifolia (ZL) 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 Zaga latifolia (ZL) 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, Zaga latifolia.

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 expected and will ascend to over and above sixty five million peoples gets 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 Zaga latifolia 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

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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 Zaga latifolia was filtered and collected for further process. The leaf powder of Zaga latifolia (100gm) was successively extracted by Soxhlet apparatus using the petroleum ether and ethanol solvents. The leaves of Zaga latifolia 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, and this extract is also used to isolate and identify the different phytoconstituents present in selected plants by gas chromatography-mass spectral analysis. These extracts were then subjected to prepare copper oxide nanoparticles.

RESULTS AND DISCUSSION

QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical screening of the petroleum ether and ethanol leaves extracts of Zaga latifolia (ZLPE and ZLE) by qualitative study showed the presence of phytochemical alkaloids, terpenoids, carbohydrates, proteins, phenolics, anthroquinones, flavonoids, glycosides, saponins and tannins as shown in the Table 1

Table 1: Preliminary phytochemical analysis of AP and OC

S. No	Test name	Procedure	Observation	ZLPE	ZLE
1	Alkaloids	Mayers test	Yellow color	+	+
2	Flavonoids	Lead acetate test	Yellow color	+	+
3	Carbohydrates	Molisch test	Violet ring	+	+
4	Terpenoids	Salkowski's text	Reddish brown	+	+
5	Proteins	Biuret test	Violet color	+	+
6	Saponins	Froth test	Froth making	-	+
7	Anthraquinones	Borntrager's test	Pink color	+	+
8	Tannins	Ferric chloride test	Green color	-	+
9	Steroids	Sulfuric acid test	Green color	+	+
10	Phenols	Lead acetate test	Yellow color	+	+

+ Presence

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- Absence

PHYSICOCHEMICAL ANALYSIS OF ZAGA LATIFOLIA

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 2 Zaga latifolia (ZL).

Table 2: Physicochemical analysis of leaves of ZL

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

FLUORESCENCE ANALYSIS OF ZAGA LATIFOLIA

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 3 for the leaves of Zaga latifolia (ZL).

Table 3: Fluorescence analysis of leaf powder of zaga latifolia

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

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DETERMINATION OF TOTAL PHENOLICS CONTENT ZAGA LATIFOLIA

The total phenolics content for the different leaves of Zaga latifolia (ZL) were carried out and tabulated (Mean±SD) as shown in the Table 4. The ethanol extracts of leaves of Zaga latifolia (ZL) have higher phenolics content.

Table 4: Total phenolic content of zaga latifolia

Extracted samples	ZL	
Ethanol	82.49±0.20	
Petroleum ether	64.89±0.28	

DETERMINATION OF TOTAL FLAVONOIDS CONTENT

The total flavonoids content for the different leaves of Zaga latifolia (ZL) were carried out and tabulated (Mean±SD) as shown in the Table 5. The ethanol extracts of leaves of Zaga latifolia (ZL) have higher flavonoids content than the petroleum ether leaves extracts.

Table 5.: Total flavonoid content of ZL and DD

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

DISCUSSION:

The standardization of medicinal plant is very much important to ensure the safetyand 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.

Zaga latifolia (ZL) have higher phenolic and flavonoids content than the petroleum ether leaves extracts.

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

IN VITRO BIOACTIVITY EVALUATIONS

IN VITRO ANTIOXIDANT ACTIVITY

The in vitro antioxidant activity for the petroleum ether and ethanol leaf extracts of Zaga latifolia was performed by DPPH (1, 1- diphenyl-2-picrylhydrazyl) scavenging activity method and the results are tabulated as shown in the Table 6.

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 Zaga latifolia (ZLE) has higher antioxidant activity than the petroleum ether leaves extract Zaga latifolia (ZLPE).

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Table 6: In Vitro Antioxidant Activity of ZL and DD

Extraxt samples	Zaga latifolia (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

IN VITRO ANTIDIABETIC ACTIVITY

The in vitro antidiabetic activity of Zaga latifolia and Dalbergia diphaca were performed by alpha-amylase enzyme inhibition method and the results are tabulated as shown in the Table 7. The ethanol extracts of leaves of Zaga latifolia (ZLE) have higher dose dependent antidiabetic activity than the petroleum ether leaves extract of Zaga latifolia (ZLPE).

Table 8: In Vitro Antidiabetic Activity of ZL

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	

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	1000	58.53 ± 0.4658	

Values are expressed in mean ± SEM for the three determinations

IN VITRO ANTI-INFLAMMATORY ACTIVITY

The in vitro anti-inflammatory activity for the petroleum ether and ethanol leaf extracts of Zaga latifolia and Dalbergia diphaca were performed by Human Red Blood Corpuscles membrane stabilizing method and the results are tabulated as shown in the Table 5.10.

Table 9: In Vitro Anti-inflammatory Activity of ZL

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 were assessed 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.

IN VITRO ANTIMICROBIAL ACTIVITY

The in vitro antimicrobial activity for the petroleum ether and ethanol leaf extracts of Zaga latifolia was performed by agar well diffusion method and the results of in vitro antimicrobial activity for the petroleum ether and ethanol leaf extracts of Zaga latifolia was tabulated as shown in the Table 10. Ethanolic leaves extract Zaga latifolia (ZLE) have higher antimicrobial activity than the petroleum ether leaves extract of Zaga latifolia (ZLPE

The ethanol extracts of leaves of Dalbergia diphaca (DDE) and Zaga latifolia (ZLE) have higher significant ($p<0.0001$) anti- inflammatory activity than the petroleum ether leaves extract of Dalbergia diphaca (DDPE) and Zaga latifolia (ZLPE).

Table 10: In Vitro Antimicrobial Activity of ZL

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

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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

DISCUSSION

In the current study, the petroleum ether and ethanol leaf extracts of Zaga latifolia was studied for different in-vitro bioactivity evaluations like antidiabetic activity, anti-inflammatory activity, antimicrobial activity and antioxidant activity because the pathological pathway aspects of Alzheimer's disease is very much complex which requires multiple functional drugs like antidiabetic, anti-inflammatory, antimicrobial and antioxidant drugs for the treatment of Alzheimer's disease. The ethanol extracts of leaves of Zaga latifolia (ZLE) have higher antidiabetic activity, anti-inflammatory activity, antimicrobial activity and antioxidant activity than the petroleum ether leaves extract of Zaga latifolia (ZLPE). The different in- vitro bioactivity evaluations proved that the ethanol extracts of leaves of Zaga latifolia (ZLE) have significant pharmacology activity than the petroleum ether extracts of leaves of Zaga latifolia (ZLPE).

PHARMACOLOGICAL ACTIVITY

ACUTE TOXICITY STUDIES

The acute toxicity study for the ethanol leaves extracts of Zaga latifolia (ZL) were studied and tabulated as shown in the Table 11. The ethanol leaves extract of Zaga latifolia (ZL) 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 Zaga latifolia (ZL) 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 Zaga latifolia (ZL) were chosen for the neuroprotective activity study.

Table 11: Individual mortality data of ZL 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

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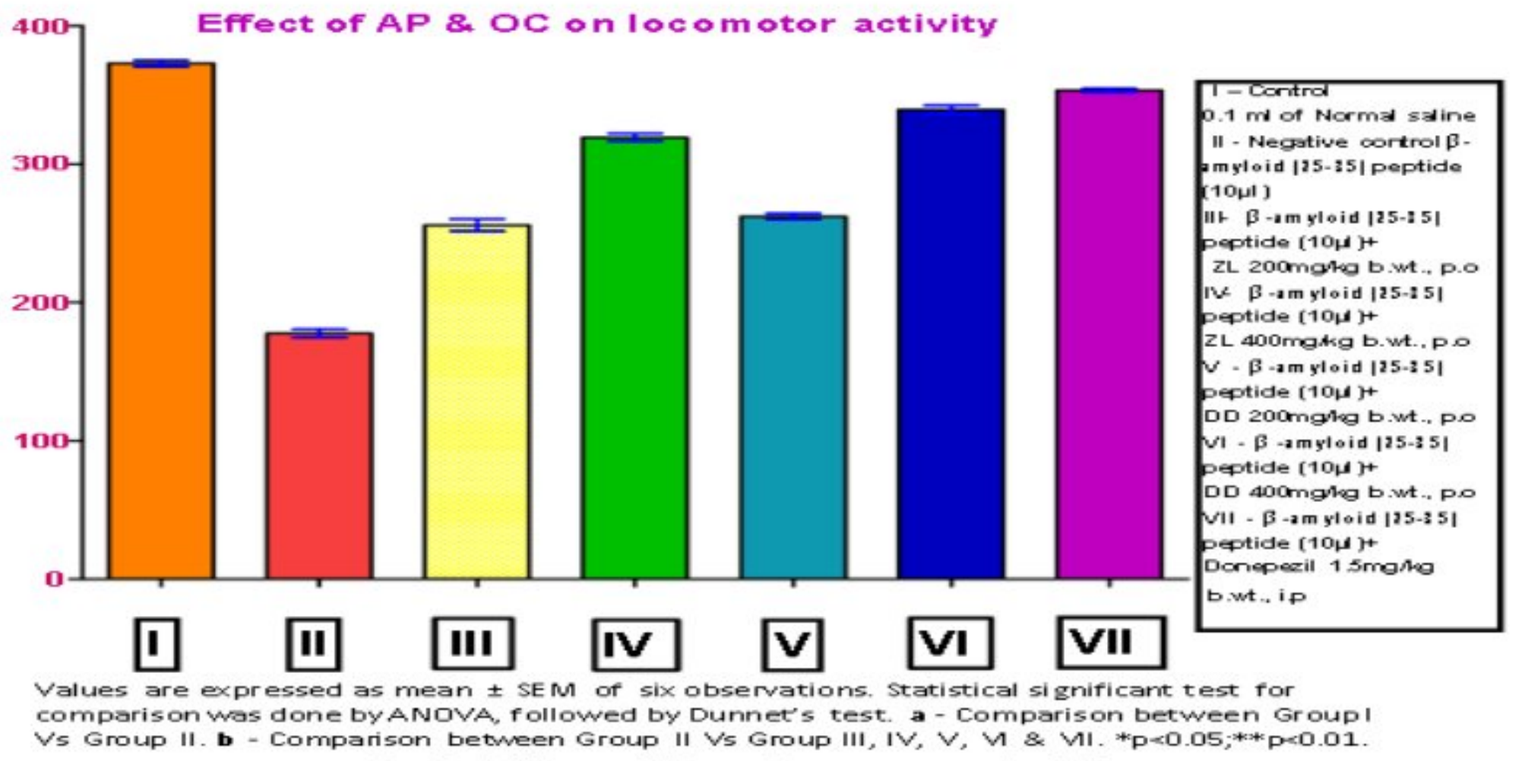
OPEN FIELD TEST

The open field test for the ethanol leaves extracts of Zaga latifolia (ZL) were studied and the results are tabulated as shown in the Table 12. 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.

Table 12: Effect of ZL 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



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.

Fig.1: Effect of ZL on Locomotor Activity

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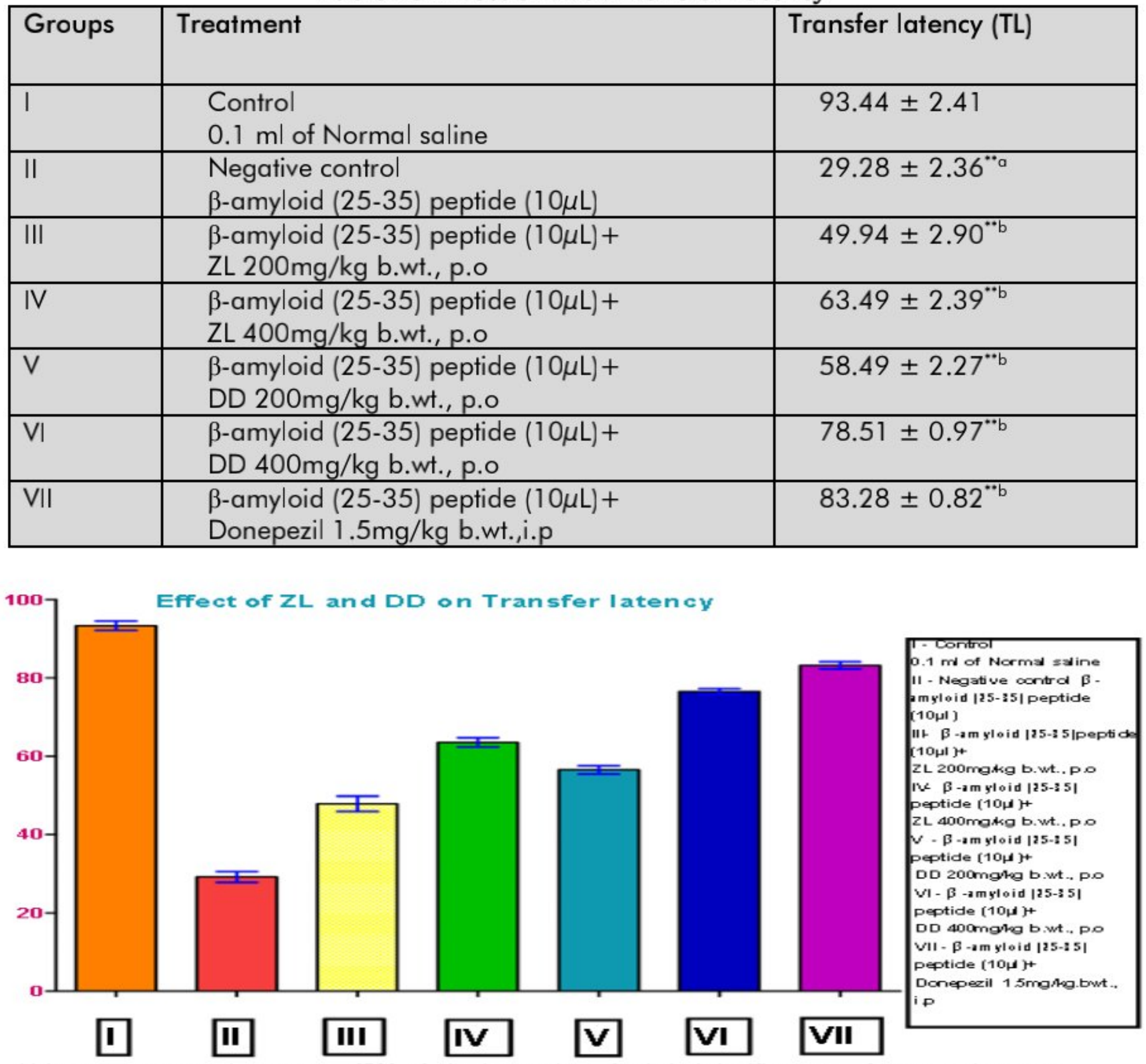
Values are expressed as mean ± SEM of six observations. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test. a - Comparison between Group I Vs Group II. b - Comparison between Group II Vs Group III, IV, V, VI& VII. *p<0.05;** p<0.01.

ELEVATED PLUS MAZE TEST

The elevated plus maze test for the ethanol leaves extracts of Zaga latifolia (ZL) were studied and the results are tabulated as shown in the Table 13. 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 Fig. 2.

Table 13: Effect of ZL 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



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 GroupIVs Group II. **b** - Comparison between Group II Vs Group III, IV, V, VI & VII. *p<0.05;**p<0.01.

Fig.2: Effect of ZL on Transfer Latency

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 Zaga latifolia (ZL) and the results are tabulated as shownin the Table .14.

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 Zaga latifolia (ZL) when compared with the other treated groups and toxic negative control group as shown in the Fig. 5.44, Fig. 5.45, Fig. 5.46, Fig 5.47 and Fig 3.

The level of antioxidant enzymes and the brain enzyme acetyl cholinesterase restored by ethanol leaves extracts of Zaga latifolia (ZL).

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Table 14: Effect of ZL and 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}

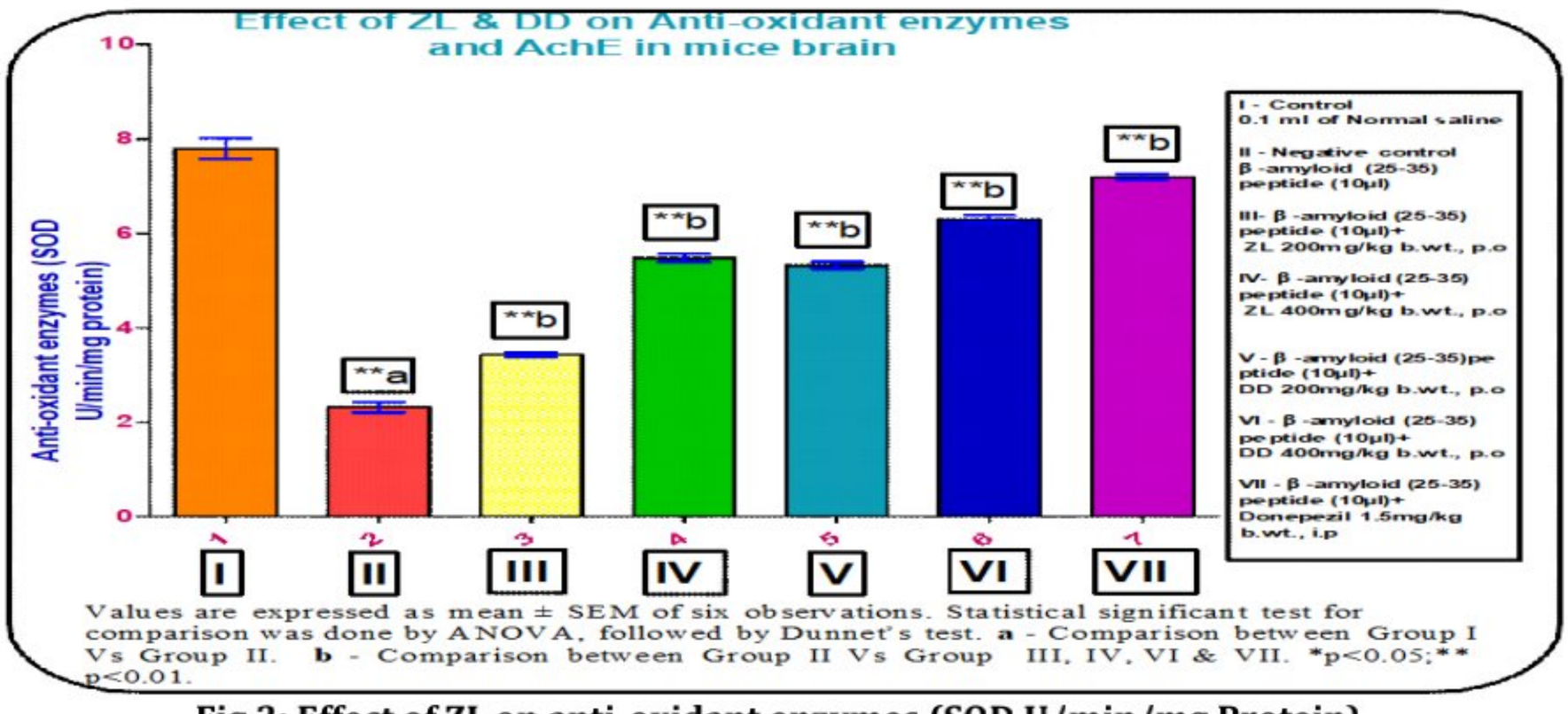

Fig.3: Effect of ZL on anti-oxidant enzymes (SOD U/min/mg Protein)

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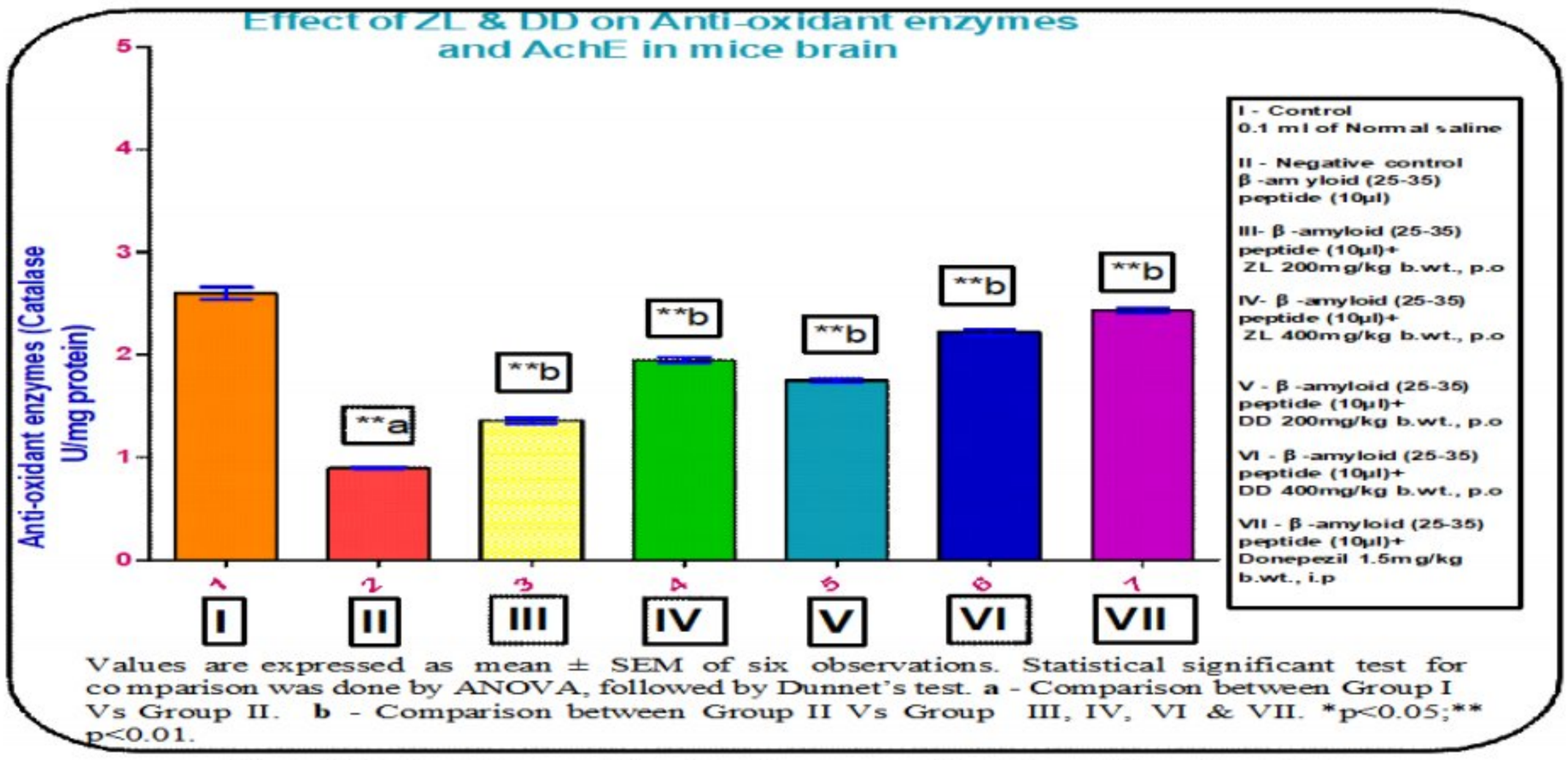

