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Research Article

A Virtual Study on the Multitarget Potential Efficacy of the Ligands, Alpha Asarone and Glabridin, in Ameliorating Behavioural Deficits due to Neurodegeneration of Hippocampus Induced by Chronic Restraint Stress

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ABSTRACT

Life span of human, a gradually increase can occur with change in diet and life style which play an important role in delaying or even block the progression of age related degenerative problems like dementia, Alzheimer's which decrease the cognitive function mainly learning and memory. The objective of the study was to find the multitarget potential efficacy of the ligands, Alpha asarone and Glabridin, in ameliorating behavioral deficits due to hippocampal damaged neurodegenerative condition induced by chronic restraint stress. Hence in the current study we analyzed polypharmacological effect of the two natural compounds on the Mitogen activated protein kinases (MAPK's) families which are mainly involved in neurodegenaration by molecular docking using iGEMDOCK software, the drug likeliness and their absorption, digestion metabolic and toxicity profile were analyzed by DruLiTo and admetSAR software. With the results of molecular docking the two natural compounds were selected and taken for experimental study.Experimental Groups received chronic restraint stress 6hrs/day for 21days. Behavioural performance, Biochemical and Histopathological analysis of rats' brain were estimated. Statistical analysis was done by one-way analysis ofvariance, followed by *post hoc* Dunnett's test. $P<0.05$ was considered statistically significant. The results suggest that both the natural compounds α asarone and Glabridin has significantly improved cognitive functions in rats subjected to chronic restrain stress. The Corticosterone concentration was decreased in rats pre-treated with α asarone and Glabridin. The results of molecular docking and further animal study in pre treatment of rats with Glabridin and αasarone before exposure to chronic restrain stress showed observable neuroprotection and improved cognition that could be due to the antioxidant action of the compounds in the rat hippocampus. Hence, these two natural compounds could be an adjuvant therapy for treatment of neurodegenerative diseases. Hence we conclude that the two natural compounds play a role in neuronal stress adaptation mechanism and have potential to prevent progression of neurodegenerative diseases.

Keywords: Molecular docking, Polypharmacolgy, Cognition,Restrain stress.

INTRODUCTION

Most of the therapeutic drugs are withdrawn at various levels of clinical trials due to their side effects or toxic profile. The multitarget perceptive drugs have shown to have less side effects when compared with single-target drugs or combined drug therapy[1,2]. Hence polypharamacology is an emerging path in developing new drugs with multi targeting effect. Further, the disease of central nervous system, cancer, various inflammatory diseases and the complication in their pathogenesis has turned the researcher's interest towards polypharmacological approach. One such study involves 'Computer aided multitarget ligand designing by virtual screening' as an effective path to find the multitarget potential of a ligand[3]. The notion of developing a ligand and Polypharmacology, mainly in treatment of neurodegenerative diseases(NDD) is essential as the pathogenesis of NDD is very complex and needs designing a drug with multitarget portrait.

Oxidative stress is the major etiology of NDD as the release of reactive oxygen species and reactive nitrogen species causes damage like nucleic acid breakage, enzyme inactivation, polysaccharides depolymerisation and other destruction leading to cell death[4] which lead to damage of the brain tissues mainly in the hippocampus and amygdala leading to loss of memory and learning[5,6]. There are many natural compounds present in the plants to counter these oxidative stress. The natural plants or herbs, their

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polyphenols and flavonoid compounds has been proved to have protection against neurodegeneration by inhibiting apoptosis and enhancement of neurocognition by facilitating the neurogenesis[7,8]. The aim of the current study is to determine ligands or compounds possessing antioxidant effects from the natural plants that have the multitarget or polypharmacology.

The selected ligands were docked with multiple targets of Mitogen-activated protein kinases(MAPK) subfamilies by molecular docking method as the computational method are time and resource saving processes. Restraint stress is accepted method to elicit physiologically significant stress model in animals[9]. Hence in the study Chronic restrain stress (CRS) rat model were created by giving stress 6hrs for 21 consecutive days. We explored the effect of repeated CRS in cognitive impairment using behavioural parameters and biochemical changes of Corticosterone level were estimated. To confirm the histological changes Haematoxylin and eosin staining technique was done. The results were compared in between groups.

MATERIALS AND METHODS

Analysis of physiochemical properties and drug likeness

The ligands, α asarone (ID – 636822) and Glabridin (ID – 124052) were downloaded from Pubchem Database. The ligands are natural antioxidants and their physiochemical properties were analysed before docking using software like admetSAR, DruLiTo. Depending upon the absorption, distribution, metabolism, excretion, toxicity profile and drug likeness, the ligands were selected for further studies. The PDB structure of target was downloaded from RCSB. The targets selected were p38 (3GCV), JNK (1JNK), ERK1/2(1TVO), ERK5 (2Q8Y) all these targets are Mitogen-activated protein kinases (MAPK) subfamilies involved in various NDD.

Molecular docking with iGEMDOCK

iGEMDOCK is used for docking and virtual screening of the target-ligand interaction profile as this docking tool is more reliable and accurate in drug discovery field. Rapid virtual screening of the ligands in iGEMDOCK V2.0 with a population size of 800 is set with 80 generation and 10 solutions for accurate docking. The results are predicted based on the interaction profiles like Electrostatic energy, Vander Waal energy, Hydrogen bond energy, Elect.[10,11]

Animals and Chemicals

The study was conducted with the approval from Institute's Animal ethical Committee. Healthy adult male Wistar albino rats weighing between 180-200g were housed in cages (45x28x20cm) with food and water available ad libitum and maintained on 12/12-h light/dark cycle at constant temperature of 22 ± 2°C. The drugs α-asarone and Glabridin was purchased from Sigma chemicals.

Stress parameters

Restraint stress (procedure)

The rats were placed in the restrainer of 25cm x 7cm without supply of food and water for 6hrs (from 8.30AM to 2.30PM) every day for 21 consecutive days. The restraint apparatus had multiple holes allowing the animals to stretch the legs but will not allow the animal to move within the restrainers[12,13].

Corticosterone (assay)

To determine the effectiveness or potency of stress application, the plasma Corticosterone level was analysed. Before sacrificing the rats, the blood samples were collected a by Cardiac puncture in heparinized tubes from all the group animals. The samples were centrifuged at 3500 rotations for 15 min and the plasma was stored at -70 ˚C until used for the corticosterone assay. Corticosterone levels were determined by an ELISA assay.

Behavioural parameters

Elevated plus maze

It is a Simple method to assess anxiety- like behaviour in rats. The apparatus is made of wood, two open arm (50x10x2cm)and two closed arm (50x10x40cm) opposite to each other elevated 45cm from the ground. The animal was placed in the centre of the maze and let to explore the maze for 5minutes.The following variables were analysed- time spent in open arm with all 4 paws, number of time animal crossed over open arm, total number of arm crossed (both close & open), number of fecal pellet, and number of rearing[14].

Open field test

Open field exploration test is a measure of emotional behaviour in animal. It is a systematic assessment of new environment exploration, locomotor activity and anxiety related behaviours in rats. The OFT apparatus is made of a large square shaped arena of 80 cm×80 cm with 40 cm high walls. The floor is marked into 25 equal square segments to allow quantification of locomotor activity. Each rat was placed at the centre of the arena and was observed for the following:- Time spent in the periphery of the arena (ambulation), Time spent in the centre of the arena (ambulation), Number of times standing on the hind legs (rearing) with or without support of wall, the grooming behaviour and the number of faecal boli (defecation) passed by the animals[15].