Fig.4: Effect of ZL on anti-oxidant enzymes (Catalase U/mg Protein)

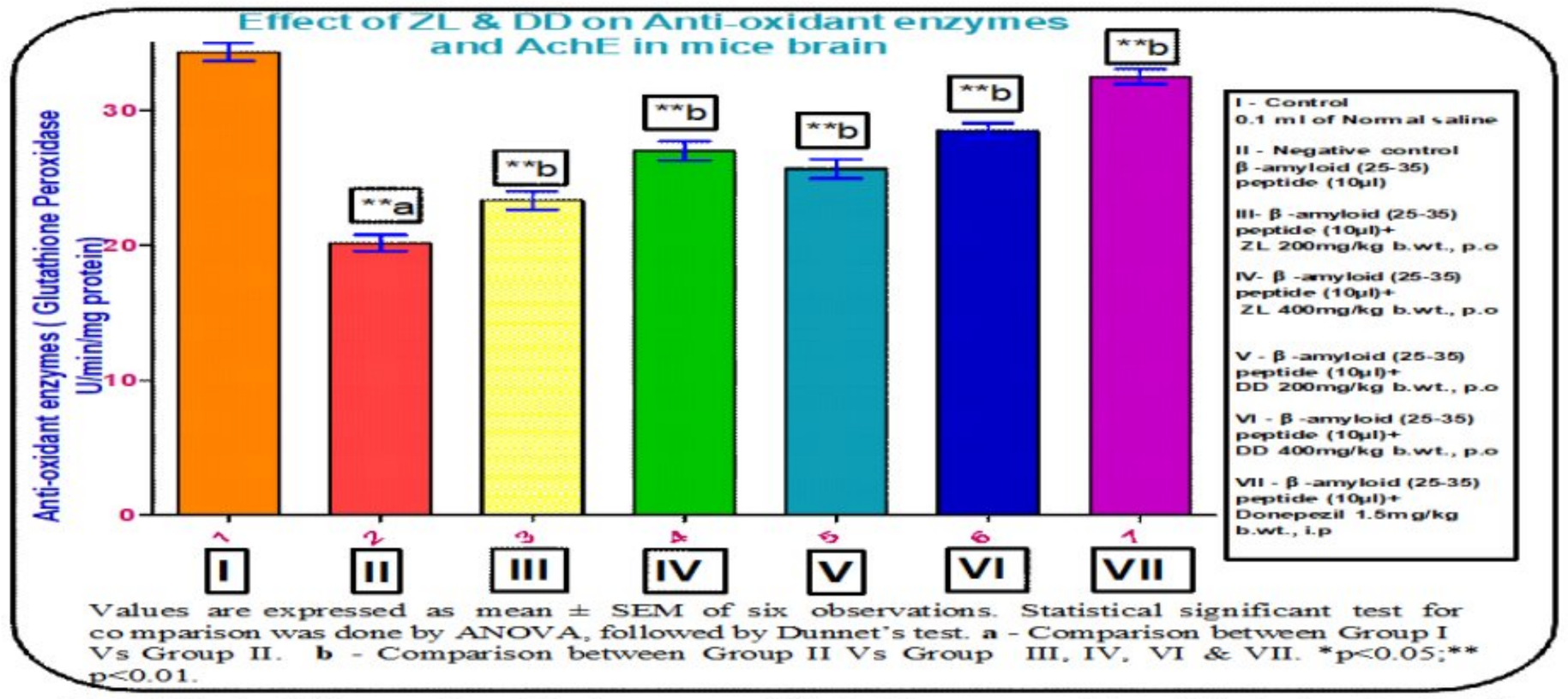

Fig.5: Effect of ZL on anti-oxidant enzymes (Glutathione peroxidase U/min/mg Protein)

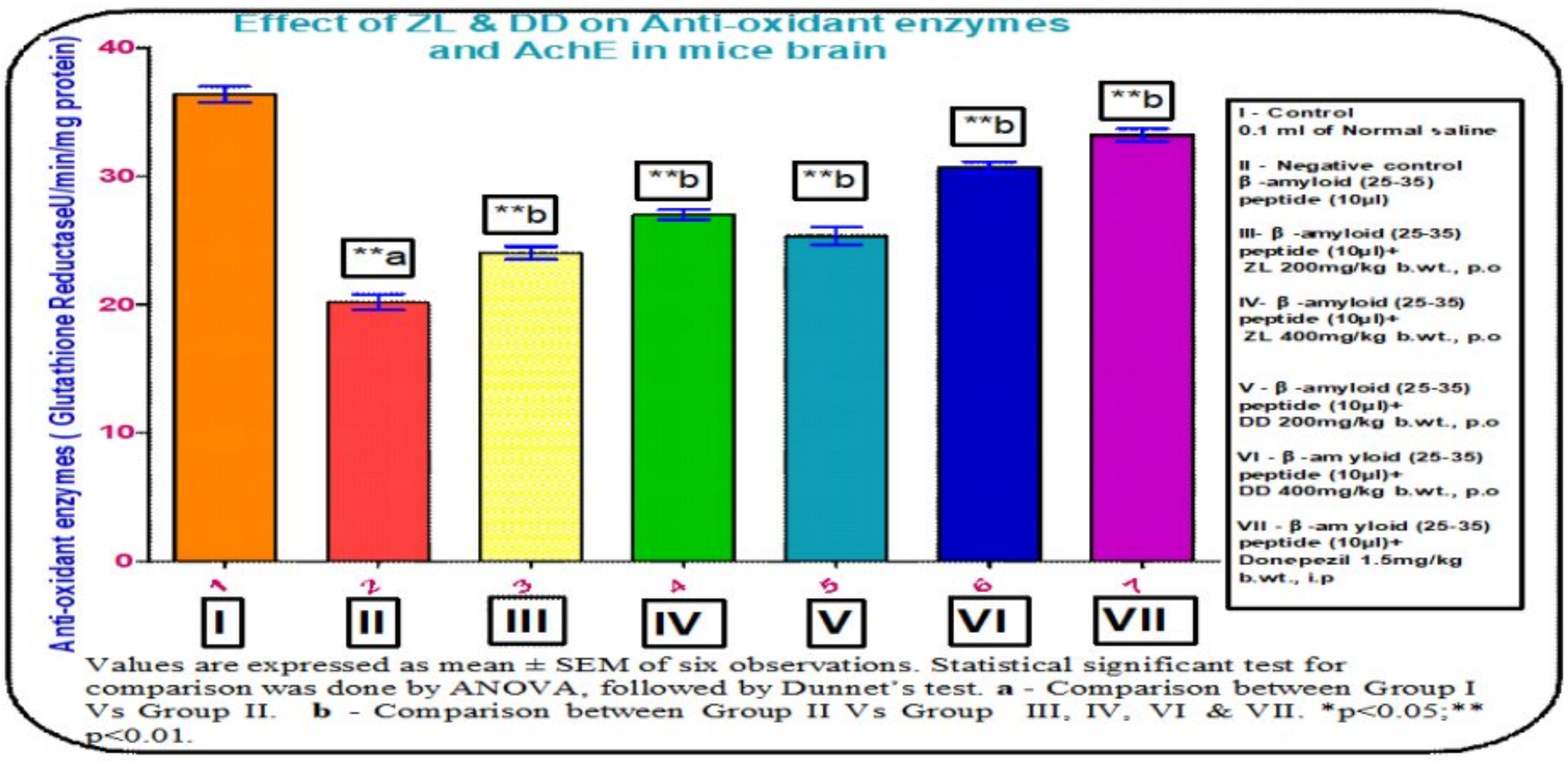

Fig.6: Effect of ZL anti-oxidant enzymes (Glutathione reductase U/min/mg Protein)

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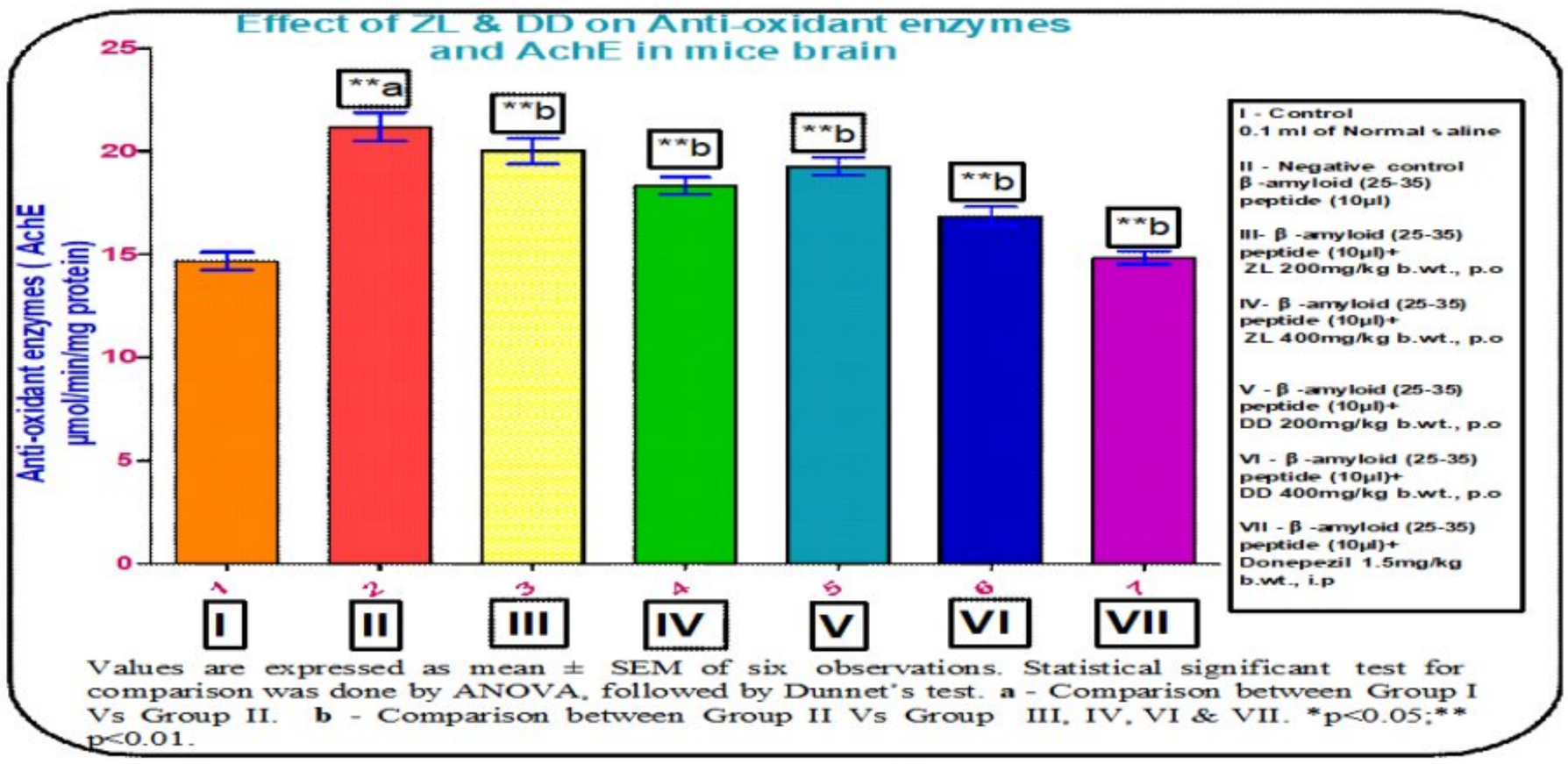

Fig.7: Effect of ZL and DD on Acetylcholinesterase enzyme

SUMMARY AND CONCLUSION

The petroleum ether and ethanol leaf extracts of Zaga latifolia 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 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 Zaga latifolia (ZLE) have higher anti-diabetic activity, anti-inflammatory activity, antimicrobial activity and antioxidant activity than the petroleum ether leaves extracts of Zaga latifolia (ZLPE). The different in-vitro bioactivity evaluations proved that the ethanol extracts of leaves of Zaga latifolia (ZLE) have significant pharmacology activity than the petroleum ether extracts of leaves of and Zaga latifolia (ZLPE).

The ethanol leaves extracts of Zaga latifolia (ZL) 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 Zaga latifolia (ZL) 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 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. Preincubation of ethanol leaves extracts of Zaga latifolia (ZL) 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 ZL have significant neuroprotective activity against the 6-hydroxydopamine on human SH-SY5Y neuroblastoma cell line.

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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}

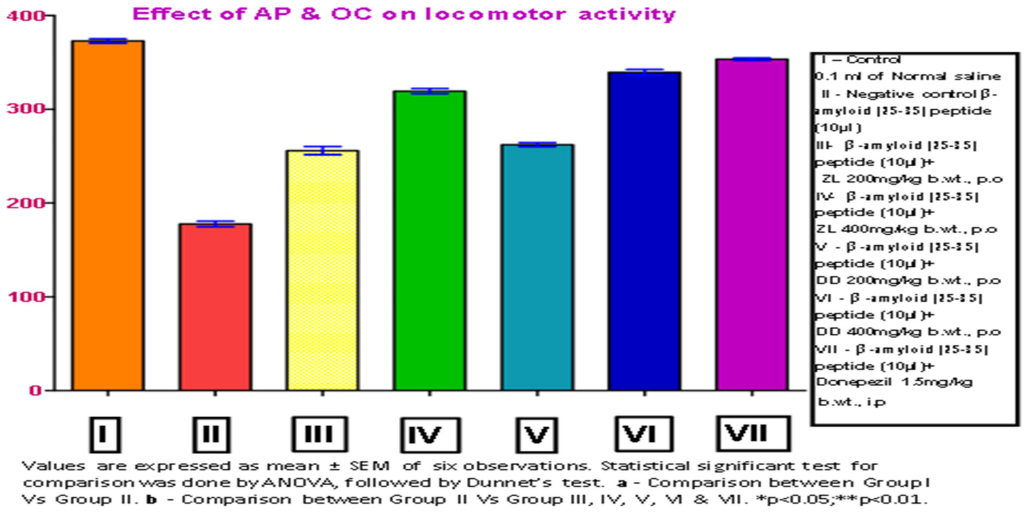

Fig 1: Effect of DD on Locomotor Activity

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}

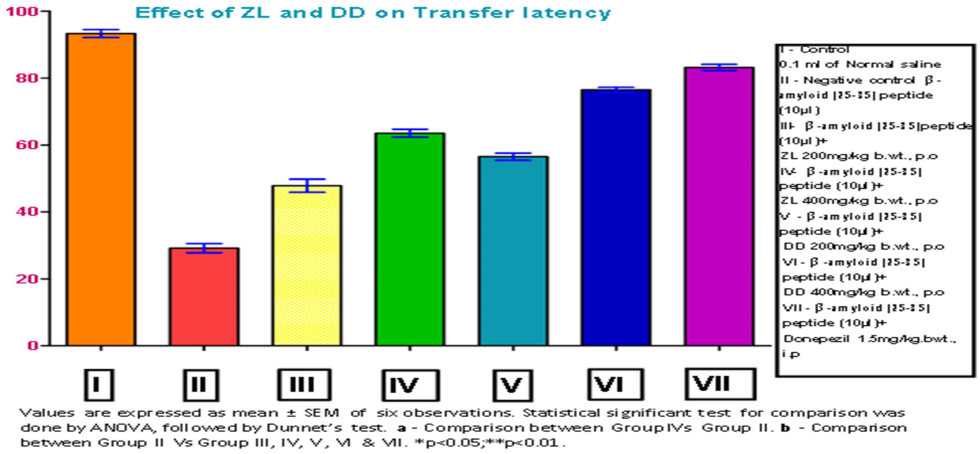

Fig 2: Effect of DD on Transfer Latency

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}

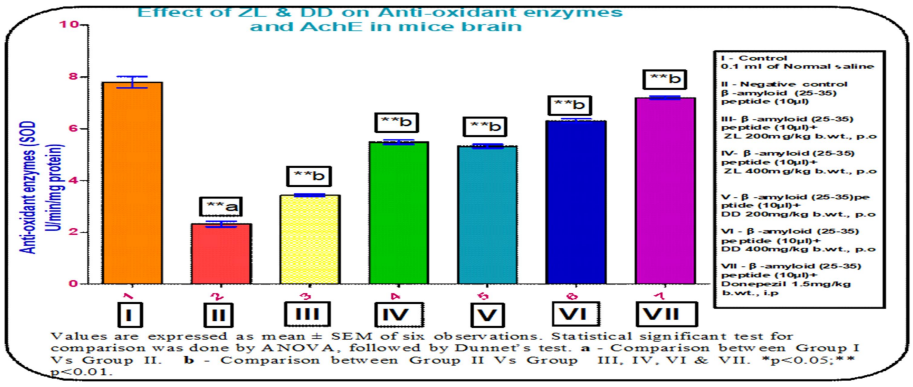

Fig 3: Effect of DD on anti-oxidant enzymes (SOD U/min/mg Protein)

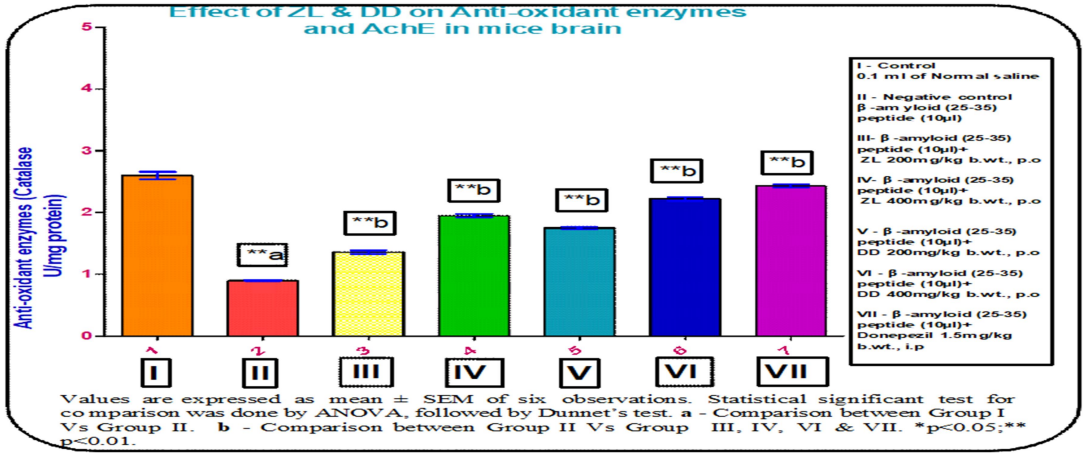

Fig 4: Effect of DD on anti-oxidant enzymes (Catalase U/mg Protein)

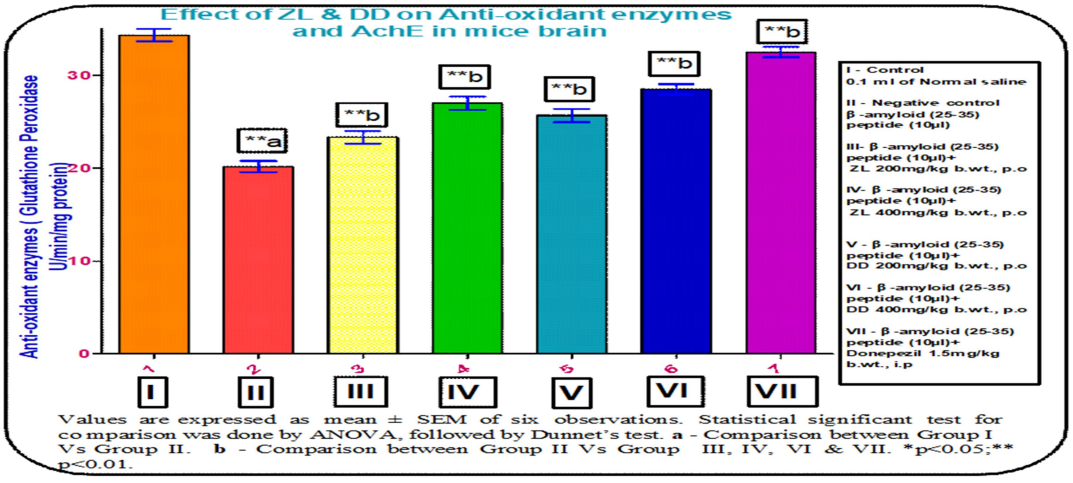

Fig 5: Effect of DD on anti-oxidant enzymes (Glutathione peroxidase U/min/mg Protein)