Eight arm radial maze

Eight arm radial maze is to test spatial learning and memory .It is designed to asses or evaluate working and reference memory. The radial arm maze consist of eight arms, numbered from 1 to 8 arms of (48x 3x 12 cm), extending radially from a central platform (40 cm in diameter), with a 5 cm edge around the apparatus. Each Radial arm is equally spaced and contains food cups at the end. Removable blocks of 9x3x13 cm were used to block the selected arm of the maze. The maze was elevated 40cm from the ground or floor. The following variables will be scored for 10 min and recorded- Number of reference memory errors (entry of animal into the non-baited arm), Number of working memory errors (re-entry of animal into already visited baited arm), Time taken to visit all four baited arm[16].

Histopathology

Brain sectioning and staining

The rats brain were collected from all the four groups at the end of experimentation with aesthetic sacrifice procedure. Each of the brain tissue was gently rinsed with saline solution 0.9%Nacl so as to remove blood and other

Table 1: iGEMDOCK result of α asarone and Glabridin against four targets.

S.No	Name of the target	Name of the ligands	Total binding energy	VdW	H bond
1.	P38 MAPK	α asarone	-116.7	-103.4	-13.3
		Glabridin	-123.7	-116.80	-6.9
2.	JNK	α asarone	-97.5	-91.1	-6.4
		Glabridin	-94.4	-91.8	-2.5
3.	ERK ½	α asarone	-102.0	-77.8	-24.2
		Glabridin	-101.8	-91.74	-10.0
4.	ERK 5	α asarone	-126.3	-80.0	-29.3
		Glabridin	-84.4	-78.4	-5.9

Table 2: ADMET Predicted Profile of GLABRIDIN.

Blood-Brain Barrier	BBB+	0.5803
Human Intestinal Absorption	HIA+	0.9892
Caco-2 Permeability	Caco2+	0.8867
P-glycoprotein Substrate	Substrate	0.6994
P-glycoprotein Inhibitor	Non-inhibitor	0.8761
	Non-inhibitor	0.6823
Renal Organic CationTransporter	Non-inhibitor	0.8787
Aqueous solubility	-2.9380	LogS
Caco-2 Permeability	1.6367	LogPapp, cm/s
CYP450 2C9 Substrate	Non-substrate	0.7476
CYP450 2D6 Substrate	Non-substrate	0.8567
CYP450 3A4 Substrate	Substrate	0.5760
CYP450 1A2 Inhibitor	Inhibitor	0.5934
CYP450 2C9 Inhibitor	Inhibitor	0.6850
CYP450 2D6 Inhibitor	Non-inhibitor	0.7926
CYP450 2C19 Inhibitor	Inhibitor	0.7623
CYP450 3A4 Inhibitor	Non-inhibitor	0.6114
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	0.7998
AMES Toxicity	Non AMES toxic	0.8678
Carcinogens	Non-carcinogens	0.9114
Fish Toxicity	High FHMT	0.9395
TetrahymenaPyriformis Toxicity	High TPT	0.9619
Honey Bee Toxicity	High HBT	0.7538
Biodegradation	Not ready biodegradable	0.9962
Acute Oral Toxicity	III	0.5318
Carcinogenicity (Three-class)	Non-required	0.6797
Caco-2 Permeability	1.1051	LogPapp, cm/s
Rat Acute Toxicity	2.9435	LD50, mol/kg
Fish Toxicity	0.5048	pLC50, mg/L
TetrahymenaPyriformis Toxicity	1.1034	pIGC50, ug/L

tissue debris sticking to the tissue. Later the tissue was fixed in 10% formalin for 24 hours before being dehydrated using ethanol (70% for 24 h, 90% for 1 h and 100% for 1 h) then cleaned in xylene and embedded in paraffin wax. By using microtome a series of 5 µm thicknesses coronal sections were cut which was mounted on glass slides and stained by using Haematoxylin-eosin stain. From the slides, the hippocampus were assessed microscopically by using 40x magnification[17,18,19].

Experimental Protocol

Molecular docking

Before starting the animal experiment a computer based molecular docking study was done using iGEMDOCK software for the two selected natural compounds; α asarone of *Acorus Calamus Linn* and Glabridin of *Glycyrrhiza Glabra* which are polyphenolic compounds from natural sources and has antioxidant effects.[][20,21] The absorption, digestion, metabolism excretion and toxicity profile was analysed using admetSAR software. The drug likeliness property of the two compounds was checked using DruLiTo software. Based on their result the compounds were further used for animals study All animals were randomly grouped into four groups (n=6 in each group).

Group I: Control group -- received normal saline (1ml/kg/day i.p)

Group II: Experimental control group – received normal saline (1ml/kg/day/ i.p) and 6 hrs/day Chronic restraint stress for 21days.

Group III: α asarone group -- received α asarone of *Acorus Calamus Linn* (9mg/kg/day/i.p.) 30min prior to 6hrs/day chronic restraint stress for 21 days.

Table 3: ADMET predicted profile of α ASARONE.

Blood-Brain Barrier	BBB+	0.9151
Human Intestinal Absorption	HIA+	1.0000
Caco-2 Permeability	Caco2+	0.8869
P-glycoprotein Substrate	Non-substrate	0.6830
P-glycoprotein Inhibitor	Non-inhibitor	0.5085
	Non-inhibitor	0.8381
Renal Organic Cation Transporter	Non-inhibitor	0.8919
CYP450 2C9 Substrate	Non-substrate	0.8020
CYP450 2D6 Substrate	Non-substrate	0.7282
CYP450 3A4 Substrate	Non-substrate	0.5551
CYP450 1A2 Inhibitor	Inhibitor	0.6330
CYP450 2C9 Inhibitor	Non-inhibitor	0.9829
CYP450 2D6 Inhibitor	Non-inhibitor	0.9545
CYP450 2C19 Inhibitor	Non-inhibitor	0.7913
CYP450 3A4 Inhibitor	Non-inhibitor	0.7836
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	0.6126
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9276
	Non-inhibitor	0.9535
AMES Toxicity	Non AMES toxic	0.7803
Carcinogens	Non-carcinogens	0.7926
Fish Toxicity	High FHMT	0.8446
TetrahymenaPyriformis Toxicity	High TPT	0.8984
Honey Bee Toxicity	High HBT	0.8760
Biodegradation	Not ready biodegradable	0.7288
Acute Oral Toxicity	III	0.7744
Carcinogenicity (Three-class)	Non-required	0.4991
Aqueous solubility	-2.9380	LogS
Caco-2 Permeability	1.6367	LogPapp, cm/s
Rat Acute Toxicity	1.9737	LD50, mol/kg
Fish Toxicity	1.3407	pLC50, mg/L
TetrahymenaPyriformis Toxicity	0.5271	pIGC50, ug/L

Table 4: Score card of the different experimental groups in open field test.

Groups	Dose(mg/kg)	Time spent in the Center(sec)	Time spent in the Periphery (sec)	Rearing (sec)	Grooming (sec)	Total number of fecal pellets
Control	Saline 1ml / rat	35.33±2.9	65.33±2.1	34.83±1.7	36.50±2.8	2.00±1.0
Experimental control CRS	Saline 1ml / rat	13.17±2.3	74.14±2.8	11.67±1.6	13.67±3.5	8.50±1.8
α asarone +CRS	9mg/kg/day	37.00±2.6	60.83±3.7	34.33±3.8	41.67±5.1	1.50±1.0
Glabridin +CRS	4mg/kg/day	36.17±2.3	60.00±5.2	34.83±4.0	45.00±3.4	1.50±.837

Group IV: Glabridin group-- received Glabridin of *Glycyrrhiza Glabra*(4mg/kg/day/i.p) 30min prior to 6hrs/day chronic restraint stress for 21 days.

On 22nd day, the rats in all four groups were subjected to Open field test, Elevated plus maze, Eight arm radial maze test. At end of the experiment blood sample and brain tissues were collected for conducting Corticosterone assay and Histopathology study in all groups as described already.

Statistical Analysis

The results were analyzed using SPSS computer software package, version 16. Data were presented as Mean±SD. Data was analyzed using One way analysis of variance (ANOVA) followed by Dunnett's test (post-hoc). Results were considered statistically significant at $p<0.05$.