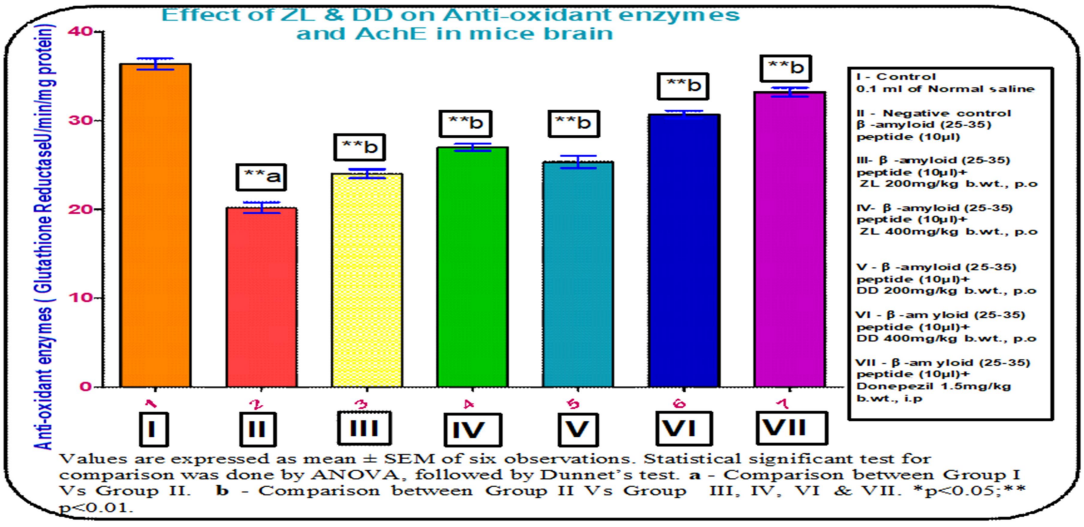

Fig 6: Effect of DD anti-oxidant enzymes (Glutathione reductase U/min/mg Protein)

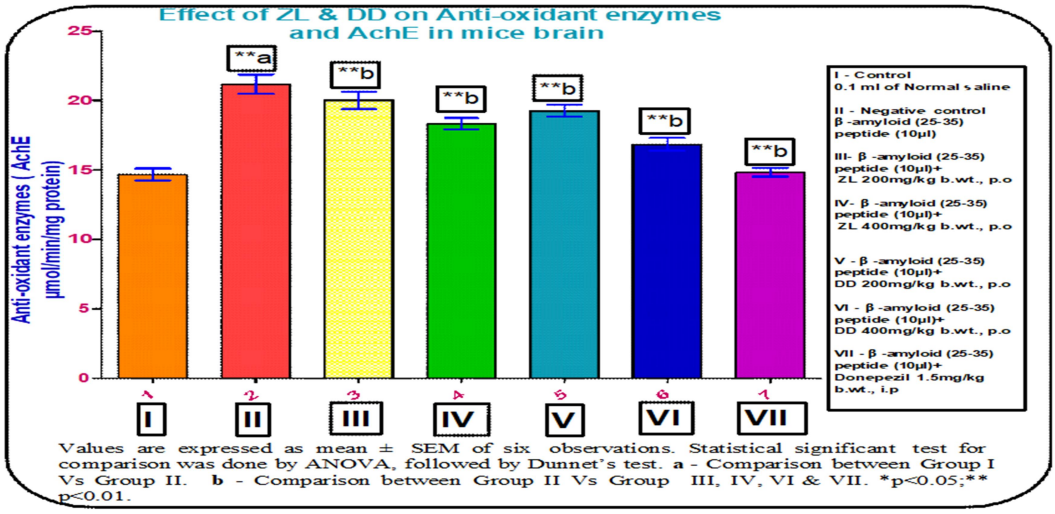

Fig 7: Effect DD on Acetylcholinesterase enzyme

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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IJBPAS, September, Special Issue, 2021, 10(9): 614-624
ISSN: 2277–4998

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

EVALUATION OF ANTICANCER ACTIVITY OF TAXILLUS TOMENTOSUS PLANT USING ALBINO MICE

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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/2021/10.9.1047

ABSTRACT

Plant based drugs are useful to treat different ailments with less side effects compare to synthetic drugs. Anticancer agents which are available in the market are having various side effects apart from their therapeutic effectiveness. For this, we have estimated the anticancer activity of *Taxillus tomentosus* against EAC (Ehrlich ascites carcinoma) induced cancer in Swiss albino mice. We have prepared the ethanolic leaf extract of *Taxillus tomentosus* (TTEE) and their ethyl acetate fraction (TTEAF) and n-hexane fraction (TTNHF). These extract and fractions were tested at 50 and 100 mg/kg body weight doses against EAC induced mice using paclitaxel (PCTXL) at 5 mg/kg dose as standard, assessed the various antitumor and hematological parameters and compared to EAC control. The test extract and fractions resulted in decreased bodyweight of animals, especially at higher dose. The test substances also exposed lowered tumor volume, viable tumour cell count and packed cell volume compared to EAC control mice. The treatments of test TTEE, TTEAF and TTNHF have enhanced the mean survival time and

percentage increased life span compared to EAC control, indicating anticancer activity. Also, the test substances returned the all-hematological parameters such as hemoglobin content, red and white blood cells, and differential leucocyte count. Hence, the test extract and fractions have shown anticancer activity against EAC induced mice which was comparable to standard drug PCTXL and TTNHF was the most promising fraction representing anticancer activity. Future research on isolation and purification of *Taxillus tomentosus* might be useful to obtain most promising agents to treat cancer.

INTRODUCTION

Medicinal plants are the source of outdated health care systems around the world. Pharmacological studies have discovered that the worth of medicinal plants acts as a potential source of biologically dynamic compounds. Phytochemical herbal formulations are a principal compound in the innovation and development of drugs. WHO clarification that more than Eighty percentage (80%) of the world's population depends on plants to meet their basic health desires **[1]**.

Cancer is primary to cause of death widespread and has become a main public health problem in developed countries. Cancer growth is a multistep process, during collect genetic abnormalities by which cells, especially in tumor suppressor genes and transforming gene, causative to uncontrolled proliferation. These abnormalities make available several enlargement advantages. Definitely, the conversion of a tumor cell from a normal cell to frequently involves mutations in the cell genome. There were 6 key changes that take place throughout the transformation to a cancer cell from a normal cell, these structures may be measured hallmarks of cancer **[2]**.

Medicines for the treatment of malignant tumors of plant derivation have been carefully studied to extend drugs for the treatment of a variety of human tumors. Medicinal plants used to treat cancer include *Acalypha fruticosa, Terminalia chebula, Catharanthus roseus, Embelia ribes,* and *Tylophora indica*. The extracts used to treat breast cancer are *Scopolendra subspinipes, Radix glycyrrhizae* and *Squama manitis*. The drugs used for treating pancreatic cancer are Emblica officinalis and Nigella sativa **[3]**.

Taxillus tomentosus is a plant belonging to the family loranthaceae. Recent research studies revealed that this plant has various pharmacological activities, such as antidiabetic, cardioprotective, hepatoprotective, anti-urolithic, neuroprotective, and antistress,

nootropic activities **[4-6]**. In the present study, we evaluated the anticancer activity of ethanolic leaf extract and, ethyl acetate and n-hexane fractions of *Taxillus tomentosus* using Ehrlich Ascites Carcinoma (EAC) inoculated mice by estimating various parameters.

MATERIALS AND METHODS

Plant material

The recent healthy, sickness-free leaves of *Taxillus tomentosus* were collected from the hills of Tirumala, Tirupati, India, and were authenticated by Dr. M. Madhava Chetty, Department of Biological Sciences, Sri Venkateshwara University, Thirupati, Andhrapradesh.

Extraction and Fractionation

The leaves of *Taxillus tomentosus* plant were shade dried for Two weeks and ground into a rough powder by using a grinder. The 100 g of powder of plant leaves were macerated for 24 hours with continuous stirring in 500 mL of ethyl alcohol employing a mover and shaker at 28^{o}C. Then, the supernatant was recovered by filtration through muslin cloth and along with Whatman paper. Further, the filtrates were totally dried by rotary vacuum evaporator. The ethanol extract were evaporated to dryness at room temperature to produce the ethanol extract **[7]**. The ethanol extract of *Taxillus tomentosus* (TTEE) were subjected to fractional process by partitioning the aqueous suspension of the drug with ethyl acetate and n-hexane to get respective fractions such as ethyl acetate fraction (TTEAF) and n-hexane fraction (TTNHF). Further, the extract and fractions were stored at -4^{o}C until further use.

Acute toxicity study

The acute toxicity studies of TTEE, TTEAF and TTNHF were conducted as per the guidelines of OECD 423 i.e., acute oral toxicity (Acute toxic class method). This acute toxicity class technique is a stepwise process with the practice of 3 female mice. The main principle of this test is constructed on a stepwise process with the use of the minimum number of animals (5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg). The constituent is directed orally to a group at one of defined doses 5mg/kg. Each step using 3 mice of single-sex (Female). Presence (or) absence of compound-related death rate of the animals dosed at 1 step will conclude the next step i.e., 50mg/kg, 300mg/kg and 2000mg/kg.

Animals

The 8 weeks old female Swiss albino mice were taken from Mahaveer Enterprises, Hyderabad, India. The mice were kept 1 week for adaptation before starting the experiments. The mice were housed in

polypropylene mice cages in a room, provide water and feed *ad libitum* with controlled temperature (25 ± 2 °C), humidity (55 ± 5%) and a 12 h light/dark cycle during the adaptation and experimental periods.

Experimental Design

The animals, weighed approximately20–25 g were chosen and randomly divided into 9 groups (*n* = 10). Group 1-Normalgroup, Group 2-EAC control, Group 3-Standard Paclitaxel drug (PCTXL) 3 mg/kg, Group 4-TTEE 50mg/kg and Group 5-TTEE 100mg/kg, Group 6-TTEAF 50mg/kg and Group 7-TTEAF 100mg/kg, Group 8-TTNHF 50mg/kg and Group 9-TTNHF 100mg/kg body weight of mice. All the test and standard drugs are injected intraperitoneally. The ascitic fluid from EAC bearing mice was withdrawn with sterile syringe and aseptically by intraperitoneal route. The normal group 1was not inoculated with tumor cells, while other groups were injected with (EAC) Ehrlich Ascites Carcinoma cells (0.2mL of 2×10^6 cells/mouse) intraperitoneally. This was taken as day 0 and the experiment was started after 24 hr. From the first day, and recorded the Bodyweight of each animal and 100 µL/mouse per day of sterile saline was directed intraperitoneally to the negative control group (EAC-bearing mice). The test drugs at doses of 50 mg/kg and 100 mg/kg were administered each day to the treated groups and the standard drug PCTXL at a dose of 5 mg/kg was administered to each animal from the positive control group by intraperitoneal route. The pharmacological treatment lasted for 9 days. 14 days after the treatment, 5 mice from each group were dissected for the study of the antitumor parameters. The remaining 5 mice from each group were reserved to check the Mean survival time (MST) of EAC tumor-bearing hosts **[7]**.

Ehrlich Ascites Carcinoma Model

The EAC cells procured from UCPSc, Kakatiya University, Warangal, were maintained and broadcasted by serial intraperitoneal transplantation of EAC cells in a germ-free environment. The EAC cells propagated for 12-14 days were used in the experiment. The tumor cell cultures for EAC were start from mouse Ehrlich Ascites with at least 1 passage *in vitro* prior to use. The ascitic fluid is withdrawn using an sterile syringe with18 gauge needle. Tumor viability was finding by trypan blue exclusion test and cells were calculated using hemocytometer. The ascitic fluid was diluted with suitable normal saline to get a concentration of $1x10^7$ cells/mL of tumor cell suspension. From this stock suspension 0.25 mL (2.5 million cells/mice)

was injected i.p to find ascitic tumor **[8]**.

Body weight of animals

The mice were weighed on the day of tumor injection and noted as 0^{th} day. Then weigh the body weight of EAC inoculated mice once in 2 days of the post inoculation period, the % increase in body weight was calculated. The formula for calculation of percentage increase in body weight was, % increase in body weight = (Animal Weight on respective day/ animal weight on day 0) -1 x 100 **[9]**.

Mean survival time (MST) and Percentage Increase in life span [%ILS]

The major parameters to evaluate the antitumor activity were mean survival time and percentage increased life span. For this purpose, total number of days a mouse survived from the day of tumor injection was counted. Subsequently the MST was calculated as Mean survival time = (Day of first death + Day of last death)/2. The %ILS was calculated as %ILS = [(Mean Survival Time of treated group/ Mean Survival Time of control group)-1]x100follows. An improvement of life span by 25% or more ended that of control was measured as current antitumor response **[10]**.

Tumor volume, Packed cell volume and Viable tumor cell count

The parameters like tumor volume, viable cell count and packed cell volume were estimated to assess the anticancer activity in EAC injected mice. On 14 day of the tumor inoculation, the animals were dissected and the peritoneal ascitic fluid was taken into a measuring cylinder and the volume of the ascitic fluid was measured and also measure the packed cell volume and compared in all the treated and EAC control groups **[11]**. The number of viable and non-viable cells present in ascetic fluid in all the groups were measured by dye exclusion method using 0.4% Trypan Blue solution **[12]**.

Hematological parameters

In order to detect the influence of extract and fractions on the hematological status of EAC bearing mice, the parameters such as white blood cell count, red blood cell count hemoglobin content and differential leukocyte count such as lymphocytes, neutrophils and monocytes. For this, on the 14^{th} day of tumor inoculation, the blood was withdrawn from animals in each group by retro-orbital puncture and different haematological parameters were estimated.

Statistical analysis

The statistical analysis was executed by one-way ANOVA subsequently bonferroni posttests. The data was represented as mean ± standard deviation (S.D.) of 3 independent experiments. Test significance was designated by *$p<0.01$ and **$p<0.001$ compared to EAC control.

RESULTS AND DISCUSSION

The present investigation assesses the anticancer activity of *Taxillus tomentosus* ethanolic extract (TTEE) and their ethyl acetate fraction (TTEAF) and n-hexane fraction (TTNHF) at the doses of 50 and 100 mg/kg body weight using EAC bearing mice by various antitumour and hematological parameters.

Acute toxicity study

Acute toxicity study was performed according to the OECD guide line 423 using 3 female mice per step. Mice were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and up to 72 hours. All the changes were systematically recorded with individual records being maintained for each mouse. Observations included changes in skin, mortality and general behavioral pattern. There was no death was observed till the end of study till the 2000 mg/kg dose for all the 3 test substances. The same was reported with the ethanolic extract of *Taxillus tomentosus* **[5]**. So, the 2 doses were selected for the anticancer activity studies viz., 50 and 100 mg/kg body weight.

Body weight

The percentage increased body weight of animals were calculated on 1, 3, 5 and 7^{th} days compared to 0^{th} day of post treatment in EAC control, PCTXL and test drugs. The observations were shown in the Table 1. All the three test substances have shown considerable reduced body weight of animals. Among the three test substances, TTAEF at 100 mg/kg dose have shown significant reduced body weight ($p<0.001$), where as TTNHF at both 50 and 100 mg/kg doses have shown significant decrease in body weight after 7 days ($p<0.001$) which are comparable to standard drug PCTXL. The ethanol extract of *Sargassum tenerrimum* have also shown the decreased body weight of EAC bearing mice should contribute the anticancer activity **[11]**.

Mean survival time (MST) and Percentage Increase in life span (%ILS)

The important parameters to anticancer activity *in vivo* were mean survival time and percentage increased life span (Sridhar *et al*, 2012). The calculated values of MST and %ILS were shown in the **Table 2**. The TTEE and TTEAF were significantly ($p<0.01$) increases the MST at 100 mg/kg dose whereas TTNHF was increased MST significantly at both doses 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$) which was almost same as with standard PCTXL. Similarly, TTEE and TTEAF increased the %ILS significantly at 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$). The TTNHF have shown

enormous increase in %ILS at both doses significaly ($p<0.001$). The PCTXL have also shown in the similar way as in case of TTNHF. The antitumour activity of ethanol extract of *Bauhinia variegata* has been evaluated up on oral administration against EAC inoculated albino mice. A significant enhancement of MST in EAC bearing mice was found with respect to the EAC control group. The test treatment was found to enhance % ILS after 14 days of injection compared to EAC control group. The ethanolic extract of *Bauhinia variegata* was originate to be a potent cytotoxic towards EAC tumor cells **[13]**.

Tumour volume, Packed cell volume and Viable tumour cell count

The results of tumour volume, viable tumour cell count and packed cell volume were shown in Table 3. The tumour volume and packed cell volume were reduced with the treatment of test extract and fractions. The treatment of TTEE and TTEAF at both doses have shown lowered tumour volume and packed cell volume significantly ($p<0.01$) compared to EAC control. The treatment of TTNHF at 50 mg/kg ($p<0.01$) and 100 mg/kg ($p<0.001$) doses lowered the tumour volume and packed cell volume when compare to EAC control group which was similar to PCTXL. The treatment of TTEAF at 100 mg/kg and TTNHF at both doses decreases the viable tumour cell count when compare to EAC control group. Among three test extract and fractions, the TTNHF have shown promising decreased activity towards all three tested parameters. The methanolic extract of *Argyreia nervosa* has been tested against EAC induced liquid tumor in mice. Significant and dose-dependant results were detected when the mice are dissected on 15th day for estimation of tumor proliferation, biochemical and hematological parameters. The extract also showed a decrease in tumor volume, packed cell volume and viable tumour cell count accounts for anticancer activity supports our study **[14]**.

Hematological parameters

Different hematological parameters such as hemoglobin, RBC, WBC, lymphocytes, neutrophils and monocytes were estimated and presented in Table 4. Compared to normal group animals, the EAC control group animals have shown abnormal changes in all the hematological parameters tested. The TTEE, TTEAF and TTNHF treated animals have brought back the values of hematological parameters to near normal values, especially at higher dose i.e., 100 mg/kg. Among these three tested substances, the TTEAF and TTNHF have shown promising results in brought back to the normal hematological parameters levels which were comparable to the standard drug

PCTXL treatment. The antitumor activity of methanol extract of *Lactuca serriola* was evaluated against EAC induced Swiss albino mice at 100 mg/kg and 200 mg/kg. Up on administration of the extract of plant, improvement in the hematological parameters such as hemoglobin content, RBC and WBC count and differential cell count and restored altered hematological parameters. It can be decided that the methanol extract of *Lactuca serriola* keeps significant antitumor activity **[15]**.

Table 1: Effect of different doses of *Taxillus tomentosus* extract and fractions on percentage increase in Body Weight in EAC inoculated mice

Treatment	Dose (mg/kg)	% Increase in Body weight as compared to '0th' day			
		Day 1	Day 3	Day 5	Day 7
EAC Control	Vehicle	1.27±0.84	3.50 ± 0.81	7.01 ± 0.63	24.22 ± 1.70
PCTXL	5	1.59±1.01	2.91 ± 2.29	5.47 ± 2.01	.7.21 ± 3.00**
TTEE	50	1.35±0.3	3.4 ± 0.8	5.32 ± 0.8	19.43 ± 0.78*
	100	1.41±0.76	3.12 ± 1.27	5.14 ± 2.67	16.23 ± 3.71*
TTEAF	50	1.45± 1.56	2.63 ± 1.62	5.26 ± 2.02	17.73 ± 4.10*
	100	1.25±0.17	2.69 ± 1.54	4.47± 2.68*	11.02 ± 3.81**
TTNHF	50	1.26±0.86	3.11 ± 1.13	5.15 ± 0.60	12.36 ± 1.24**
	100	1.77±0.75	3.03 ± 1.50	4.97 ± 1.32*	9.73 ± 2.50**

All the values are mean ± S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

Table 2: Effect of different doses of *Taxillus tomentosus* extract and fractions on (MST) Mean survival time and (%ILS) percentage increased life span in EAC inoculated mice

Treatment	Dose (mg/kg)	MST	% ILS
EAC Control	Vehicle	15.67±0.76	-
PCTXL	5	45.00±3.21**	187.17 ±14.75**
TTEE	50	26.00 ±2.29	65.92 ±14.44*
	100	30.17 ±2.08*	92.53 ±10.79**
TTEAF	50	28.17 ±2.35	79.77 ±13.53*
	100	31.33 ±2.48*	99.93 ±13.45**
TTNHF	50	32.83 ±3.08*	109.50 ±14.38**
	100	36.67 ±3.63**	134.01 ±11.13**

All the values are mean±S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

Table 3: Effect of different doses of *Taxillus tomentosus* extract and fractions on tumour volume, packed cell volume and viable cell count in EAC inoculated mice

Treatment	Dose (mg/kg)	Tumour Volume	Packed Cell Volume (mm)	Viable cell count (x 10^7 cells/ml)
EAC Control	Vehicle	16.65 ± 1.21	3.23 ± 0.03	7.33 ± 0.61
PCTXL	5	4.35 ± 0.45**	0.26 ± 0.01**	0.51 ± 0.08**
TTEE	50	10.92 ± 1.01*	2.12 ± 0.09	5.52 ± 0.21
	100	8.53 ± 0.79*	1.23 ± 0.06*	4.13 ± 0.18*
TTEAF	50	9.87 ± 0.83*	1.97 ± 0.08	4.17 ± 0.28*
	100	6.93 ± 0.49*	0.73 ± 0.05*	2.54 ± 0.16**
TTNHF	50	7.53 ± 0.68*	1.13 ± 0.06*	3.43 ± 0.11**
	100	4.85 ± 0.36**	0.45 ± 0.04**	1.58 ± 0.12**

All the values are mean ± S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

Table 4: Effect of different doses of *Taxillus tomentosus* extract and fractions on Hematological parameters in EAC inoculated mice

Treatment	Dose (mg/kg)	Hematological parameters					
		Haemoglobin (gm%)	RBC(million /mm^3)	WBC (10^3cells/mm^3)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
Normal Control	--	14.10 ± 0.86	4.62 ± 0.38	7.86 ± 0.12	65 ± 3.31	28 ± 1.96	1 ± 0.31
EAC Control	Vehicle	5.35 ± 0.55	1.94 ± 0.08	23.60 ± 1.84	21 ± 0.67	69 ± 4.53	2.61 ± 0.91
PCTXL	5	12.31 ± 1.12**	4.23 ± 0.37**	9.92 ± 0.83**	61 ± 0.52**	32 ± 2.24**	1.32 ± 0.13**
TTEE	50	6.96 ± 0.71	2.43 ± 0.24	20.02 ± 1.73	38 ± 3.14	54 ± 5.13	2.08 ± 0.21
	100	8.36 ± 0.62*	3.27 ± 0.29*	16.38 ± 1.31*	45 ± 4.12*	45 ± 4.08*	1.75 ± 0.16*
TTEAF	50	8.44 ± 0.82*	3.17 ± 0.27*	17.74 ± 1.52*	42 ± 3.87*	48 ± 4.24*	1.95 ± 0.16*
	100	10.75 ± 1.22**	3.82 ± 0.36**	13.42 ± 1.26**	51 ± 4.76**	41 ± 3.82**	1.52 ± 0.15*
TTNHF	50	9.75 ± 1.11*	3.54 ± 0.33**	15.38 ± 1.34**	48 ± 4.24*	45 ± 4.05**	1.76 ± 0.14*
	100	11.21 ± 1.34**	4.05 ± 0.41**	10.13 ± 1.02**	59 ± 4.12**	36 ± 3.15**	1.39 ± 0.12**

All the values are mean ± S.D. of six samples, *p<0.01 and **p<0.001 compared to EAC control

CONCLUSION

The TTEE, TTEAF and TTNHF were administered intraperitoneally at 50 mg/kg and 100 mg/kg to EAC inoculated mice and assessed the different antitumour and hematological parameters. The results have clearly described that the treatment of test extract and fractions were significantly decreases the body weight, tumour volume, packed cell volume and viable tumour cell count compared to EAC control group. And also the MST and %ILS were enhanced significanltly up on the treatment of test extract and fractions against EAC control. All the hematological parameters were also brought back to near normal values and almost restored. In conclusion, the test extract and fractions showing anticancer activity against EAC induced mice. Among the three test substances, the TTNHF showed highest anticancer activity followed by TTEAF and TTEE, which were comparable to the standard PCTXL.

ACKNOWLEDGEMENTS

The first author is grateful to SJJT University, churela, Jhunjhunu, Rajasthan.

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ADVANCEMENTS IN MAGNETIC MICROSPHERES AS TARGETED DRUG DELIVERY VEHICLES: A COMPREHENSIVE REVIEW

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Abstract- Currently, non-uniform external magnetic fields generated by rare-earth permanent magnets or electromagnets are used to target tumors in vivo with magnetic microspheres. Magnetic embolization therapy, which uses magnetic particles containing anticancer agents like chemotherapeutic drugs or therapeutic radioisotopes, is one application for this method. One approach to local or regional antitumor treatment is drug targeting. One possibility for drug targeting is magnetically controlled drug targeting. This technology works by binding an anticancer drug to ferrofluids, which then use magnetic fields to concentrate the drug in the area of interest (the tumor site). The creation of a drug delivery system with a magnetic target has received a lot of attention. These medication conveyance frameworks plans to convey the medication at a rate coordinated by the necessities of the body during the time of treatment, and focus on the movement element to the site of activity. In addition to discussing the mechanism of magnetic targeted delivery, drug release rate in vitro, as well as the advantages and disadvantages of magnetic targeting, this paper provides an overview of the current application of magnetic microspheres (ferrofluid) in conjunction with magnetic fields to the most recent advancements in medical application, particularly to anticancer therapy.
Keywords: Cholangiocarcinoma, Ferromagnetic, Magnetic microspheres, drug targeting using magnetic fields.

1 INTRODUCTION

One of the most exciting challenges in medicine is precisely delivering drugs to the desired target sites with minimal side effects. One approach to accomplishing such focusing of medications is by the utilization of attractive microspheres in mix with an outer attractive field. Microspheres are free-flowing powders that can be injected with an 18 or 20-needle needle and are composed of encapsulated, spherical drugs of ideally less than 125p. Microspheres with a magnetic substance inside that can be easily targeted by applying a magnetic field from the outside.

In order to improve target site specificity and reduce renal clearance, magnetic microspheres were created. They are capable of trapping a wide range of drugs. The treatment of localized tumors in areas with well-defined blood supply holds great promise for this system. Each particle is essentially a drug matrix embedded in a polymer, from which a first-order process causes the drug to be released.

They can be made from a wide range of carriers. Serum albumin from humans or other appropriate species is one of the most frequently used. Various stabilization techniques, which typically involve chemical or thermal cross-linking of the protein carrier matrix, can maintain or control drug release from albumin microspheres. Polyacryl, polylactide, polyglycoside, and other biocompatible and biodegradable polymers are used.

In the ideal scenario, magnetic microspheres would be injected into a specific artery. As the microspheres would

be specifically and attractively limited at the fine level, they would have free stream access through the enormous corridors. As a result, when placed in the desired location, the microspheres would function as a time-release capsule system.

The particular slender restriction of the microspheres can be accomplished by exploiting the physiological distinction in the straight stream speed of blood at the fine level (0.05 cm/sec). Clearly, a much lower attractive field strength is important to limit the microspheres at the more slow moving stream speeds of blood in vessels. The microspheres remained at the target site even after the magnetic field was removed, possibly because they had entered the interstitial space through the vascular endothelium and been retained there.

Drug targeting is the process of delivering drugs exclusively to receptors, organs, or any other particular part of the body. Despite their poor site specificity and rapid clearance by the RES (reticuloendothelial system), magnetic microspheres are successfully utilized for drug targeting. These magnetic microspheres will then be able to be captured in the tumor by applying an external non-uniform magnetic field. However, these treatments have been associated with severe complications. As a result, one of the most active areas of cancer research at the moment is the development of methods that could selectively deliver drug molecules to the diseased site without simultaneously increasing their level in healthy tissues. The fundamentals of drug targeting, with an emphasis on magnetically controlled anticancer chemotherapy, are the focus of this overview.

2 IMPORTANT CHARACTERISTICS OF MICROSPHERES AND MAGNETIC MICROSPHERES ARE

- The degree of drug entrapment can be affected by the size of a drug carrier's particles.
- The hydration-induced increase in albumin microsphere size may alter the distribution of the protein in the body.
- Using microspheres that are less than one micron in size reduces the risk of pulmonary embolism because these microspheres frequently come into contact with particles larger than seven microns or with particles that clump together during in vivo administration.
- The carrier's magnetic content and the magnitude of the applied magnetic field influence the retention of magnetic microspheres at the target location.
- Despite the fact that a high magnetic content makes it possible to employ smaller magnetic fields, it reduces the effective space within the carrier that is available for drug entrapment.
- When employing MM for targeting, the carrier's magnetic content and the magnitude of the applied magnetic field are crucial.
- The field gradients were calculated after gaussmeter measurements of the magnetic fields.

2.1 Factors Regulating Drug Release from Microspheres

- The size of the microspheres, drug content, magnetic content, Hydration State, and drug release characteristics of the carrier all influence the amount bind rate or drug delivery via magnetically responsive microspheres.
- Each factor is connected to another. Size determines the amount of drug. The solubility properties of the drug and how it was prepared determine the drug content.
- The distribution of magnetic microspheres throughout the body is affected by their hydration state. The retention of microspheres at the target site is governed by the applied

magnetic field's magnitude and magnetic content.

- In microspheres with high attractive substance, the outer attractive field strength required is less, yet on the off chance that high attractive substance is available than the space for drug accessible is less and consequently the size of attractive substance and medication ought to be carefully adjusted to have viable restorative framework.

2.2 Benefits of Magnetic Microspheres

- Because magnetic microspheres are site-specific, the issue of their rapid clearance by RES can also be resolved by locating them in the intended area.
- Because the linear blood velocity of capillaries is 300 times lower than that of arteries, a significantly smaller magnetic field is sufficient to keep them in the target area's capillary network.
- Controlled release within the target tissue for 30 minutes to 30 hours, avoiding acute toxicity to endothelium and normal parenchymal cells. Adaptable to any body part, as desired.
- In the case of targeting a tumor, the microsphere's significantly higher phagocytic activity compared to that of normal cells makes it possible for tumor cells to internalize it.
- The issue of drug resistance that arises from drugs' inability to cross the cell membrane can be resolved.

3 PREPARATION METHOD

Phase separation emulsion polymerization (PSEP) and continuous solvent evaporation (CSE) are the two main methods for making magnetic microspheres. For lipophilic drugs, these microspheres are made by mixing water-soluble drugs and 10 nm magnetite (Fe3O4) particles in an aqueous matrix material. They are about 1.0 m in size, making them small enough to inject intravenously without occluding the micro vascular. These microspheres do not have any side effects and do not react with blood components. They can be balanced out by warming or synthetically cross connecting egg whites to accomplish a wide range of medication discharge energy. These are injected into a target site-supplying artery. A magnet of adequate field strength is then positioned remotely over the objective region to limit the microspheres at the slender bed around here. In most cases, a stronger field is required to localize microspheres in a fast-moving arterial system. Preparing microspheres is typically accomplished through one of two methods.

4 STORAGE

Because freezing is likely to result in aggregation that is impossible to undo, microsphere suspensions should not be frozen. To prevent the growth of microorganisms, cold storage at temperatures between 0 and 8 °C is recommended for microspheres. Most as-provided 'standard' (non-protein covered) microsphere suspensions don't contain an antimicrobial specialist. It is suggested that every suspension be handled in an aseptic manner. To keep microspheres in suspension without producing foam (foam may cause particle loss through bead entrapment), continuous rolling (e.g., 3-5 rpm on a cell culture roller) is recommended whenever possible. Particles should be thoroughly resuspended prior to use if continuous rolling is not possible. According to our experience, rolling at a higher speed (30-60 rpm for 2-4 hours) is effective in resuspending settled material. Again, the purpose of the rolling speed is to effectively resuspend the beads without producing foam.

5 CONCLUSION

In a variety of scientific fields, magnetic vesicular systems have been realized as extremely useful microsphere systems. Due to their superior tumor targeting, magnetic microspheres have been the subject of research into targeted drug delivery over the years, particularly magnetic targeted chemotherapy. An efficient strategy for assisting the drug molecule in reaching the desired location is targeted drug delivery. The primary benefit of this method is the reduction in drug dose and adverse effects. The magnetic targeted chemotherapy is less toxic, more effective, and better at targeting tumors. Despite some drawbacks, such as the ferrofluid's need for a strong magnetic field and the deposition of magnetite, magnetic microcarriers still play a significant role in the selective targeting and controlled drug delivery of a variety of drugs. Since this is a difficult area for future drug targeting research, further studies, long-term toxicity studies, and characterizations will guarantee the advancement of the magnetic drug delivery system. Magnetic microspheres hold a lot of promise for the future, and with more research, they will be developed into an innovative and effective method for targeted drug delivery systems.

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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF HPTLC FOR THE SIMULTANEOUS DETERMINATION OF LOPINAVIR AND RITONAVIR IN A TABLET DOSAGE FORM

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Abstract- The HPTLC method was used to develop and validate simultaneous tablet quantification of Lopinavir and Ritonavir. A Chloroform mobile phase was used to create the chromatograms: 1, 4, and dioxane (7:3 %v/v) were measured by densitometric absorbance mode at 210 nm on a silica gel GF aluminum TLC plate that had been pre-coated. Llopinavir and ritonavir had Rf values of 0.74 and 0.58, respectively. Lopinavir's linearity was found to be between 160 and 960 ng/spot, while Ritonavir's was found to be between 40 and 240 ng/spot. Lopinavir's detection and quantification limits were 9.56 ng/spot and 28.96 ng/spot, respectively, and Ritonavir's detection limits were 6.82 ng/spot and 20.66 ng/spot. Additionally, the method was checked for accuracy, specificity, and recovery. A Lopinavir and Ritonavir fixed-dose tablet sample from Cipla Ltd. was analyzed using this newly developed method.
Keywords: HPTLC, Ritonavir, and Lopinavir.

1 INTRODUCTION

Lopinavir as [1S-[1R*, (R*), 3R*, 4R*]]-N-[4-[[(2, 6-dimethylphenoxy) acetyl] amino]-3-hydroxy-5-phenyl-1-phenylmethyl) pentyl] tetrahydro - alpha-(1-methylethyl)-2-oxo-1(2H)- pyrimidineacetamide. (Fig. 1) Ritonavir and 10-hydroxy-2-methyl-5-(1-methylethyl)-1- [2-(1 methylethyl)-4-thiazolyl] -3, 6-dioxo-8, 11-bis (phenylmethyl)-2,4,7,12-tetraazatridecan-13- oic acid, 5-thiazolylmethyl ester, [5S-5R*,8R*,10R*,11R*)] 2) are medicines that fight HIV (HIV protease inhibitors). Lopinavir and Ritonavir have been accounted for to be evaluated exclusively or in mix by spectrophotometric methods1-3 and HPLC4-7.

According to the literature review, there are analytical methods for determining Lopinavir and Ritonavir from biological matrices, bulk drugs, and dosage forms, as well as RPHPLC/MS8–12 for determining Lopinavir and Ritonavir in combination with other antiviral drugs. Patel D. et al. reported using Merck TLC aluminium sheets of silica gel 60F-254 as a stationary phase and ethyl acetate for the HPTLC method to analyze Lopinavir and Ritonavir: ethanol: toluene: diethylamine, which can be detected at 266 nm, is used as the mobile phase (7:2.0:0.5:0.5 %v/v). In this study, we report the HPTLC method for the analysis of Lopinavir and Ritonavir using a solvent system of chloroform, with a linear range of 8-20 mg/mL: 1, 4, dioxane (7:3 percent v/v).

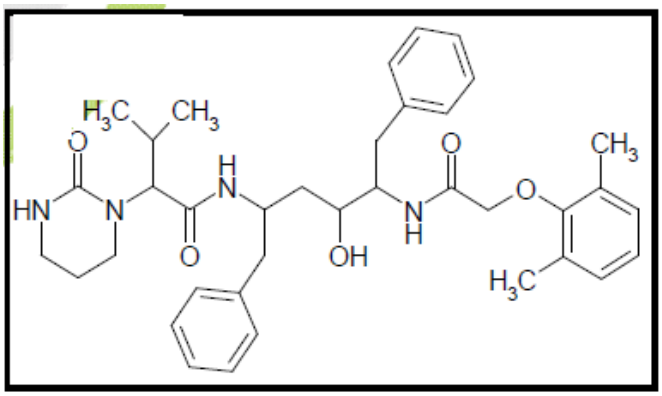

Figure 1 Lopinavir

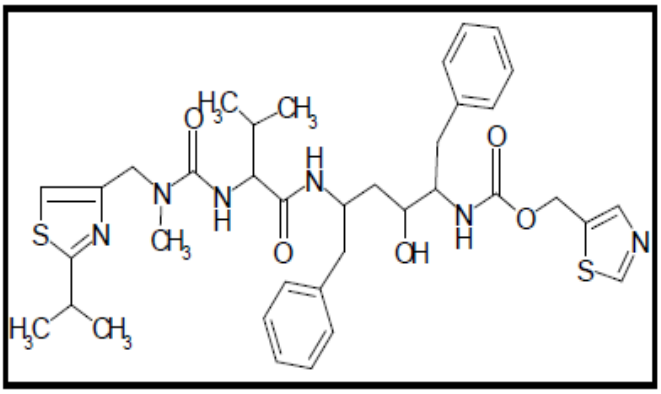

Figure 2 Ritonavir

2 EXPERIMENTAL CHEMICALS AND REAGENTS

Emcure Pharmaceuticals Ltd., based in Pune, India, generously provided powders of pure Lopinavir and Ritonavir. The study utilized commercial Lopinavir (200 mg) and Ritonavir (50 mg) tablets from CiplaLtd. The analytical-grade chloroform, 1, 4-dioxane, and methanol used were from E.Merck, Mumbai, India. (E.Merck, India) All of the other chemicals used were also analytical grade.

2.1 Instrumentation and Conditions

Merck supplied HPTLC plates (10 cm x 10 cm) pre-coated with silicagel GF aluminum TLC plate. A CAMAG TLC Scanner3 equipped with win- CATS 1.4.0 planar chromatography management software was used for densitometry. Under nitrogen gas flow, samples were sprayed onto HPTLC plates with the CAMAG LINOMAT V spray-on method and developed in twin CAMAG 10 cm X 10 cm trough chambers.

2.2 Standard Preparation

Both Lopinavir (200 milligrams) and Ritonavir (50 milligrams) were accurately weighed, transferred to a volumetric flask of 100 milliliters, and dissolved in methanol. Methanol was used to achieve the required volume. To calibrate both drugs, the final concentrations of 200 mcg/mL Lopinavir and 50 mcg/mL Ritonavir were obtained by diluting the resulting stock solution ten times with methanol.

2.3 Preparation of Sample Solution

Twenty tablets, each containing 50 milligrams of ritonavir and 200 milligrams of lopinavir, were weighed and their average weight was calculated for the purpose of analyzing the tablet dosage form. The tablets were ground into a fine powder, and powder containing 50 mg

Ritonavir and 200 mg Lopinavir was accurately weighed before being transferred to a 100 mL volumetric flask. It was shaken for 30 minutes before 60 milliliters of methanol were added. Methanol was used to achieve the required volume. The solution was filtered through the Whattman No. 41 filter paper after being sonicated for 30 minutes. Methanol was used to further dilute this solution to the same concentration as the final standard solution.

2.4 Chromatographic Conditions

Methanol was used as the solvent in the preparation of the Lopinavir and Ritonavir reference standard solution. Using LINOMAT V, aliquots of 0.8, 1.6, 2.4, 3.2, 4.0, and 4.8 L of the prepared standard solution were applied to the HPTLC plates in the form of spot bands of 6 mm. The application positions were at least 15 mm from the sides and 10 mm from the bottom of the plates. Before each run, the development chamber was saturated with mobile phase vapors for ten minutes and the components of the mobile phase were mixed before use. The ascending method was used to develop the plate to a migration distance of 7 cm. After that, a hot plate was used to dry the plates. All of the analysis was done in a controlled laboratory with temperatures between 20 and 240 C. Using a deuterium lamp, densitometry scanning was carried out in absorbance mode at 210 nm. The data resolution was set at 100 mm/step, the scanning speed was 10 mm/s, and the slit dimensions were set at 6 mm x 0.30 mm. Since only the main components were analyzed, single wavelength detection was carried out.

2.5 Method Validation

The guidelines established by the International Conference on Harmonization (ICH) regarding linearity, range, specificity, precision, accuracy, limit of detection, and limit of quantification were used to validate the developed method.

3 RESULTS AND DISCUSSION

3.1 Linearity and Range

Methanol was used to make a ten-fold diluted stock standard solution that contained Ritonavir and Lopinavir. The diluted standard solution was applied in aliquots of 0.8, 1.6, 2.4, 3.2, 4.0, and 4.8 L to the HPTLC plate, delivering 160, 320, 480, 640, 800, and 960 ng of Lopinavir per spot and 40, 80, 120, 160, 200, and 240 ng of Ritonavir per spot, respectively. This was finished in three-fold and rehashed for three days. To reduce potential variation along the silica layer, the applied spot bands were distributed uniformly across the plate for each concentration. Table 1 summarizes the findings.

Table 1 Linearity results

Components	Concentration range (ng/spot)	Equation for regression line	R2
Lopinavir	160-960	y = 10.46x + 2162	0.993
Ritonavir	40-240	y = 24.68x + 289.0	0.999

3.2 Limits of Detection and Quantification

The equations LOD = 3.3 x/s and LOQ = 10 x/s were used to determine the detection and quantitation limits, respectively. Lopinavir's and Ritonavir's respective limits of detection were found to be 9.56 and 6.82 ng/spot, respectively. For Lopinavir and Ritonavir, the limit of

quantification was determined to be 20.66 ng/spot and 28.96 ng/spot, respectively. The amounts recovered from samples of marketed antiretroviral tablets containing Lopinavir 200 mg and Ritonavir 50 mg were expressed as a percentage of the label claims. Lopinavir and Ritonavir had a recovery rate of 98.66 1.48 percent and 98.76 1.80 percent, respectively, meet the assay specifications for active drugs in the United States of Pharmacopoeia, which most drug formulations must meet (90.0–110.0%).