RESULTS

'Computer aided multitarget ligand designing by virtual screening' study

Post docking analysis of test drugs α asarone and Glabridin:

The post docking analysis of the two compounds in iGEMDOCK software reveals that both the compounds follows Lipinski's rule shown in Table :-1. According to the rule the probability of using the compound as a therapeutic drug is more when the-

Table 5: Score card of the different experimental groups in Elevated plus maze test.

Groups	Drug &Dose(mg/kg) P/O	Time spent in open arm (sec)	Time spent in closed arm (sec)	Total number of entry in open arm	Total number of entry in closed arm	Total number of Rearing	Total number of fecal pellets
I- Control	Saline 1ml / rat	100.17±10.4	230.0±23.6	38.33±2.5	33.00±2.8	11.50±1.8	2.50±1.8
II- Experimental control CRS	Saline 1ml / rat	37.67±9.9	285.0± 18.7	11.17±1.4	41.83±2.9	4.33±1.6	8.50±1.0
III-Drug 1 α asarone +CRS	9mg/kg/day	129.83±23.1	241.0±22.2	37.17±5.2	33.83±3.4	13.17±2.3	2.17±1.4
IV-Drug 2 Glabridin +CRS	4mg/kg/day	123.33±18.7	241.83±21.7	37.67±2.4	32.83±2.3	12.17±2.6	2.50±1.7

Table 6: Score card of the different experimental groups Eight Arm Radial Maze in Rats.

Groups	Dose(mg/kg)	Number of Working Memory Error (Biated)	Number of Working Memory Error (UnBiated)	Number of Reference Memory Error	Latency
Control	Saline 1ml / rat	3.83±1.0	3.67±1.0	2.67±1.6	268.33±37.1
Experimental control CRS	Saline 1ml / rat	12.00±2.0	13.00±2.3	12.00±2.3	693.33±62.8
α asarone +CRS	9mg/kg/day	2.33±1.0	3.50±1.0	1.83±.983	240±32.8
Glabridin +CRS	4mg/kg/day	2.33±1.0	4.33±1.0	1.50±1.0	240±30.3

H- bond donors is less than 5(in this study it was 2 for α asarone and 3 for Glabridin),
H-bond acceptor is less than 10(here it was 4 and 3 for α asarone and Glabridin respectively).
Molecular weight is less than 500 dalton (α asarone 324.14,Glabridin 228.08) and
Moriguchi's Log P is less than 5(α asarone 0.981,Glabridin 1.194)
The results are shown in Table:- 2 & 3.
admetSAR profile for Drug delivery, absorption and side-effect prediction
As per the probability value in admetSAR profile test -
The maximum penetration through blood brain barrier of the drug α-asarone is predicted to be higher of about 0.9151 and Glabridin is 0.5803. The main strategy in drug delivery particularly in brain is its ability to cross the BBB, so that it can inhibit or act on the target. Higher the values better the drug of choice.
The admetSAR profile also showed positive and no side effect on absorption as the human intestinal absorption (HIA) score of α-asarone is highest (1.0000) and Glabridin is 0.9892. Both the test ligands are non-substrate and non-inhibitor; hence the compound can be metabolized by cytochrome P450 enzymes and so no hepatotoxicity effect. Thus, the results of iGEMDOCK, DruLiTo and admetSAR software clearly established that the two natural compounds chosen for the study as a rule passed the eligibility test to be used as drugs for further animal study.
Behavioural experiment
Open field test
The Open Field Test was performed for 5min and results shown in Table-4. The result showed that both the test drugs treated groups III & IV spent more time in open arm and their total number of entry in open arm also more which were statistically significant $F(3,20) = 38.72$, $p \leq 0.001$ when compared with that of Group II (Experimental control rats which received only chronic restrain stress alone for 6hrs) but was comparable with the group I. The same result was repeated in Time spent in closed arm and Total number of entry in closed arm were significantly decreased $F(3,20) = 13.33$, $p \leq 0.001$. In chronic restrained stressed rats treated with α asarone (group III) and Glabridin (group IV) when compared to rats subjected only to restrain stress. Likewise, the total number of rearing was less and number of faecal pellets passed was more in experimental control (group II) when compared with other three groups but the values of which were not significant among themselves.
Rats activity in Elevated Plus Maze (EPM) apparatus was recorded for 10 minutes. The rats were subjected to chronic restrain stress for 6hrs after the administration of normal saline for Group II, α asarone for Group III and Glabridin for Group IV . The data are expressed as Mean ±SD (n=6/group).
The Elevated Plus Maze test
The Elevated Plus Maze test was performed for 5 min and results are shown in Table 5. The statistical analysis of the result showed the time spent in centre of the maze was increased ($F(3,20) = 121.5$, $p < 0.001$) and total time spent in Periphery was decreased ($F(3,20) = 18.66$, $p < 0.001$) in both drugs treated groups III and IV when compared group II but not with group I. Similarly, rearing and grooming was less and number of faecal pellets expelled was more in group II ($F(3,20) = 87.07$ $p < 0.001$) in comparison of

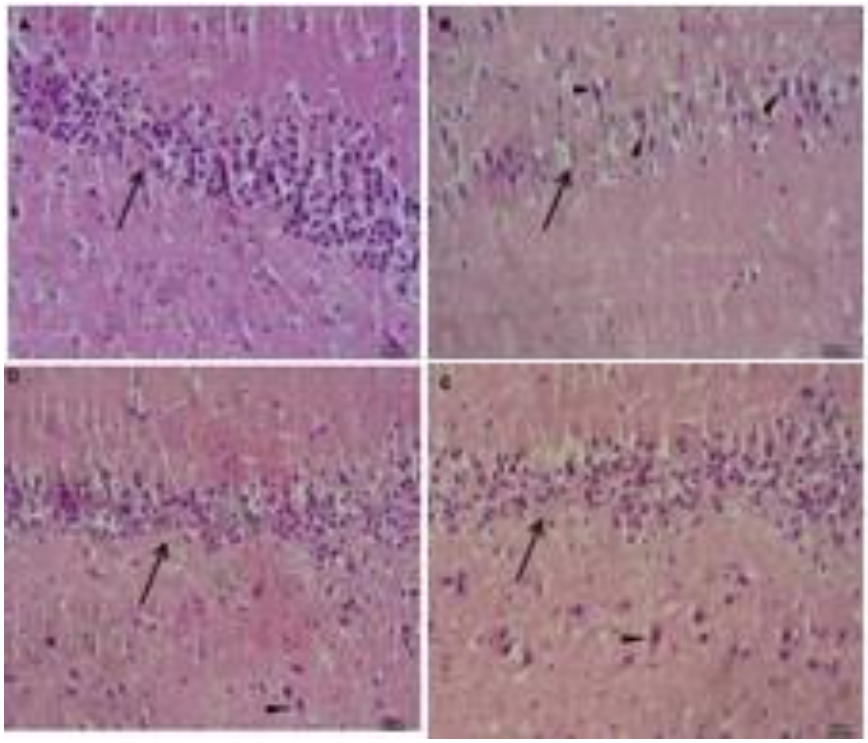

Figure 1: A, B, C, D: Histological changes in CA1 region of hippocampus in animals groups. A-Group I,B- Group II, C- Group III,D-Group IV.Disorganization of granular layer (moderate thick arrow), Pyknosis(arrow head) The scale bars in the figure indicate 100 µm.