4 CONCLUSION

For the routine analysis of Lopinavir and Ritonavir in fixed-dose combination tablets, a quick, precise, and accurate HPTLC method has been developed. The method's linearity, precision, accuracy, and specificity were all confirmed. It enjoys the upper hand over HPLC techniques overall. It consumed under 35mL of versatile stage per run (8 examples for each plate), though HPLC techniques would consume more than 50mL per runs of comparative number of tests. The new method took an average of one hour from sample preparation to densitometric evolution for a single plate, whereas HPLC methods typically take more than two hours for the same number of samples. It is suitable for routine analysis of Lopinavir and Ritonavir in fixed-dose combination tablets because it is inexpensive, quick, and does not use chloroform. The developed HPTLC method is faster and cheaper than the reported HPLC method for determining Lopinavir and Ritonavir mixtures in bulk and tablet dosage forms. It can be used to analyze lopinavir and ritonavir separately, both in bulk and as tablets.

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ADVANTAGES AND CHALLENGES OF MUCOADHESIVE BUCCAL DRUG DELIVERY SYSTEMS

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Abstract - When it comes to overcoming issues with the previous method of administration, the buccal region of the oral cavity is an appealing target for the drug of choice. Issues, for example, high first-pass digestion and medication debasement in the gastrointestinal climate can be avoided by managing the medication by means of the buccal course. A state known as mucoadhesion is one in which interfacial forces hold two components, one of which is biological, together for an extended period of time. The buccal dosage forms of the mucosa will be discussed, with a focus on bioadhesive polymeric delivery systems. The theories and properties of polymers, in addition to the structural characteristics of mucosal tissues, provide an explanation for the mucoadhesive interaction. Adhesive mucosal dosage forms, such as adhesive tablets, adhesive gels, adhesive patches, and numerous other dosage forms containing various combinations of polymers and absorption enhancers, were suggested for oral delivery to prevent accidental swallowing of drugs. In addition, research has been done on the creation of controlled or slow-release delivery systems for the systemic and local treatment of oral diseases.
Keywords: Mucoadhesive; Buccal; Polymers; Time to retain; System for delivering drugs.

1 INTRODUCTION

Mucoadhesion is characterized as the capacity of material sticks to organic tissue for a drawn out timeframe. The buccal mucosa was found to be the most convenient and easy-to-access transmucosal site for the delivery of therapeutic agents for both local and systemic administration in retentive dosage forms. Since the beginning of the 1980s, mucoadhesive polymers have been the focus of interest for buccal drug delivery. Mucoadhesives are natural or synthetic polymers that interact with mucin molecules, which make up the majority of mucus, and the mucus layer that covers the mucosal epithelial surface. Mucoadhesive medication conveyance framework use the property of bioadhesion of specific water solvent polymers which become glue on hydration and consequently can be utilized for focusing on a medication to a specific district of the body for expanded timeframe.

Mucoadhesion has been widely promoted as a method for providing blood and lymph vessels with site-specific drug delivery; underneath this is a flimsy layer of smooth muscle tissue.

Therapeutic peptides and other drugs that undergo first-pass metabolism or are unstable in the rest of the gastrointestinal tract have been tested for local and systemic delivery in the buccal mucosa. A suitable buccal drug delivery system should have good bioadhesive properties so that it can be retained in the oral cavity for the desired duration. Buccal delivery offers a safer mode of drug utilization because drug absorption can be promptly terminated in cases of toxicity by removing the dosage form from the buccal cavity. To elicit the necessary therapeutic response, it should also release the drug in a controlled and

predictable manner in a single direction toward the mucosa.

1.1 Rationale for Buccal Mucosal Drug Delivery

It possesses a number of characteristics that make it ideal for drug delivery:

1) A substantial supply of blood that flows directly into the jugular vein, bypassing the liver and avoiding first-pass metabolism for the drug.
2) Ease of administration of the medication, even in patients who are unconscious and cannot consume anything.

1.2 Advantages of Mucoadhesive Buccal Drug Delivery System

It enjoys a few benefits:

- Oral mucosal drug delivery systems are simple to use, do not cause any discomfort, and the patient readily accepts them.
- It is possible to precisely localize the dosage form, and delivery can be stopped when necessary.
- Adaptability to changes in physical state, size, shape, and surface.
- The oral cavity is a useful site for drug delivery for upper symptoms in patients who are unconscious, have an upper gastrointestinal tract disease, or have surgery that affects oral drug absorption.
- Augmented ingestion rate because of personal contact with the retaining layer and diminished dispersion boundaries.
- A superior route for the systemic administration of a drug with a high first-pass metabolism that increases bioavailability
- Dose-dependent side effects can be reduced by a significant dose reduction.
- This method allows for the administration of drugs that are either destroyed by the enzymatic or alkaline environment of the intestines or that are unstable in the acidic environment of the stomach.
- It provides a drug absorption system that is passive and does not require activation.
- It considers the neighborhood alteration of tissue penetrability, restraint of protease action or decrease in immunogenic reaction. As a result, therapeutic agents such as peptides, proteins, and ionized species can be utilized selectively.
- Because the oral mucosa lacks prominent goblets cells that secrete mucus, there is no issue with limited mucus buildup beneath the applied dosage form.
- In contrast to the rectal and transdermal routes, the presence of saliva ensures a relatively large amount of water for drug dissolution.
- It satisfied a number of the controlled release system's requirements.
- It can be made to only ensure absorption through the mouth.
- The buccal mucosa is more permeable than the skin because it has more blood vessels.
- Bioadhesion improves bioavailability and dosing interval by extending the residence time at the site of drug absorption.
- Action takes place quickly.

1.3 Limitations of Buccal Drug Administration

- Drugs that irritate the mucosa, have a bitter or unpleasant taste, or have an unpleasant odor cannot be administered through this route.
- This method is not suitable for administering medications that are unstable at buccal pH.
- Just medications with a little portion necessity can be managed.
- The benefits of the buccal route are lost because the drug in saliva travels through the mouth.

- Only drugs that are absorbed through passive diffusion can be given through this method.
- Eating and drinking could be limited.
- There is always a chance that the patient will swallow the tablet.
- Bioadhesive polymer swelling and hydration may disrupt the formulation's structural integrity and cause a slippery surface if it is overhydrated.

2 MECHANISMS OF BIOADHESION

2.1 The Bioadhesive Interface

The surface of the potentially bioadhesive polymer must contribute to the formation of adhesive bonds between a polymer and soft tissue. The interfacial layer between the adhesive and the natural tissue, which is the first layer. When exposed to external media, mucus, a highly viscous product, covers the lining of hollow organs. Glycoproteins or mucins, inorganic salts, proteins, lipids, and muco polysaccharides are the main components of the mucous layer, and their composition varies depending on the source. The pathological conditions also influence the composition of mucin. It was discovered that the mucins produced by normal tissues and those produced by abnormal tissues differ histochemically.

3 THEORIES OF BIOADHESION

The hypothetical system for polymerpolymer bond can be effectively stretched out to depict the bioadhesion of polymeric materials with organic surfaces. Appropriate speculations incorporate the electronic, the adsorption, the wetting, the dissemination and the crack hypothesis.

A. Electronic Theory According to the electronic theory, when the bioadhesive polymer and the glycoproteinic network, which have distinct electronic structures, come into contact, there is likely to be an electron transfer that results in the formation of a double electrical charge at the bioadhesive interface.

B. Adsorption Theory Bioadhesive systems adhere to tissue due to vander walls, hydrogen bonding, and related forces, according to the adsorption theory.

C. Wetting Theory The development of a strong adhesive bond necessitates intimate molecular contact, which necessitates examining the wetting equilibrium and dynamic behavior of the bioadhesive candidate material with the mucus.

4 FACTORS AFFECTING MUCOADHESION IN THE ORAL CAVITY

Mucoadhesive qualities are a variable of both the bioadhesive polymer and the medium where the polymer will dwell. The molecular weight, flexibility, hydrogen bonding capacity, cross-linking density, charge, concentration, and hydration (swelling) of a polymer are just a few of the factors that affect their mucoadhesive properties.

4.1 Hydrogen bonding capacity

Hydrogen holding is one more significant calculate mucoadhesion of a polymer. According to Park and Robinson's findings, desired polymers must possess functional groups that are capable of forming hydrogen bonds for mucoadhesion to occur. In addition, they have established that the polymer's flexibility is crucial to increasing this hydrogen bonding potential. Polymers with a high capacity for hydrogen bonding include poly(vinyl alcohol), hydroxylated methacrylate, and poly(methacrylic acid), as well as all of their copolymers.

4.2 Cross-linking Density

A polymer network's three crucial structural parameters—the average pore size, the number of cross-linked polymers with an average molecular weight, and the density of cross-linking—are all interconnected. As a result, it makes sense that as crosslinking density increases, water diffusion into the polymer network slows down, resulting in

polymer swelling that is insufficient and mucin penetration rates that slow down as well. The general property of polymers that the degree of swelling at equilibrium is inversely correlated with the degree of cross-linking has been described by Flory.

4.3 Charge

There have been some generalizations about the charge of bioadhesive polymers in the past. Nonionic polymers appear to adhere less strongly than anionic polymers. Peppas and Buri have shown areas of strength for that charge on the polymer is one of the expected attributes for mucoadhesion. In a neutral or slightly alkaline medium, it has been demonstrated that some cationic polymers are likely to exhibit superior mucoadhesive properties. Additionally, it has been demonstrated that some cationic high-molecular-weight polymers, like chitosan, have excellent adhesive properties.

5 BUCCAL MUCOADHESIVE DOSAGE FORMS

Based on their geometry, there are three types of buccal mucoadhesive dosage forms. Type I drugs are released in multiple directions and have a single layer. Due to swallowing, this kind of dosage form loses a lot of drug. A double-layered device is created by superimposing an impermeable backing layer on top of the drug-loaded bioadhesive layer in type II devices, which prevents drug loss from the dosage form's top surface into the oral cavity. Due to the fact that the drug is only released from the side closest to the buccal mucosa, Type III is a one-way release device with minimal drug loss.

6 CONCLUSION

The buccal mucosa has a number of advantages for long-term controlled drug delivery. Vascular and lymphatic drainage provide ample supply to the mucosa, avoiding first-pass metabolism in the liver and pre-systemic elimination in the gastrointestinal tract. The patient appears to be satisfied with the location, which is ideal for a retentive device. The mucosal permeability and local environment can be controlled and manipulated to accommodate drug permeation with the appropriate dosage form design and formulation. Buccal drug delivery offers a feasible and appealing alternative for the non-invasive delivery of potent peptide and protein drug molecules and represents a promising area for ongoing research with the goals of systemic administration of drugs that are ineffective when taken orally. However, for a possible future in the field of buccal drug delivery, it is essential to have safe and effective enhancers for buccal permeation and absorption.

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EXPLORING THE PROS AND CONS OF COSMETIC SURGERY: A COMPREHENSIVE REVIEW

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Abstract - The world in which we live has undergone significant transformations in recent years as a result of advances in science and technology. One such shift has been the mainstreaming of cosmetic surgery, which has gone from being reserved for the wealthy and famous to becoming an increasingly popular option for the average person. The medical correction of a person's form and structure is the focus of cosmetic surgery, a distinct subfield of surgery. People groups tend to mistake plastic medical procedure for restorative medical procedure, yet there is really a contrast between the two. In fact, cosmetic surgery is a subspecialty of plastic surgery and refers to procedures performed solely for cosmetic reasons. The following articles discuss the most common cosmetic surgery procedures. People appear to be becoming more aware of their body image all over the world in recent years, and an increasing number of people are looking for the ideal body. Therefore, if a person believes that their body isn't quite up to par, cosmetic surgery can help them fix their issues, and an increasing number of people are taking advantage of this option.

1 INTRODUCTION

A special kind of surgery called plastic surgery can affect a person's ability to function as well as their appearance. Cosmetic surgery and reconstructive plastic surgery are the two subspecialties of plastic surgery. While cosmetic surgery focuses on enhancing a person's physical appearance, plastic surgery may only focus on reconstruction (reconstructive surgery). The goal of reconstructive plastic surgery is to make the body work better; However, its primary purpose is not to attempt to mimic a normal appearance; it may also involve this. The term "reconstructive plastic surgery" is frequently used interchangeably with "reconstructive surgery."

Reconstructive procedures address flaws in the body or face. Physical birth defects like cleft lips, palates, and ear deformities, traumatic injuries like burns or dog bites, and the effects of disease treatments like reconstructing a woman's breast after breast cancer surgery are all examples of these. In some parts of the world, cosmetic surgery and plastic surgery are completely different procedures, and cosmetic surgery is referred to as elective surgery, non-essential surgery, or surgery that a patient chooses to have; whereas the term "plastic surgery" refers to procedures performed to reshape or enhance

one's appearance after an injury or illness.

1.1 What is Cosmetic Surgery?

The medically unnecessary surgical procedures known as "cosmetic surgery" include, but are not limited to, plastic surgery aimed at preserving beauty. Medical procedure/therapy given to address/remake a deformity caused from a physical issue or illness. Lasers have opened up new realms that cosmetic surgeons had previously shied away from, whether it's removing a baby's birthmark or a liver spot from an aging hand. Surgery to improve one's appearance that involves repairing or replacing body parts. The goal of cosmetic surgery is to make your body look better. These procedures are carried out for cosmetic purposes. Surgery and related medical treatment performed for aesthetic purposes rather than health-related reasons. Surgery to reshape normal body structures in order to boost a patient's self-esteem and appearance.

Cosmetic (procedures that alter a part of the body that the person is unhappy with (also known as aesthetic procedures). Normal corrective methods incorporate making the breasts larger (expansion mammoplasty) or more modest (decrease mammoplasty), reshaping the nose (rhinoplasty), and eliminating pockets of fat from explicit spots on the body (liposuction). Some cosmetic procedures, like cutting and stitching, are not even considered surgical by the majority of people. Two examples of such treatments are using special lasers to get rid of unwanted hair and sanding the skin to get rid of severe scarring.

2 TYPES OF COSMETIC SURGERIES:

Hair Transplant: Beginning in the third decade of life, male baldness is a common problem. Permanent hair root loss results in baldness. The dead roots will not be revived by any oil, massage, or medication. By putting in new hair roots, hair transplantation (HT) offers a scientific solution. Due to a genetic tendency to remain permanent, it is common knowledge that hair on the back (occipital) region of the head almost never falls out. The idea behind HT is that if hair roots taken from the back are transplanted to the area where there is baldness, they will continue to grow hair there for as long as they would have in their original location after a short period of effluvium. To achieve the desired hair density, follicular micro or mini grafts are used. The newly transplanted hair is from the patient's own body, so it will naturally grow over time and can be washed, trimmed, or even shaved.

The hair typically begins to recede (thin) at the front at first. At the same time, the hair on the crown of the head typically becomes thinner. In the middle of the scalp, a bald patch gradually forms. The top bald spot and the receding front gradually expand and join together. The back and sides of the scalp are frequently left with a fringe of hair. This fringe of hair also thins out in some men, eventually leading to a completely bald scalp. Local anesthesia is used during the outpatient procedure for hair transplantation. Rhinoplasty: A bandage is used overnight after the procedure. The cosmetic surgery

known as rhinoplasty can change the shape of your nose. Modifications can be performed to abbreviate a long nose, limited a wide nose, decrease a wide tip, bring down a high nose, fix a warped nose, and work on the taking in instances of nasal stodginess. The surgical procedure known as septorhinoplasty is used to improve nasal breathing. The bridge should not be lowered too much or the nose should not be turned up. This idea is in line with the goal of reshaping the nose so that it looks natural and un-operated and in harmony with the other facial structures.

Chemical Peel: Skin that has been damaged by the sun, brown "age spots," fine lines and wrinkles, dry or flaky skin; Problems like adult acne, rough skin texture, uneven skin tone, superficial facial or acne scars, and excessive oil on the face affect millions of people. Chemical peels are a solution that is becoming more and more popular. Chemical peels are a type of advanced clinical skin rejuvenation treatment that helps repair damaged skin caused by acne, sun exposure, aging, and other factors. By removing the skin's damaged outer layers with a chemical solution, chemical peels improve and smooth the texture of the skin. It has been shown to be very effective for people who have wrinkles, uneven skin pigmentation, and other facial imperfections.

Post Burn Surgery: Burns are devastating injuries that have the potential to kill, disable, and wreak havoc on the victim. Because there is still healthy skin at the bottom of the burn, first and superficial second degree burns are partial thickness burns that may heal without the need for skin grafting. To restore skin coverage, full thickness or third-degree burns and deep second-degree burns typically necessitate skin graft surgery. The plastic surgeon will typically need to make multiple trips to the operating room in order to remove the nonviable skin from a patient who has burned a significant portion of their body and replace it with skin grafts. Burns that occur across joints may impede joint movement and tighten the skin as they heal. The term for this is joint contracture. In poorer nations, joint contracture occurs frequently 19. Even when skin grafting is an option, plastic surgeons are frequently called upon to provide reconstruction after burn injuries. When skin grafting is not available, additional skin is provided with a combination of skin flaps and grafts.

Deformity Correction: A deformity, whether acquired or congenital, leaves a deep emotional and mental scar as well as an unacceptable physical disfigurement. Only those who have been directly impacted or their loved ones can comprehend this. The majority of these people eventually come to terms with it and accept their fate with a sigh of bitter defeat. It is ironic and unfortunate that they are unaware of a potential solution to all of their physical and emotional problems.

Obesity Surgeries (Bariatric): Worldwide, morbid obesity—defined as a BMI greater than 35—is on the rise. Obesity predisposes to diabetes, high blood pressure, heart issues, and

difficulty breathing, all of which have very clear effects on one's health. Additionally, there is the obvious blemish to be dealt with. Most of the time, a two-pronged approach is needed to treat morbid obesity.

A healthy diet and regular exercise are essential. Additionally, bariatric surgery will assist in the rapid loss of a significant amount of weight. Any coinciding or contributing clinical issues likewise should be taken care of, hormonal lopsidedness specifically.

Face Sculpting: A sharp stunning, unmistakable cheeks and an etched look are profoundly wanted facial highlights today. Fat stores on the face will generally collect beneath the jaw, on the cheeks and over the stunning making a plump look. Through minimally invasive surgery, facial features can be safely defined and enhanced.

The majority of cases respond well to the following treatments, despite the fact that each situation is unique and requires an in-depth evaluation before a treatment plan can be developed:

Cheek fat removal: Fat bulge below the cheeks can be removed from the inside of the mouth without external scars.

1. **Liposuction:** Liposculpting is a very effective method for getting rid of fat deposits under the chin and over the lower jaw. Another option for removing fat from the jaw line and under the chin is injection lipolysis. However, injection lipolysis may necessitate multiple treatments.

A chin implant will also strengthen the chin and give the face a more defined appearance. Together, these three procedures result in a significant alteration to the facial profile. All of this can be done without having to stay in a hospital as a day care surgery.

Gynaecomastia: As many as 40 to 60 percent of men suffer from gynecomastia, also known as excessive breast tissue development. Boys and men who suffer from this condition may experience extreme embarrassment, social inhibition, and self-consciousness. The excess fat, skin, and breast tissue will be removed by the cosmetic surgeon by cutting around the dark skin under your arm or around the nipple (the areola). To get rid of extra fat in the area, liposuction can also be done at the same time.

Masteopexy: When the nipples are drooping, the skin has stretched and become saggy, and the breasts have lost their youthful volume, a breast lift (mastopexy) is needed. After significant weight loss or pregnancy, this is frequently the case. To bring the entire body into harmony, mastopexy involves removing excess skin, lifting the breast gland, and reshaping the breast.

Mammoplasty: Bosom decrease a medical procedure, in fact called mammaplasty, is normally performed for actual help as opposed to for restorative reasons. In order to reshape and lift the breasts, this procedure requires the removal of excess breast tissue. The surgery makes you feel better in your neck, shoulders, and upper back. less strain on the shoulders from bra straps; improved capacity to engage in

physical activity and exercise; and a more positive view of oneself Additionally, the surgery may facilitate easier breathing and sleep. As the surgeon removes fat, glandular tissue, and skin from the lower part of the breast, the procedure has a high satisfaction rate among women. To create a smaller breast, the tissues are closed and the nipple is moved upward.

Cleft lip and palate can be associated with a number of significant signs and symptoms, including

3 ATYPICAL APPEARANCE-

The appearance of the face is noticeably unusual and deformed.

- **Feeding problems**- A baby with a cleft lip or palate may have difficulty sucking or swallowing milk because newborn babies are typically breastfed. This is on the grounds that they can't create a vacuum in their mouths.
- Consequently, they consume too much air while feeding. When a cleft is very big, the baby may even have to be fed through a nasogastric tube (a tube that goes into the nose) until the reconstructive surgery is scheduled and done.
- This is due to the development of glue ear in cleft children; a tacky liquid develops behind the eardrum. In most cases, the Eustachian tube, which connects the ear to the throat, can allow this sticky fluid to drain away. However, a cleft palate can cause this tube to become distorted. In order to allow the fluid to drain out during surgery, surgeons frequently insert a small plastic tube known as a grommet into the eardrum.
- **Speech and language problems** - our lips and sense of taste are fundamental parts for the expression of appropriate sounds for clear discourse. Speech development issues are common in children with cleft lip and palate. Their voices may frequently have a nasal quality, and they frequently have trouble correctly uttering consonants.
- **Dental health** - Children who have a cleft lip and palate are more likely to get tooth decay because it alters how the mouth develops and can cause issues with tooth development. Teeth in close proximity to the cleft may be missing or grow out in different ways.

4 CONCLUSION

Many people still don't know the difference between plastic and cosmetic surgery. However, recent research in this area has made significant progress in the treatment of many skin, body contour, and inborn deformities-related conditions. As a result, we hope that additional research will be conducted on this site in the future to provide a useful treatment option for a variety of deformities, enhance body contour, and foster confidence in individuals.