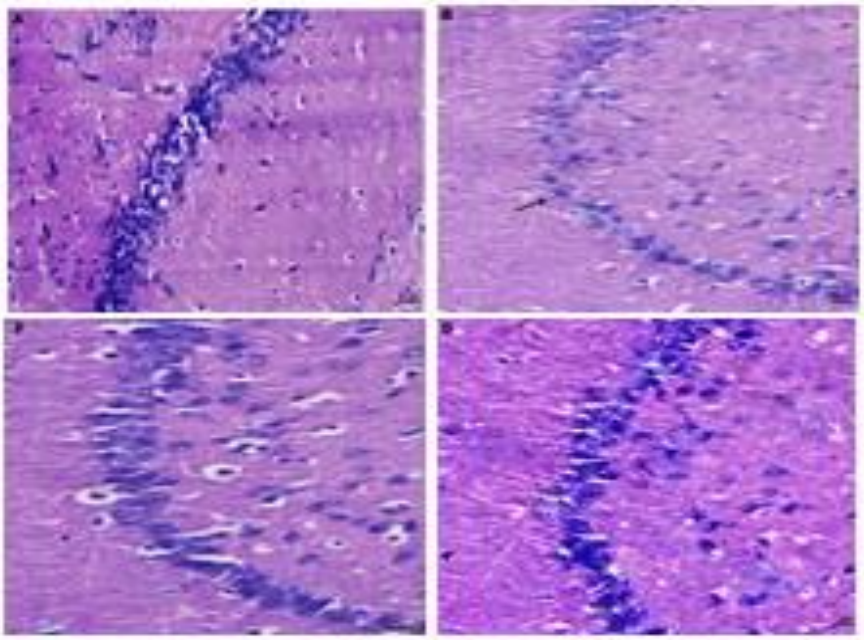

Figure 2: A,B,C,D: Histological image of CA3 region of hippocampus in animals in group. A-Group I, B- Group II, C- Group III,D-Group IV,.The scale bars in the figure indicate 100 µm.

other three groups whose these values were not significantly different.

Rat's activity in open field apparatus was recorded for 5 minutes. The rats were subjected to chronic restrain stress for 6hrs after the administration of normal saline for Group II, α asarone for Group III and Glabridinfor Group IV. The data are expressed as Mean ±SD(n=6/group).

The Eight Arm Radial Maze test

The Eight Arm Radial Maze test was performed for 5 min and results shown in Table -6.

The statistical analysis of the result here showed that all the parameters, the number of working memory error ($F(3,20) = 65.33$, $p < 0.001$), reference memory error ($F(3,20) = 58.62$, $p < 0.001$), number of reference memory error and latency were significantly less in experimental control group II than rest of three groups. Among the three

Table 7: Concentration of Corticosterone (ng/ml) in different experimental groups.

Measured parameter	Control Saline 1ml / rat	Experimental controlCRS +Saline 1ml / rat	αasarone 9mg/kg/day +CRS	Glabridin 4mg/kg/day +CRS
Corticosterone (ng/ml)	2.59±.31	12.9±.41	6.20±.41	6.98±.12

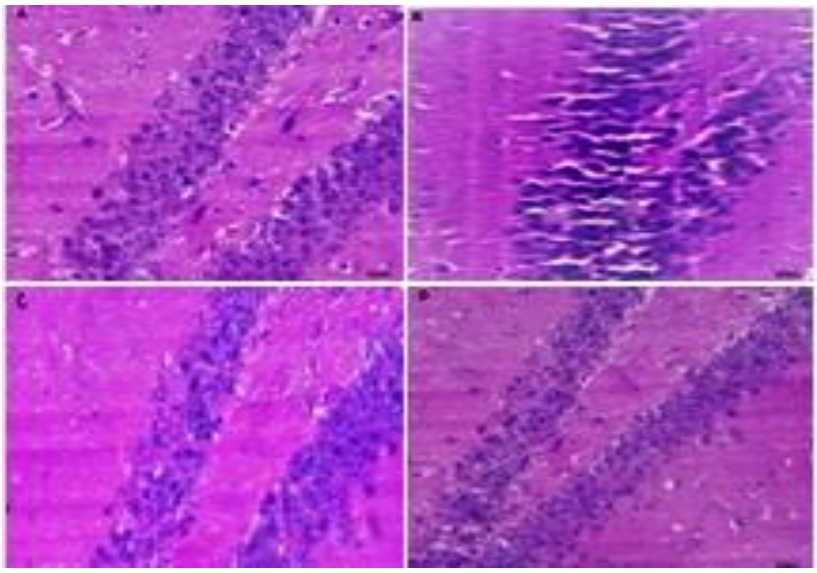

Figure 3: A,B,C,D,E,F: Histological image of DG region of hippocampus in animals in group .A-Group I, B- Group II, C- Group III,D-Group . The scale bars in the figure indicate 100 µm.