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EXPLORING THE EFFECTIVENESS OF HERBAL REMEDIES IN MANAGING HYPERTENSION: A COMPREHENSIVE REVIEW

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Abstract- Numerous people today are struggling with hypertension. Despite billions of dollars spent annually on cardiovascular disease treatment and detection, conventional treatments have not reduced the number of hypertensive patients. The rising number of people with high blood pressure can be effectively reduced with alternative medicine. Research has viewed various elective treatments as fruitful in diminishing hypertension including diet, work out, stress, the board, enhancements and spices. Herbal treatments for high blood pressure are the subject of increasing numbers of research studies each year. Punarnava, Barberry, Rouwolfia, Garlic, Ginger, Ginseng, and Arjuna are just a few of the herbal medicines that can be safely used to treat hypertension. The herbs that have been scientifically proven to treat hypertension are highlighted in this review.

1 INTRODUCTION

The treatment of human disease has been based on natural products made from plants, animals, and minerals. It is estimated that approximately 80% of people in developing countries still rely on traditional medicine for their primary health care, which is largely based on species of plants and animals. The demand for herbal medicines is currently high, and their popularity is steadily rising. In ancient literature, approximately 500 medicinal plants are mentioned, and approximately 800 plants have been utilized in indigenous medical practices. The vast repository of medicinal plants that are utilized in traditional medical procedures can be found in India. Due to the increased risk of adverse effects associated with allopathic medications, Western nations have seen an increase in demand for Ayurvedic phytopharmaceutical products. The production of herbal and phytopharmaceutical products is the current focus of numerous pharmaceutical companies]. Around 20,000 medicinal plants have been identified in India. Chemical principles derived from natural sources are now much simpler and have significantly aided in the creation of new medicines derived from medicinal plants. There are numerous home grown drugs which are utilized for the treatment of hypertension.

1.1 Chemical Classification of Antihypertensive Herbs:

- Terpenoids—Inula helenicum and jatamansi.
- Alkaloids include Rauwolfia, Papaver, Avis tolochladebis, Loptis, jayonica, Withenia, Golden seal, and Bhringaraj.
- Steroids include Veratrum, Holarrhena pubescens, Satavari, Bhringaraj, and Clerodendroon trichotomum.
- Flavonoids include Devis scandens, Mitragyna ciliate, Yaroow, Olive leaf, Hawthorn, Arjuna

1.2 Pharmacological Classification of Antihypertensive Herbs:

- **Withania (CNS acting):** centrally acting Catcholamine depeleters;

Rauwolfia Dopamine and norepinephrine reuptake inhibitors, hypericum;

- **Vasodialators:** garlic (via hyperpolarization via H2S); black cumin seed (antioxidant and acting on the central nervous system). (a direct muscle relaxant) ginseng Olive leaf, Hawthorn, Vitis (endothelium-dependent vasodilation); Lotus, forskolin (via the Adenyl cyclase pathway).

2 SPECIFIC BOTANICALS FOR TREATMENT OF HYPERTENSION:

Arjuna bark (*Terminalia arjuna*): India is home to the deciduous tree Terminalia arjuna. Ayurvedic medicine has used its bark for more than three centuries. Tanning, triterpenoid saponins, flavonoids, gallic acid, ellagic acid, OPCs, phytosterols, calcium, magnesium, zinc, and copper 5 are the active components of terminalia. The effects of Terminalia on various cardiac conditions like hypertension, coronary artery disease, and congestive heart failure have been the subject of numerous studies. It was found to be effective for stable angina patients, with a 50% reduction in angina episodes and a significant decrease in systolic blood pressure, according to a study on its effects on stable and unstable patients 6.

Olive Leaf (Olea africana and Olea europea): The leaves of the olive tree are the source of olive leaf extract. Oleuropein, a complex structure of flavonoids, esters, and multiple iridoid glycosides, which acts as a vasodilator, lowers blood pressure, and prevents angina attacks, is one of several phytochemicals in the entire leaf extract. In addition, it is becoming clear that oleeuropein is a potent antioxidant23,24. Olive leaf's hypotensive effects have been studied for two decades. Two groups of hypertensive patients participated in a clinical study of Olea europaea aqueous extract: 12 patients who were consulting for the first time and 18 patients who were receiving conventional antihypertensive treatment. After 15 days of taking a placebo, an aqueous extract was given for three months. For all patients, researchers found no adverse effects and a statistically significant drop in blood pressure (p 0.001).

Garlic (Allium Sativum): Several ailments are commonly treated with the garlic bulb. Earaches, chronic fatigue syndrome (CFS), menstrual disorders, hypertension, hyperlipidemia, coronary heart disease, age-related vascular changes and atherosclerosis, and garlic is used to treat all of these conditions. Garlic is viewed as a powerful platelet conglomeration inhibitor. Allicin, ajoene, and other organosulfur components like S-allyl-L-cysteine are responsible for many of the pharmacological effects of garlic. New garlic contains around 1% alliin 55.One milligram of alliin is changed over completely to 0.458 mg allicin which is viewed as the significant dynamic compound in garlic. Ajoene is produced by further conversion. The method of preparation determines the amount of allicin in garlic preparations. Garlic powder taken orally at 300 milligrams per day appears to slow the age-related decrease in aortic elasticity. When administered over a four-year period, higher doses of 900 mg per day appear to slow the progression of atherosclerosis in both the femoral and aortic arteries 56. Garlic is thought to lower blood pressure by causing smooth muscle relaxation and vasodilation by activating production of endothelium-derived relaxation factor (EDRF, nitric oxide). After four weeks of treatment, there is evidence that garlic can be taken orally.

Bhringraj (*Eclipta Alba/Eclipta prostrate*): Wedelolactone and dimethyl

wedelolactone in the herb have powerful antihepatotoxic properties. Ascorbic acid is abundant in the herb. It additionally contains an alkaloid, ecliptine. It is interesting that this species contains acetylenes of mono-, di-, and trithiophenes in addition to a-terthenyl. Besides stigmasterol and ß- sitosterol, the petroleum ether extract of aerial parts contains ecliptal, a trithienyl aldehyde. Thiophene acetylenes are very abundant in the roots. Eclipta works well to reduce inflammation. It prevented up to 58.67 percent of the higher levels of histamine caused by chronic inflammation. On anesthetized rats, the ethanolic extract of the dried whole plant E.prostrata and its active component, culumbin, had remarkable antihypertensive effects. Neither the histopathology of the heart, kidneys, spleen, or liver nor biochemical parameters like SGOT, SGPT, or BUN have revealed any significant side effects or toxicities. In addition, Long Evans rats' body weight and specific organ weight have not changed significantly during the investigation.

Alpinia (*Alpinia zerumbet*): Alpinia zerumbet is a West Asian medicinal plant that is used as a diuretic, antihypertensive, and antiulcerogenic in infusions or decoctions in the northeast and southeast of Brazil. A hydroalcoholic extract made from the leaves of Alpinia zerumbet (AZE) was tested for its ability to reduce blood pressure in rats with DOCA-salt hypertension and to cause vasodilation in the mesenteric vascular bed (MVB). In MVB that has been precontracted with norepinephrine, AZE causes a vasodilation that is endothelium-dependent and lasts for a long time, and indomethacin doesn't stop it.

Cat´s Claw (*Uncaria tomentosa*): Uncaria tomentosa multiplies immediately all around the Amazon rainforest, particularly in the upper Amazon district of Peru and adjoining nations, and other tropical areas of South and Focal America, including Peru, Colombia, Ecuador, Guyana, Trinidad, Venezuela, Suriname, Costa Rica, Guatemala, and Panama. It has also been reported as far south as Paraguay and as far north as Belize. This plant is related to at least 60 species. The water extract of Uncaria tomentosa contains a variety of phytochemicals that have been shown to have distinct effects on the heart and blood. The extract's alkaloids have been shown to have hypotensive and vasodilatory effects. Mitraphylline, hirsutine, and rhynchophylline are these alkaloids.

Platelet aggregation and thrombosis inhibition have also been demonstrated by rhynchophylline. Rhynchophylline's ability to prevent and reduce blood clots in blood vessels, relax endothelial cells' blood vessels, dilate peripheral blood vessels, slow the heart rate, and lower blood cholesterol are all demonstrated by the analyses carried out there. Three sterols—beta sitosterol (80%), stigmasterol, and campesterol—have been identified and demonstrated to be mild inhibitors of cholesterol synthesis in vitro[80]. This indicates that they may also assist in the prevention of atherosclerosis by preventing the formation of the atherosclerotic plaque that occurs as the disease progresses. It is known that it contains a number of chemicals that help the body lose water, relax smooth muscles, and widen small blood vessels in the hands and feet. Blood pressure may be reduced by all of these effects. Due to its ability to lower C-reactive protein activity, it has also been proposed that the water extract of Uncaria tomentosa could assist in the prevention of heart attacks, circulatory system diseases, and strokes.

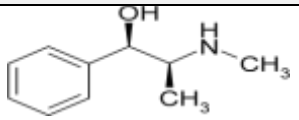
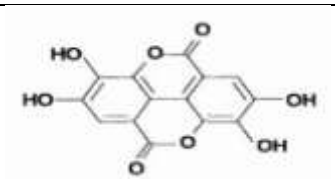
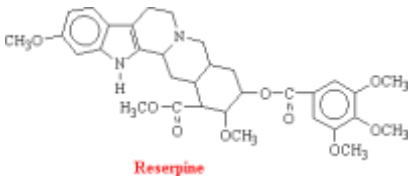
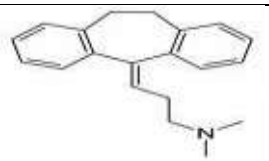
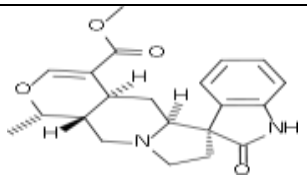
3 CONCLUSION

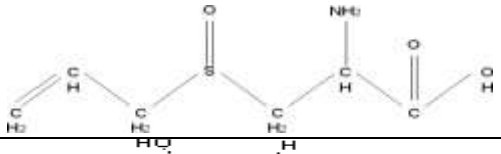
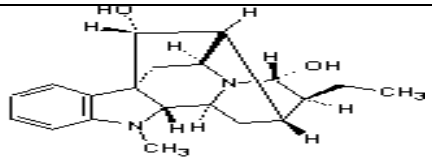
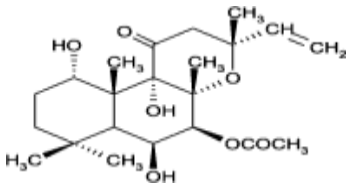
Changing one's diet, exercising, and managing one's stress can all have a significant impact on blood pressure

reduction. In the treatment of cardiovascular disease, including hypertension, supplements like potassium, magnesium, CoQ10, omega-3 fatty acids, amino acids Aarginine and taurine, and vitamins C and E have proven to be effective. They have demonstrated successful in bringing down circulatory strain and further developing heart capabilities. Hawthorne, Arjuna, Olive leaf, European mistletoe, Yarrow, Black cumin seeds, Forskolin, Indian snakeroot, and Garlic are among the most researched and frequently used for hypertension.

The full potential of alternative medicine in the treatment of hypertension requires further investigation. Alternative therapies offer hope in the face of the rising number of people with hypertension and the inability of conventional medicine to effectively treat the condition.

CHEMICAL CONSTITUENTS AND THEIR STRUCTURE:

Plant	Chemical constituents	structure
Ephedra sinica/ Ephedra intermedia	Ephedrine	
Hawthorn	ProcynadinB-3 R=H prodelfinidinB-3 R=OH	 Procyanidin B-3 (R = H) Prodelphinidin B-3 (R = OH)
Terminalia arjuna	Ellagic acid	
Rouwolfia serpentine	Reserpine	 Reserpine
Panax Ginseng	Amitryptylin	
Uncaria tomentosa bark	Mitraphylline	

Alium sativum	Alliin	
Rouwolfia serpentine	Ajmaline	 (17*R*,21*R*)-Ajmalan-17,21-diol
Coleus forskohlii	Forskolin	

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UNDERSTANDING ANTIBIOTIC RESISTANCE: A COMPREHENSIVE OVERVIEW OF MECHANISMS AND IMPLICATIONS

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Abstract - It is well known that antibiotics work well to treat existing infections as well as to prevent infections in patients with compromised medical conditions. Utilization of antibiotics is thought to be a significant risk factor for the emergence of antibiotic resistance. Antimicrobial resistance is developing at an alarming rate, reducing treatment options for nearly every human pathogen. Numerous microscopic organisms presently show various systems that assist with safeguarding them during antimicrobial openness. Penicillin-binding protein modifications, multidrug efflux pumps, transferable vancomycin resistance, and DNA gyrase gene mutations are just a few examples. The new era of antimicrobial therapeutics, as well as innovative approaches to eradicating antibiotic resistance, are discussed in this article.

1 INTRODUCTION

In the fight against infectious bacteria, antibiotics have demonstrated themselves to be an effective class of drugs. One of the greatest current obstacles to effective infection treatment is antibiotic resistance. In addition, there is every indication that the problem of antibiotic resistance will only get worse in the future. Changes in bacteria that reduce or eliminate the effectiveness of drugs, chemicals, or other agents intended to treat or prevent infection are the cause of antibiotic resistance. Our ability to effectively treat bacterial infections is in jeopardy due to the rise of antibiotic resistance.

Treatment with antibiotics promotes drug-resistant bacteria's growth, facilitates the transfer of resistance mechanisms among them, and selects for resistance mutations.

Antibiotic resistance is a natural phenomenon that is well-known. When it is made worse by human misuse and neglect, it has a significant impact on public health. Due to the rapid spread of organisms from one region of the world to another, the threat is now global. It is no longer a problem that only affects developing nations. Even with all the advancements in medicine and the widespread availability of antibiotics, a person can still die in a developed nation from an infection with resistant bacteria.

Concern about ensuring adequate and proper use of these potent agents is justified by the astonishing effects of antibiotics, the prevalence of antibiotics, and the substantial resources spent on antibiotics worldwide. The majority of all therapeutic agents, antibiotics

typically account for 15-30% of total drug expenditure. Antibiotic resistance has been around for as long as antibiotics have been around. Shortly after Penicillin became widely used in the 1940s, antibiotic resistance became a problem. The discovery and introduction of broad-spectrum antibiotics like Streptomycin, chloramphenicol, and tetracycline marked the beginning of the antibiotic chemotherapy era in the late 1940s and early 1950s. By the last part of the 1980s even Methicillin safe Staphylococcus aureus had become pervasive in numerous emergency clinics and hard to treat. Vanomycin was a reliable treatment for Enterococci infections caused by multidrug-resistant Enterococci until recently. However, Vanomycin resistance first appeared in the middle of the 1980s. Vanomycin resistance had increased more than 20 times between 1989 and 1995, according to a Gaynes study. Penicillins were the treatment of choice for gonorrhea for a while, but in 1976, plasmid-mediated beta lactamase from E. coli was discovered in Neisseria gonorrhoeae isolates from Asia and Africa 8. Improvement of anti-infection obstruction was first detailed in quite a while in 1940s 9 and emotionally revealed among patients during the 1970s.

Antibiotics are given to humans for the purpose of treating and preventing disease. Ninety percent of antibiotics are used on patients and as a reminder in hospitals. According to estimates provided by the Centers for Disease Control and Prevention in the United States, approximately 50 million of the 150 antibiotic prescriptions issued to patients each year are unnecessary. Antibiotic use in excess contributes to resistance formation. The following are some of the antibiotics' rates of resistance: Nalidixic acid is followed by ampicillin, tetracycline, sulpha-trimethoprim, streptomycin, chloramphenicol, cephradine, kanamycin, and ampicillin.

Causes of Antibiotic Resistance: Anti-toxin opposition was just brought about by the disappointment of recommended drug regimens and human mistakes additionally add to the advancement of anti-microbial safe microorganisms.

Mechanism of Antibiotic Resistance: Organisms can develop resistance to antimicrobial agents in two basic ways.

2 GENETIC MECHANISMS OF TRANSMISSION

The ease with which a microorganism can acquire DNA from other microorganisms and the degree of simplicity with which the microorganism's DNA becomes resistant are typically linked to the development of antibiotic resistance. In order for antibiotic resistance to develop, two essential factors must combine: the presence of an antibiotic that can inhibit the majority of the bacteria in a colony, as well as a diverse colony of bacteria in which at least one bacterium carries the genetic determinant that can express antibiotic resistance.

Antibiotic resistance can be acquired or natural (intrinsic), and it can spread horizontally or vertically. Normal type of anti-infection opposition is brought about by an

unconstrained quality change in the absence of particular tension because of the presence of anti-microbials and is far significantly less normal than the gained one. However, the most common cause of antibiotic resistance in bacteria is the micro-ecological pressure caused by the antibiotic's presence, which is a powerful stimulus for bacterial adaptation.

2.1 Conjugation:

The most significant and widespread method by which bacteria transmit resistance is conjugation. Plasmids, which are circular DNA fragments that are simpler than chromosomal DNA and can replicate independently of the chromosome, typically serve as the conduit for this mechanism. The formation of a "pilus," which is a hollow tubular structure that forms between bacteria when they are next to one another, allows the passage of these DNA fragments and is the mechanism by which plasmids are transmitted among them.

Transformation: When free DNA—also known as "naked DNA"—is directly transferred from one cell to another, transformation—another form of transmission of bacterial resistance genes—occurs. Most of the time, the "naked DNA" comes from other bacteria that died and split up close to the receiving bacteria. The receiving bacteria simply incorporate the unrestricted DNA into their own DNA by introducing it into their cytoplasm.

Transduction: The utilization of a "vector," the majority of which are "bacteriophages," is required for transduction, the third method of genetic transfer. The "resistant DNA" (the bacterial gene that codifies antibiotic resistance) is introduced into the receiving bacteria after the virus infects the new bacterial cell. Most of the time, the infecting bacteriophage also gives the receiving bacteria its own viral DNA. After that, the bacteriophage takes control of the bacterial replication system and forces the cell to produce more copies of the virus until the bacterial cell dies and these new bacteriophages can infect other cells.

2.2 New era of antimicrobial therapeutics:

The new therapeutic options for preventative medicine. The most plausible strategies are described here:

- Bacterial interference—inoculate hosts with bacteria that aren't pathogenic.
- Bacteriophage therapy: Bacteriophages are viruses that infect bacteria and take control of the machinery that makes proteins in the host.
- Bacterial vaccines: With the discovery of virulence regulatory mechanisms and the complete genomic sequence, the development of bacterial vaccines has gained popularity.
- Cationic peptides: These a variety of peptides are natural compounds with hydrophilic and hydrophobic properties.
- There are a number of ways cationic peptides work, all of which involve killing cells by interacting with the bacterial cell membrane.

2.3 New Strategies to Eliminate Antibiotic Resistance:

This strategy is made up of three main components:

1. During prescribing, the emphasis on drugs whose use has been shown to strongly correlate with resistance should be decreased.
2. New drug formulations with pharmacodynamic parameters better suited to deal with highly resistant strains are being developed; and
3. Promotion of the use of antibiotics with the greatest potential for bacterial elimination. We believe that employing such a strategy would slow the spread of opposition.

3 CONCLUSION

Antibiotic resistance is a significant issue in healthcare that can vary significantly from place to place, from region to nation to global. One important way to lessen the selective pressure that facilitates the emergence of resistant organisms is through prudent antibacterial drug use—using the appropriate drug at the appropriate dosage and duration. For -lactams, aminoglycosides, and quinolones, restricted permeability and efflux are fundamental properties of the organism and common components of the resistance phenotype. The development of inhibitors of resistant enzymes as co-drugs, for example, is one way to combat antibiotic resistance and increase antibiotics' delivery or accessibility to their sites of action (liposomal preparation of hydrophobic antibiotic). Expanded knowledge of the molecular mechanisms of antibiotic resistance, their origins and evolution, and their distribution across bacterial populations and genomes is required for all alternative strategies to overcome resistance.

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ASSESSMENT OF ANTIBIOTIC PRESCRIBING PRACTICES FOR WOMEN DURING AND AFTER DELIVERY IN A NON-TEACHING TERTIARY CARE HOSPITAL IN UJJAIN, INDIA: A PROSPECTIVE CROSS-SECTIONAL ANALYSIS

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Abstract-
Objectives: In order to reduce maternal mortality, antibacterial medications, also referred to as antibiotics, are essential for treating infections during delivery and the postpartum period. Although extensive use of antibiotics contributes to the development and spread of antibiotic resistance, institutional deliveries have the potential to save the lives of many women. In a non-teaching tertiary care hospital in the city of Ujjain, Madhya Pradesh, India, the purpose of this study was to document antibiotic prescribing among inpatients during and after delivery.
Methods: Women who had undergone a hospital cesarean section or vaginal delivery were included in a prospective cross-sectional study. Using a specific form attached to each patient's file, trained nursing staff collected the data daily. The data was analyzed using logistic regression, both bivariate and multivariate.
Results: 566 (53%) of the 1077 women gave birth naturally, while 511 (47%) had a cesarean section. Antibiotics were prescribed to 87% of women who had a vaginal birth and 98% of women who had a cesarean section. Women who delivered via vaginal delivery spent an average of 3.1 (1.7) days on antibiotics in the hospital, while those who delivered via cesarean section spent an average of 6.0 (2.5) days. At discharge, antibiotics were prescribed to 28% of women who had cesarean sections as well as vaginal deliveries. The most regularly recommended anti-toxin bunch in the clinic for both the ladies that had a vaginal conveyance and the ladies that had a cesarean segment were third-age cephalosporins (J01DD). For women who had a vaginal delivery, the total number of defined daily doses (DDD) per 100 bed days was 101, while for women who had a cesarean section, the number was 127.
Conclusions: Concerns include the high rate of women who delivered via vaginal birth who received antibiotics and the hospital's disregard for cesarean section recommendations. The rational use of antibiotics and improved maternal health are intertwined. In health care facilities, it is necessary to have a specific policy and set of guidelines for how to prescribe antibiotics during delivery. Additionally, a system for monitoring antibiotic prescribing and resistance must be developed and put into action.
Keywords: Prescription of antibiotics, vaginal birth, c-section, non-teaching hospital in Ujjain, Madhya Pradesh, India.

1 INTRODUCTION

In order to reduce maternal mortality, it is essential that antibacterial medications—also referred to as antibiotics—be made available for the treatment of infections that occur during labor and after delivery. Worldwide, approximately 350 000 maternal deaths occur annually. Infections are one of the leading causes of maternal mortality. The World Health Organization (WHO) says that infections are directly responsible for 15% of all maternal deaths worldwide. Other studies

say that infections are responsible for as many as 30% of all maternal deaths worldwide. Between 1990 and 2008, India's national maternal mortality rate (MMR) decreased significantly from 570 to 230 per 100,000 live births. In any case, the general typical speed of the decrease in MMR shows that India won't arrive at the Thousand years Advancement Objective (MDG) of 108 out of 2015. The MMR is predicted to be around 135 by 2015, according to recent estimates. The majority of all maternal deaths worldwide take place during labor and postpartum. Increasing access to emergency obstetric care, which frequently necessitates having access to antibiotics, is one of the single most significant interventions that can be implemented to reduce maternal mortality.

Increased access to health care interventions, including antibiotics, is necessary to prevent infection-related maternal deaths. However, due to the emergence of antibiotic resistance, caution must also be exercised when prescribing antibiotics. Antibiotics are widely used in India because they can be easily obtained without a prescription and are frequently prescribed by medical professionals. Antibiotic prescribing rates have been found to be higher in primary and secondary health care facilities in India [8-10]. In the Indian context, antibiotic prescription rates during and after delivery are unknown, but it is likely that both over- and under-prescribing occurs.

In a tertiary care hospital in the city of Ujjain, Madhya Pradesh, India, the purpose of this study was to report on the prevalence, types, and duration of antibiotics prescribed to women during and after vaginal delivery or caesarean section.

2 METHODS SETTING

Madhya Pradesh, home to Ujjain, is one of India's most populous and largest states, both geographically and in terms of population. Madhya Pradesh's maternal health indicators rank among the lowest in India. 47% of deliveries take place at a health facility, ranging from 13% in Dindori district to 79% in Indore district, according to data from the district level household and family survey that was conducted in 2007–2008. Sixty-six percent of women in Madhya Pradesh had experienced at least one complication during labor, and forty-one percent had experienced complications following delivery, such as a high fever and abdominal pain. Ninety percent of women in the Ujjain district where this study's data were gathered received antenatal care, and sixty-eight percent gave birth in a hospital. In Madhya Pradesh, there is no general surveillance system to keep an eye on antibiotic prescribing or antibiotic resistance. However, studies that were carried out in Madhya Pradesh and the Ujjain district revealed that overall, high prescribing rates were observed among both admitted and outpatient patients. Neither anti-toxin recommending rules overall nor explicit rules for endorsing of anti-toxins during vaginal conveyance or for obstetric medical procedure were accessible at the clinic at the hour of the review.

3 DATA COLLECTION

At the VD Gardi Charitable Trust Hospital and Research Centre, data were collected in a prospective cross sectional design from April 2008 to December 2010. The hospital has 350 beds and is run by the Ujjain Charitable Trust, a non-profit organization. Patients pay a small fee for consultation and treatment at the hospital, which is a non-educational facility in the city of Ujjain. The hospital serves both the urban and rural populations of the cities' neighboring villages. This study drew on a substantial set of data compiled by the research team regarding antibiotic prescriptions at this hospital. Using a specific form attached to each patient's file, trained nursing staff

collected the data daily. The procedure for gathering data has previously been described in detail.

3.1 Data Management and Analysis

Epi info (version 3.1) and Excel were used to enter the data, and SPSS (version 21.0) and Stata (version 12.1), both from Texas, USA, were used to conduct the analyses. The fundamental variable, recommending of anti-microbials, was examined independently for the gathering of ladies who had a vaginal conveyance and for the gathering of ladies that had a cesarean segment. The mean number of days for which antibiotics were prescribed, as well as the prescribing by age group, residence, and number of days spent in the hospital, were all determined using descriptive statistics. For the vaginal deliveries, a bivariate and multivariable logistic regression was used to examine the relationship between the binary outcome of antibiotic prescriptions (yes or no) and the following variables: age (18–20, 21–30, or older than 31), residence (Ujjain city, nearby city, Ujjain district villages, other districts, cities in the nearby district, other district villages), and number of days in the hospital (1–2, 3–5, or more than 5). The term 'OR' has been utilized for the chances proportion of bivariate and 'adj. In both the text and the tables, the term "OR" stands for the odds ratio of multivariate logistic regressions.

The prescribed antibiotic was classified according to the Anatomical Therapeutic Chemical (ATC) classification system and the defined daily dose (DDD). The DDD is the assumed average maintenance dose per day for a drug when used for its primary indication in adults, and the ATC system categorizes the active substances into groups and subgroups. The DDD provides a fixed unit of measurement that is independent of things like strength and price, making it possible to study drug prescribing patterns. The total DDD and DDD/100 bed days were used in this study to show that antibiotics were being prescribed.

3.2 Methodological Considerations

One of the strength of this study is the detailed record of prescribing data on individual patients throughout their hospital stay. In addition, the data includes discharge prescription. The data collection process, with data collected daily by trained hospital staff, is an additional strength. The topic of this study was multifaceted and for this purpose the composition of the group of researchers included competence in drug use, obstetrics, statistics and policy science. The lack of data on socioeconomic status limits the possibilities of comparing antibiotic prescriptions between different economic and social classes. Lack of information on proportion of the assisted or non-assisted vaginal deliveries is a further weakness of the study.

4 CONCLUSIONS AND POLICY IMPLICATIONS

Concerns include the high rate of antibiotic prescriptions for vaginal delivery patients and hospital deviations from cesarean section recommendations. Antibiotics are routinely prescribed for prophylactic purposes to women who have both normal and operative vaginal deliveries in this setting, as evidenced by the widespread use of antibiotics in vaginal delivery. Both the benefits of prophylactic antibiotic prescribing during assisted vaginal deliveries and the perceived benefits of antibiotic prescribing during non-assisted vaginal deliveries among health professionals require additional investigation into this practice.

The rational use of antibiotics and improved maternal health are intertwined. As a means of reducing maternal mortality, the Indian government is advocating for institutional delivery. The number of hospital inpatients will significantly rise as a result of this

strategy; This highlights the need for a specific policy in healthcare facilities regarding when and how to prescribe antibiotics during and after delivery. Additionally, a system for monitoring antibiotic prescribing and resistance must be developed and put into action. Several interventions are intertwined, as is typical of policy development and implementation. Policy on antibiotic prescribing must be linked to policy on interventions, such as improving postpartum care, where a large number of infections occur, and infection control measures like hand hygiene.

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DEVELOPMENT AND VALIDATION OF A HPLC METHOD FOR QUANTITATIVE ANALYSIS OF DIACEREIN IN CAPSULES: A STABILITY-INDICATING APPROACH

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Abstract - For the quantitative determination of diacerein in capsule dosage forms, a stability-indicating HPLC method was developed and tested. A perfectsil target ODS-3, 2504.6 mm in diameter, was used to achieve an isocratic separation. Utilizing a UV detector to measure the eluate at a wavelength of 254 nm and 5 m particle size columns with a flow rate of 1 ml/min Phosphate buffer made up the mobile phase: phosphoric acid-adjusted acetonitrile (40:60, v/v) with a pH of 4.0. Thermal degradation, oxidation, hydrolysis, and photolysis all affected the drug. Diacerein was found to break down under neutral conditions as well as under acidic, basic, and oxidative stress. The parent compound was completely separated from the degraded products. The parent compound, diacerein, eluted after approximately 4.9 minutes, with all degradation products taking approximately 10 minutes to analyze.a The technique was straight over the focus scope of 1-10 µg/ml (rS = 0.9996) with a restriction of recognition and quantitation of 0.01 and 0.05 µg/ml separately. To assay diacerein in capsules, the method possesses the necessary accuracy, selectivity, sensitivity, precision, and robustness. The stress studies' degradation products did not prevent diacerein from being detected, so the assay indicates stability.

1 INTRODUCTION

Diacerein 1,8-diacetoxy-3-carboxy anthraquinone is an original osteoarthritis drug which specifically hinders the IL-1. It is a semisynthetic derivative of anthraquinone that comes from certain plants. It directly inhibits IL-1 synthesis and release, which is crucial to the pathophysiology of osteoarthritis and the destruction of cartilage. In human osteoarthritis chondrocytes, IL-1 increases the expression of inducible nitric oxide synthase, increases the release of PGE2, and stimulates matrix metalloproteinases, IL-6, and IL-8, which aid in the breakdown of the joint. Thus, by hindering IL-1 diacerein impedes all obsessive incline started OA. Additionally, IL-1-induced expression of enzymes that degrade cartilage is inhibited by diacerein. Diacerein has no gastrodeodenal toxicity because it does not inhibit prostaglandin synthesis, unlike NSAIDS. It also plays a role in preventing the degradation of proteoglycans and hydroxyproline in joint cartilage. One HPLC method was only reported after a thorough literature review. However, due to the long retention time for pure drug, the reported method was found to take

more time and use more solvent. When it comes to the quantitative determination of diacerein in pharmaceutical dosage form, the validated method that has been proposed is more cost-effective, precise, and specific.

2 MATERIALS AND METHODS

Glenmark Pharmaceuticals, Kurkumbh, India, generously provided the diacerin pure compound, which was utilized without further purification. Diacerein cases, (Artodar) containing 50 mg diacerein according to mark guarantee were bought from nearby drug store. Analytical-grade chemicals were all used. All of the solutions were prepared with HPLC-grade purified water that was obtained through double distillation and filtered through a filter (Millipore, Milford, MA).

2.1 HPLC Instrumentation and Conditions:

The injecting facility for the HPLC system, a Jasco PU- 2080 intelligent HPLC pump with a capacity of 20 l per injection, was utilized. The identifier comprises of an UV/Vis (Jasco UV 2075) worked at a frequency of 254 nm. Jasco Borwin, LC-Net II/ADC system, version 1.5, was the software that was used. A Perfectsil® ODS-3, 5 mm, 250 4.6 mm i.d., was used for the chromatographic separation column. A mobile phase of phosphate buffer-acetonitrile solution (40:60 v/v) at a flow rate of 1 ml/min was used for separation. UV detection at a wavelength of 254 nm was used to keep an eye on the eluent. The injection volume was 20 l, and the column was kept at room temperature. Before use, the mobile phase was filtered through a 0.45 m micron filter.

2.2 Preparation of Stock and Standard Solutions:

By accurately weighing approximately 10 mg of diacerein into a 10 ml volumetric flask, 5 ml of DMSO was dissolved in the diacerein, and HPLC-grade acetonitrile was used to make the stock solution (1 mg/ml). The stock solution was stored for a week and found to be stable after being shielded from light by aluminum foil. A-grade bulb pipettes were used to transfer aliquots of the diacerein standard stock solutions into 10 ml volumetric flasks. The mobile phase was used to make the solutions up to the volume, giving the final concentrations of 1-10 g/ml.

2.3 Estimation of Diacerein from Pharmaceutical Dosage Form:

To decide the substance of diacerein in cases (name guarantee: 50 mg diacerein) 20 containers were opened and the items were gauged and blended. The powder was accurately weighed before being transferred to a 100 ml volumetric flask, where it was dissolved in 5 ml of DMSO and filled up to the required volume with HPLC-grade cetonitrile. To achieve complete dissolution, the volumetric flask was sonicated for 30 minutes. A nylon filter with a size of 0.45 m was used to filter the solutions. A volumetric flask was filled with mobile phase and suitable aliquots of the filtered solution were added, resulting in concentrations of 3, 5, and 7 g/ml. Under the aforementioned conditions, a 20-l volume of each sample solution was injected six times into HPLC. The samples' concentrations were

determined by comparing the sample's area to the standard's. The peak areas were measured at 254 nm.

2.4 Forced Degradation Studies

In order to carry out forced degradation studies, Diacerein pure drug was subjected to a variety of stressors in order to ascertain whether the analytical method and assay were stability-indicating. DMSO was used to dissolve diacerein because it was insoluble in water, chloroform, and methanol. Acetonitrile was used to make up the volume. By dissolving 10 mg of diacerein in 5 ml of DMSO and creating a volume of up to 100 ml with acetonitrile, a stock solution of 100 g/ml was created. For forced degradation studies, this solution was used to remove the stability that indicated the property and specificity of the proposed method. The average peak area of the standard diacerein and degradation sample after application (20 g/ml for HPLC) was determined in all degradation studies across six replicates.

2.5 Oxidation:

Separately, 2 ml of 1% hydrogen peroxide was added to 2 ml of stock solution. The solutions were kept at room temperature for 30 minutes. For the HPLC study, the resulting solution was diluted to a concentration of 20 g/ml, 20 l were injected into the system, and chromatograms were taken to determine the sample's stability.

2.6 Acid Degradation Studies:

2 ml of 0.01 N hydrochloric acid was added to the stock solution. At room temperature, the solution was kept for 15 minutes. After diluting the resulting solution to a concentration of 20 g/ml, 20 l was injected into the system, and chromatograms were taken to determine the sample's stability.

2.7 Alkali Degradation Studies:

2 ml of 0.01 N sodium hydroxide was added to the stock solution. At room temperature, the solution was kept for 15 minutes. After diluting the resulting solution to a concentration of 20 g/ml, 20 l was injected into the system, and chromatograms were taken to determine the sample's stability.

2.8 Neutral Degradation Studies:

The drug was refluxed in water for three hours at a temperature of 70o for stress testing under neutral conditions. For the HPLC study, the resulting solution was diluted to a concentration of 20 g/ml, 20 l was injected into the system, and chromatograms were recorded to determine the sample's stability.

2.9 Dry Heat Degradation Studies:

The standard medication was set in stove at 80º for 6 h to concentrate on dry intensity debasement. For the HPLC study, the resulting solution was diluted to a concentration of 20 g/ml, 20 l were injected into the system, and chromatograms were taken to determine the sample's stability.

3 PHOTO STABILITY STUDIES:

The drug's photochemical stability was also studied by keeping the stock solution (1 mg/ml) on a wooden plank on a terrace for 360 hours. In order to conduct an HPLC study, the resulting

solution was diluted to yield solutions containing 20 g/ml. Twenty 1 were then injected into the system, and chromatograms were taken to determine the sample's stability.

4 CONCLUSION

Stress testing studies revealed that this approach is highly selective for diacerein. From the assay of pure and stressed samples, typical chromatograms were obtained. Six replicates revealed an average retention time standard deviation of 4.9 0.018 for diacerein. The resulting peaks were clear and had a sharp baseline separation.

Diacerein has an anthraquinone moiety with a hydrophilic side chain that includes an acetoxy group and a carboxylic acid group. The parent compound and all of the main degradation products were separated[6,7]. Diacerein was found to be stable under dry heat and no decomposition was observed when the solid drug powder was kept in daylight for 360 hours. However, the drug was unstable when kept at room temperature for 15 minutes under basic stress conditions. The drug was reduced to about 90% degradation. It was also unstable when kept at room temperature for 15 minutes in an acidic environment. The drug was roughly degraded to 48%. The drug was degraded to approximately 54% when kept at room temperature for thirty minutes under oxidative stress conditions with one percent H2O2. When the drug was refluxed with water for three hours in neutral conditions, approximately 30% degradation was observed. Diacerein was measured in stock solution and compared to a freshly prepared standard to determine its stability. In comparison to the freshly prepared standard, the stock solution's response did not significantly alter.

Diacerein in Artodar capsules was determined using the proposed method. The capsules' label claim was 99.68 percent 0.62 percent (%RSD=0.86) as a result of these assays. The assay shows that it is selective for the assay of diacerein and unaffected by the excipients in these capsules, as shown by the results.

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NANOSUSPENSION TECHNOLOGY: A PROMISING STRATEGY FOR IMPROVING BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

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Abstract - Due to their low water solubility, the majority of the novel chemical entities discovered through high-throughput screening in the drug discovery process fail. When drugs that are poorly soluble in water are prepared in conventional dosage forms, many issues arise. The excessively low bioavailability of poorly soluble drugs is one of the most serious issues. Since drugs in the BCS CLASS II category are poorly soluble in both aqueous and organic media and have a log P value of 2, the issue is even more complicated. To address the issues of low bioavailability and low solubility, a variety of formulation strategies are available. Because of their limitations, these methods for improving solubility have limited application in improving solubility. The issues that arise from these conventional approaches to improving solubility and bioavailability can be solved with the help of nanotechnology. The practice of science and engineering at the 10-9 meter nanoscale is referred to as nanotechnology. The current article depicts the insights concerning nanosuspensions. The pure, poorly water-soluble drug suspended in dispersion in nanosuspensions lacks any matrix material. The pharmaceutical applications, characterization and evaluation parameters, and preparation methods with their advantages and disadvantages are all discussed in the review article. Not only does a nanosuspension resolve the issues of low solubility and bioavailability, but it also alters the drug's pharmacokinetics, increasing its safety and effectiveness.

1 INTRODUCTION

Due to their low solubility in water, the majority of the new chemical entities discovered through high-throughput screening (roughly 40%) fail in the drug discovery process. According to a recent report 2, 46% of all New Drug Applications (NDAs) submitted between 1995 and 2002 were BCS class IV drugs, while only 9% were BCS class I drugs. This indicates that the majority of approved new drugs were water insoluble. Because of their poor solubility, it will be more difficult to incorporate them into conventional dosage forms, which will reduce the drugs' bioavailability.

Because they are poorly soluble in both aqueous and organic media and have a log P value of 2, drugs like Glibenclamide, which is part of BCS CLASS II and is classified by BCS System 4, make the issue even more complicated. For class II medications, the rate restricting variable in their digestive retention is disintegration/ dissolvability and in this manner the presentation of these medications is

disintegration rate-restricted and is impacted by the fed/abstained condition of the patient. The drug's shape and particle size have a significant impact on its dissolution rates for sparingly soluble drugs. As a result, the rate of dissolution increases when particle size decreases. There are number of plan moves toward that can be utilized to determine the issues related with the low solvency and low bioavailability of these class II medications. Micronization, solubilization with co-solvents, use of permeation enhancers, oily solutions, surfactant dispersions, salt formation, and precipitation techniques are all methods of increasing solubility.

The majority of these methods for improving solubility have some benefits but also drawbacks, so their use in improving solubility is limited. Microspheres, emulsions, microemulsions, liposomes, supercritical processing, solid-dispersions, and inclusion complexes made with cyclodextrins are just a few of the other methods for improving solubility, but they don't work for all drugs. These procedures are not relevant to the medications, which are not dissolvable in both fluid and natural Media.

Particle engineering technologies capable of formulating poorly soluble drugs to improve their efficacy and optimize therapy in terms of pharmacoeconomics still need to be provided to the pharmaceutical industry. One such original innovation is nanosuspension innovation. Sub-micron colloidal dispersions of nanosized drug particles stabilized by surfactants are known as nanosuspensions. The poorly water-soluble drug is suspended in dispersion in nanosuspensions with no matrix material. These can be used to make drugs that are hard to dissolve in aqueous or lipid media more soluble. The active compound floods at a faster rate as a result of the compound's increased solubility, speeding up the process that leads to its maximum plasma level.

When compared to other methods for increasing solubility, this is one of its distinct advantages. This approach is helpful for particles with unfortunate dissolvability, unfortunate porousness or both, which represents a critical test for the formulators. Because of the smaller particle size, poorly soluble medications can now be administered intravenously without clogging blood vessels. The suspensions can also be made into a solid matrix by lyophilizing them. It also has the advantages of liquid formulations over others, in addition to these advantages. The various preparation methods, critical parameters, and evaluation of the nanosuspension are the primary focus of this review.

2 PREPARATION OF NANOSUSPENSIONS

Nanosuspensions have been shown to be a more cost-effective and technically simpler option than liposomes and other conventional colloidal drug carriers, particularly for drugs that are poorly soluble, and to produce a product that is physically more stable. Micronization by colloid or jet milling is the simplest way to prepare nanosuspensions. This method increases the rate of dissolution but has no effect on

saturation solubility. Preparation methods like precipitation, high pressure homogenization, emulsion, and milling are currently utilized in nanosuspension engineering processes. In the sections that follow, these methods and the compounds that were produced are briefly discussed. There are primarily two ways to make nanosuspensions. "Bottom Up technology" refers to the conventional approaches to precipitation. Disintegration techniques, also known as "Top Down Technologies," are favored over precipitation techniques. High-Pressure Homogenization in nonaqueous media (Nanopure), Media Milling (Nanocrystals), High-Pressure Homogenization in water (Dissocubes), and Precipitation and High-Pressure Homogenization in combination (Nanoedege) are a few examples. Emulsion as a template, microemulsion as a template, and so forth are a few additional methods utilized in the preparation of nanosuspensions.

- Precipitation
- Templates for Lipid Emulsions and Microemulsions
- Homogenization at High Pressure
- Milling Techniques

3 APPLICATIONS

The drug's bioavailability, dissolution rate, and saturable concentration all rise when it is made into nanosuspensions. These nanosuspensions are being used in oral, parenteral, topical, ophthalmic, mucoadhesive, pulmonary, and targeted drug delivery applications. Because of their improved adhesion properties, nanosuspensions can be administered orally as a drug delivery strategy to target gastrointestinal bacterial and parasitic infections in addition to improving bioavailability. Nanosuspension innovation is considered as reasonable new colon conveyance frameworks for the therapy of colon malignant growth, helminth contaminations, gastrointestinal aggravation or GIT related infections like sprue (zoeliaki). Parasites in the MPS's macrophages are the cause of infections like tuberculosis, listeriosis, leishmaniasis, and toxoplasmosis. Because of this, they are relatively simple for I.V. injected particles to get to. In the event that they absorb uptake-promoting proteins like apolipoproteins, the MPS cells rapidly and heavily absorb the particles that have been injected with an IV. However, the CNS is also home to some parasites. If not treated, the brain-localized parasite typically results in recurrent infections. Consequently, it would be crucial to target drug nanoparticles at the brain through surface modification. Kreuter et al. reported that Tween 80® surface-modified polyisobutyl - cyanoacrylates nanoparticles were able to successfully deliver the peptide dalargin to the brain. Amphotericin B nanosuspension developed by Kayser et al. exhibited a significant increase in oral absorption when compared to standard commercial formulations. Particle sizes of less than 5 m are preferred when administering IVs. The nanoscale size of the particles will make it easier for the drug to get into the body's small blood vessels without being blocked. To stop omeprazole from degrading when taken orally, a stable formulation that can be injected intravenously has been made.

Watery suspensions of the medication can be effectively nebulised and given by pneumonic course as the molecule size is exceptionally less. Nebulizers of various kinds are available for administering liquid formulations. Budesonide, ketotifen, ibuprofen, indomethacin, nifedipine, itraconazole, interleukin-2, gene, leuprolide, doxorubicin, and others have all been successfully tried through the pulmonary route. 58 Because the surface of the particle can be appropriately altered to make it target-specific, nanosuspensions can also be used for targeted delivery. To improve drug targeting against leishmania-infected macrophages, Kayser created a nanosuspension of Aphidicolin. Scholer and co an improved drug targeting to the brain in the treatment of toxoplasmic encephalitis in a new Toxoplasma gondii-infected murine model was demonstrated by preparing a nanosuspension formulation of Atovaquone.

4 CONCLUSIONS

Nanosuspensions, which are primarily regarded as means of administering drugs that are poorly soluble in water, have largely resolved dissolution issues to enhance drug absorption and bioavailability. Traditional dosage forms can be combined with nanosuspension technology: tablets, cases, pellets, and can be utilized for parenteral items. As colloidal carriers for the targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents, or diagnostic agents, they have recently received a growing amount of attention. They are easy to target at the tumor area due to their submicron size. Additionally, the potential for surface functionalization with a targeting moiety has made it possible to deliver drugs, genes, photosensitizers, and other molecules precisely where they are needed. As oral formulations and non-oral administration develop in the future, nanosuspensions will continue to be of interest in order to take advantage of nanosuspension drug delivery, simple formation technologies, and a variety of applications. It is normal that future innovative work will be done soon for clinical acknowledgment of these designated conveyance vehicle.

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EXAMINING THE IMPACT OF ACCESS TO ANTIBIOTICS ON ANTIBIOTIC POLICY

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Abstract -
Objective: Using standardized WHO/HAI methodology, the current survey was conducted to investigate the price and availability of a basket of 24 essential antibiotics and eight high-end antibiotics at various levels of public and private health care in the National Capital Territory of Delhi, India.
Methods: Three public health care providers in the state provided information on procurement prices and availability: the Municipal Corporation of Delhi (MCD), the state government, and the federal (central) government. The survey included 83 public facilities, 68 primary care facilities, 10 secondary care facilities, and 5 tertiary care facilities. A leading corporate house's private retail (n = 40) and chain pharmacies (n = 40) were also the sources of the data. The prices were compared to a global reference price, which was represented by the median price ratio, or MPR.
Results: Sector public: The Delhi state government uses the Delhi state essential medicine list (Delhi state EML) for procurement; The procurement lists for the other two agencies were distinct. Except for injections, primary care facilities had access to all of the acquired antibiotics, including those of the second and third generations. The antibiotics that were available were chosen based on supply rather than rationality or the Delhi state EML, and none of them were always readily available. Some essential antibiotics were scarce, while others that weren't essential were readily available. In tertiary care facilities, antibiotic availability was also subpar. Sector private: It was good that antibiotics were available. The most expensive and well-known brand names were frequently available for the majority of the antibiotics. Meropenam, gemifloxacin, moxifloxacin, and other high-end antibiotics were commonly available. Some non-essential antibiotics of a newer generation, like gemifloxacin, cost less in retail pharmacies than the most expensive generics of azithromycin.
Conclusions: Newer generation antibiotics, which can currently be purchased without a prescription, are likely to result in overuse and an increase in resistance if they are not made available at appropriate prices. The essential medicine concept should be followed by all providers, who should follow the EML of whichever of the three concerned Delhi public sector agencies it falls under. Drug schedules and pricing policies that will prevent unintentional access to the latest generation of antibiotics must be taken seriously immediately by Indian regulatory authorities.
Keywords: Primary care, the public health sector, private retail pharmacies, procurement prices, antibiotics, availability, and price.

1 INTRODUCTION

Because antimicrobial resistance (AMR) is spreading rapidly across the globe, appropriate policies and actions to combat AMR are urgently required. Over-prescription and inappropriate use of antibiotics are widely cited as reasons for the rapid rise in AMR. As a result of this consensus, there is a need for an antibiotic policy that should ensure the appropriate selection and use of antibiotics. It will be necessary to have a nationally or locally coordinated antibiotic policy that is contextualized to the particular country or region in order to have any significant impact on appropriate antibiotic use because health systems and prescribing behavior are diverse and complex. Measuring antibiotic access and use is the first step in combating inappropriate antibiotic use to determine the scope of the problem and establish a baseline against which any intervention can be compared and evaluated. In order to investigate patterns of antibiotic use as well as guidelines for antibiotic use, extensive surveillance programs have been established in a number of developed nations. However, in settings with limited resources, such surveillance is not possible. In developing nations, the issue of AMR—the appropriate availability and inappropriate use of antibiotics—has received relatively little attention. A few collaborative studies have recently been conducted in India to develop a validated method for measuring antibiotic consumption and use in the hospital and community. In both public and private settings, overprescribing, antibiotic misuse, and a lack of adherence to treatment guidelines were observed. On the other hand, there is a lack of literature in India regarding the accessibility, availability, and cost of various antibiotics at public and private retail pharmacies. As a result, the purpose of the current survey, which was conducted between July and October 2011, was to measure the availability of various antibiotics in the public and private sectors of New Delhi using a standard approach developed by Health Action International (WHO/HAI). The survey was conducted with the ultimate goal of advancing an antibiotic policy that will promote the availability of appropriate antibiotics (rather than ineffective ones) and their rational use at various healthcare levels.

2 METHODS

State background: The National Capital Territory of Delhi (NCT Delhi) is the name given to Delhi, India's capital. In NCT Delhi, the three primary providers of public health care are: the Focal (national) Government under the Service of Wellbeing and Family Government assistance (MoH&FW), the Directorate of Wellbeing Administrations (DHS) in the Public authority of NCT (GNCT) Delhi and one more open area supplier in Delhi city - the Civil Company of Delhi (MCD). The facilities of the three public healthcare providers offer free services to all citizens. The essential or procurement list of medicines is unique to each of these public sectors' procurement systems. Chain pharmacies have recently entered the Indian retail market from the private sector. As a result, the survey included both types

of retail pharmacies: traditional private retail pharmacies and leading corporate house chain pharmacies.

Sampling: This survey was carried out in accordance with the WHO/HAI methodology and surveyed five facilities across the entire state of Delhi in each of the eight districts of Delhi.

Public sector: All three centralized public sector procurement agencies and the department of healthcare providers provided prices for central procurement. To supplement the supply from the central government procurement agency, tertiary care facilities under the CG also conduct independent medicine procurement. These tertiary care facilities also provided prices for procurement; Specifically, Safdurjung Hospital (SH) and Ram Manohar Lohia Hospital (RML), both of which use pooled procurement (referred to as CG1), and Lady Hardinge Medical College (LH), which uses independent procurement (referred to as CG2).

Since all public facilities provide free medicines, only antibiotic availability was gathered from the various public facilities. Four primary health care (dispensaries) hospitals and one secondary care hospital were chosen at random for each district. A tertiary care rather than a secondary care hospital was enrolled in one of the districts. Generally a sum of 83 offices (40 offices under GNCT, Delhi, 40 under MCD and 3 tertiary consideration offices of CG) were overviewed.

Private Sector: By selecting one retail pharmacy in each sector that is geographically closest to each public outlet, the private sector samples were identified. Five retail chain pharmacies and five retail pharmacies were included in each district. Consequently a sum of 80 offices were studied.

Antibiotics surveyed: The WHO/HAI method identifies a basket of 30 core medicines, 14 of which are essential medicines based on the global disease burden and 16 that are specific to South East Asia. Seven of these thirty drugs come in a variety of dosage forms, including antibiotics. Eight additional high-end antibiotics were also surveyed in public and private pharmacies for secondary and tertiary care. According to the WHO/HAI approach information was gathered for both the originator brand, and the lowestpriced conventional comparable found at each medication outlet. Since process patents were not recognized by the Indian regulatory system until 2005, all medicines manufactured in India are generic versions. Nevertheless, every product bears a brand (or trade name). In India, originators brands (OBs) do not have any additional recognition as originator brands. Often, OBs are not available, but branded generics, the same molecules produced by different companies with different trade names, are. As a result, a third version, highest-priced generic (HPG), was added to this survey in addition to the originator brand and lowest-priced generic (LPG) to determine the price and availability of seven essential antibiotics at each facility. At each private facility, two versions, highest-priced and lowest-priced generics of

seventeen supplementary antibiotics and eight high-end antibiotics were surveyed. Since each medicine is only available in one version in the public sector, only the lowest-priced generic (LPG) was gathered for price and availability.

With a standardized form, trained data collectors went to enrolled facilities and recorded the cost and availability of each antibiotic. The price for public sector procurement was collected from three central agencies: the Delhi state government's central procurement agency (CPA; GNCT, Delhi); MCD's procurement department; and the CG maintenance division of the Medical Stores Organization (MSO). Obtainment cost was additionally gathered from two decentralized destinations of focal government emergency clinics - CG1 (RML/SH) and CG2 (LH). The WHO/HAI workbook's automated analysis and double-entry feature were used to enter medicine unit prices into Excel spreadsheets. Antibiotic prices are expressed as median price ratio (MPR) in order to facilitate international comparisons. The MPR compares a medicine's local median unit price to the median unit price in the Management Sciences for Health (MSH) Price Indicator Guide, 2010.

3 ETHICAL APPROVAL

Ethical approval of the study was obtained from Vallabhbhai Patel Chest Institute, University of Delhi, India. Permission for data collection was obtained from Health Department, Directorate Health Services (DHS) of Government of NCT Delhi, Municipal Corporation of Delhi, and from Ministry of Health & Family Welfare, Government of India.

4 CONCLUSION

While other non-essential antibiotics are readily available in both the public and private sectors, the availability of some essential antibiotics is subpar. An EML was only partially followed in procurement and distribution by one public sector agency. Some more recent reserve antibiotics are cheaper than older essential non-reserve antibiotics and are freely available in the private sector. A situation like this is likely to contribute to AMR and the inappropriate use of antibiotics. To ensure that all public sector procurement follows an evidence-based EML and that newer antibiotics are not available over-the-counter in the private sector at inappropriately low prices, urgent managerial and regulatory interventions must be initiated.

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EXPLORING THE PROS AND CONS OF TRANSGENIC ANIMAL PRODUCTION AND ITS WIDE-RANGING APPLICATIONS

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Abstract - Organic entities containing coordinated successions of cloned DNA (transgenes), moved utilizing strategies of hereditary designing (to incorporate those of quality exchange and quality replacement) are called transgenic creatures. The rapid expansion of biotechnology research has included the creation of transgenic animals. In addition to producing organs to meet the demand for organ transplantation, transgenic animals were created with the intention of producing breeds of higher quality and better quality, increased milk yield, and both. Animals that have been genetically modified are becoming increasingly important in the research and development of new treatments and cures for numerous serious diseases. Transgenesis is a revolutionary new method for introducing foreign genetic material into animals to alter their characteristics. Numerous methods, including pronuclear micro-injection, ES cell manipulation, Cre-lox technique, viral vectors, cytoplasmic injection, primordial germ cells, nuclear transfer, and spermatogonial manipulation, are now available for the production of transgenic animals. Lentiviral vectors and chimera generation by injecting pluripotent cells are becoming important tools for transgenesis, and they have contributed to human welfare in numerous ways, including agriculture, medicine, food, disease model, industrial Transgenic animals demonstrated that genetically modified animals will play a significant and important role in the biomedical field in the next five to eight years, particularly through the supply of xenografts and the production of valuable pharmaceutical proteins. The goal of this paper is to provide some insight into how transgenic animals develop and how they can benefit human welfare.
Keywords: Stem Cell, DNA Microinjection, Lenti Virus, and Transgenic Animal.

1 INTRODUCTION

The only practical method for studying the regulation and function of genes in mammals prior to the development of molecular genetics was to observe specific traits. This method was utilized by farmers to increase milk production. In 1970, the first chimeric mouse was made. The first transgenic creatures were delivered right around quite a while back by utilizing microinjection of unfamiliar deoxyribonucleic corrosive into the pronuclei of zygotes.

Sperm-mediated gene transfer4 laid the groundwork for the production of transgenic animals, and in the 1980s, the most widely used microinjection method was used to produce transgenic mice. In 1985s6, a number of transgenic animal creations were reported. When creating transgenic animals, a variety of methods are utilized. The microinjection technique is frequently used, but it has a few disadvantages, such as low efficiency and variable expression patterns. Therefore, sperm-mediated DNA transfer, intracytoplasmic injection of sperm heads containing foreign DNA, injection or infection of oocytes and/or embryos by various viral vectors, ribonucleic acid (RNA) interference technology, and the utilization of nuclear transfer are examples of alternative methods.

Transgenic animals are organisms that have integrated sequences of cloned

DNA (transgenes) that have been transferred through the use of genetic engineering methods like gene transfer and gene substitution. There are a few sorts of transgenic creatures like transgenic sheep, birds, chickens, pigs, bugs and so forth. In addition to producing organs to meet the demand for organ transplantation, transgenic animals were created with the intention of producing breeds of higher quality and better quality, increased milk yield, and both. Animals that have been genetically modified are becoming increasingly important in the research and development of new treatments and cures for numerous serious diseases. Transgenesis is a revolutionary new method for introducing foreign genetic material into animals to alter their characteristics. A few significant advantages and dangers of transgenic creatures.

2 METHODS FOR PRODUCING TRANSGENIC ANIMALS

The introduction of a foreign gene or genes into an animal—the inserted genes are referred to as transgenes—is the fundamental step in the production of transgenic animals. To ensure that all animal cells, including germ cells, contain the same modified genetic material, the foreign genes must be passed down through the germ line. Mice were the first transgenic animals produced in 1980. Transgenic animals can be produced in a variety of ways.

DNA Microinjection: Pronucleus microinjection was first depicted by Gordon and Associates. Several hours after the sperm enter the oocyte, microscopically visible male and female pronuclei appear. It is possible to microinject the transgene into either of these pronuclei The Pronucleus microinjection method was used to create Big Blue animals and Muta mice 18. Only 0.5–3 percent of the microinjected embryos that produce transgenic offspring are able to integrate the transgene into the founder animal's genome using this method.

All transfection methods work with cultured animal cells, but microinjection is usually not used because it is time-consuming and only allows for a small number of cells to be handled 20. In order to guarantee that transgenic DNA is present in all host cells, the method makes it possible for the transgene to be incorporated into the host DNA as soon as possible. Before harvesting newly fertilized eggs before the pronuclei fuse, first isolate the required piece or pieces of DNA and clone them into a vector, such as a plasmid. The nucleus of the sperm cell (for males) or the egg cell (for females) before they join to form the fertilized zygote is called a pronucleus. The DNA fragment that has been isolated is then injected into the sperm cell's pronucleus using a syringe.

After the pronuclei of the new zygote fuse to form the nucleus, the cells are allowed to divide into two embryonic cells before the embryo is transferred into the uterus of a pseudopregnant mouse, which is a female mouse that has been mated with a vasectomized male mouse. This is done so that the hormones in a pseudopregnant mouse's body can make her uterus more receptive to the embryo that will be

Because the microinjection of the transgene's DNA is a random process, not all of the pups that are born will have this gene expressed. A sample of the pup's tail tissue will be taken and DNA will be analyzed because this could occur if the gene inserts itself in an area of DNA that is not normally expressed.

Retrovirus mediated gene transfer: Male germ line stem cells are used to create transgenic mice through retroviral transduction. Since male germ line stem cells are capable of self-renewal, genetic modification of these cells would assist in

the biology of their intricate self-renewal and differentiation processes as well as the generation of numerous transgenic animal species. A retrovirus is one in which the genetic material is transmitted via RNA rather than DNA. Using retroviruses as a vector, genetic material is transferred into the host cell, resulting in the generation of chimera (an organism with distinct genetic makeup in different parts or tissues). Chimeras are inbred for up to generations before producing homozygous transgenic offspring, which carry the desired transgene in every cell. In 1974, a simian virus was successfully injected into the embryos of mice, resulting in mice containing this DNA.

Nuclear Transfer Method: The transgenic goats were created using nuclear transfer of fetal somatic cells in this method. Benefactor karyoplasts were gotten from an essential fetal substantial cell line got from a 40-day transgenic female hatchling created by planned impregnation of a nontransgenic grown-up female with semen from a transgenic male. Two different methods of nuclear transfer were used to produce live offspring.

Transfection of Gametes: Beginning in the early 1960s, the first transfection procedures were performed, and subsequent experiments with various tissues and cell types are now common. Various transfection techniques have been used:

a) The process of introducing foreign genes into cultured cells or tissues in vitro
b) The in vivo method, in which genes are directly injected, sprayed, etc., into the tissue;
c) The ex-vivo method, in which transfected cells are introduced into a living organism after being grown in a laboratory.

In order to check for transfection, gametes are placed in a solution containing the gene constructions for brief periods of time before being used for insemination or in vitro fertilization. Naked DNA has been used to good effect in a few instances, but DNA-Liposome complexes or electroporation procedures have also been used. On account of the female gamete in vitro transfections utilizing liposomes or retroviruses have been applied effectively. Electroporation, high-velocity microprojectiles, and particle guns are other methods that have been used. Autoradiography, immunocyto chemistry, and fluorescent in situ hybridization have all been used to locate the foreign gene in spermatozoa. Eighty percent of the spermatozoa that were transfected using the in vitro or in vivo transfection procedures did so. Most of the time, these results showed that the foreign gene got into the spermatozoa's nucleus, and molecular tests like Southern Blot, PCR, and gene sequences have shown that the transgene is in the DNA of the gametes.

3 APPLICATIONS OF TRANSGENIC ANIMALS:

As disease model: Due to their physiological, anatomical, and genomic similarities to humans, mice have historically been utilized as disease models. In order to study effective treatments for diseases like Alzheimer's, cancer, and AIDS, transgenic animals are used as disease models. These animals have their genes changed to show symptoms of the disease. Scientists can learn more about how specific diseases are caused by genes by studying transgenic animals. The development of disease models in mice rather than dogs or non-human primates, as well as the degree of discomfort experienced by parent animals during the experimental procedures, are among the advantages of using transgenic animals. Another benefit is the possibility of replacing higher species with lower species. It has been discovered that transgenic animals like

mice are useful for investigating gene function and analyzing various hereditary diseases.

As food: The Food and Drug Administration (FDA) advised that humans could consume cloned animals and their products 40. Due to their muscle hypertrophy, they have some disadvantages, such as difficulty calving that necessitates Caesarean sections, low calves' viability, and low fertility.

Drug and Industrial production: Transgenic animals are used to make proteins like alpha-1-antitrypsin, which is made in the liver and is used to treat emphysema and cystic fibrosis. Compared to using human cell culture to make protein, this method is cheaper. Foreign substances like bacteria, spores, and dust constantly affect the human lungs. Neutrophils release the enzyme elastase to prevent these, but this enzyme damages the elastin in the lungs, which keeps the lungs supple. As a result, sheep successfully expressed a protein 1 proteinase inhibitor that the human body releases. Human recombinant proteins made in transgenic animals' mammary glands. Drug proteins are presently utilized for business reason. The spider genes were inserted into the cells of lactating goats by two researchers at Nexia Biotechnologies in Canada. Goats are used to produce silk, milk, and bucketfuls of tiny strands that are secreted from their bodies. by taking the polymer strands out of the milk and weaving them into thread, a light but durable material that can be used to make military uniforms, medical micro sutures, and tennis racket strings. Sixty percent of Americans support the use of transgenic animals. Monoclonal antibodies are produced from transgenic goats' mammary glands. The blood of transgenic cattle is used to make a recombinant bispecific antibody.

4 CONCLUSION

Human health and well-being have been significantly improved by transgenic animals throughout history. Animal breeding now has a new dimension thanks to recent advancements in reproductive technologies like in vitro embryogenesis, sperm sexing, somatic nuclear transfer, lentiviral transfer of oocytes and zygotes, and chimera generation by injecting pluripotent cells. Transgenic animals demonstrated that genetically modified animals will play a significant and important role in the biomedical field in the next five to eight years, particularly through the supply of xenografts and the production of valuable pharmaceutical proteins. Intriguing strategies being created will keep on extending this significant and valuable area of trial and error.

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