groups control (group I), drug treated groups α asarone group III and Glabridin group IV there was no significant difference in these parameters.

Biochemical test

Analysis of Plasma Corticosterone level of the different groups

The plasma concentration of Corticosterone of the rats was estimated in ng/dL and given Table 7. The result showed that the Corticosterone level in experimental control group II was more (133.2±2.0which was significantly high F (3,20) =40.03, p <0.001) when compared with drug treated groups III and IV and also control group I. Though the Corticosterone level between the drugs treated groups III (120.8±1.6) and IV (121±2.3) were comparable it was significantly high when compared with control group (70.4±1.7)The rats were subjected to chronic restrain stress (CRS)for 6hrs after the administration of normal saline for Group II, α asarone for Group III and Glabridin for Group IV. The concentration of Corticosterone (ng/ml) The data are expressed as Mean ±SD (n=6/group).

Histological analysis

Histopathology of rat brain (Hippocampus) showed less and hypochromic cells in group IIof CA1, CA3, DG regions compared with Control, Glabridin and αasarone treated groups. (Shown in Fig 1, 2,3A, B, C, D). Larger Perinuclear (PN) space which is an indication of swelling and onset of necrosis of the brain tissues which can be observed clearly in Fig1,2,3 B in all three regions of experimental control subjected to chronic restrain stress alone. No PN space in control group and less in Glabridin and α asarone treated groups with slightly Disorganization of granular layer, Pyknosis. Surprisingly α asarone treated groups has less inflammatory cellular infiltration in H and E staining when compare with Glabridin treated group.

DISCUSSION

In this study, the natural compounds extracted from the plant source, α asarone of *Acorus Calamus Linn* and Glabridin of *Glycyrrhiza Glabra* with proven antioxidant effects were selected as the aim of the study was to find out a drug that can ameliorate stress induced neuronal degeneration. It was well known that the stress release free radicals that cause damage at the cellular level. Literature survey indicating that the oxidative damage was induced by activating MAPK signalling cascade and was presumed that these natural compounds selected can act as an antioxidant and by inhibiting the MAPK signalling cascades reduce or slow down the progression of the pathogenesis in various neurodegenerative diseases[23,24,25]. Before administering the drug, to find out the suitability of the drug for animal study‘Computer aided multitarget ligand designing by virtual screening’ approach was employed. First by molecular docking method, it was showed that natural polyphenolic compound α asarone and Glabridin has good druglikeliness and polypharmacological properties. The main criterion in drug delivery is that the compound or the drug has to cross the blood brain barrier (BBB) so that it can act on the target protein in the brain. The results of admetSAR analysis revealed that both the compounds have good BBB score. The results of admetSAR predict that the human intestinal absorption HIA is also better for these compounds. Both the compound is non-inhibitor /non substrate of

cytochrome p450 so that the biotransformation of the drug metabolized by CYP450 microsomal enzymes will not be affected or halted. Thus, the two compounds are proved to be non-carcinogenic, no hepatotoxic and safe for oral administration. The study was further conducted on animal model as to verify whether these two selected compounds can halt or slow down the neurodegenerative changes created by induced chronic stress akin to which was seen in various degenerative diseases like Alzheimer's, dementia.

In the present study, to produce neuro-degeneration the rats were subjected to 6hrs chronic restrain stress for 21 days. At the end of the experiment, extend of neuronal damage was studied in the three groups underwent stress condition. The experimental control group which underwent stress without any drug treatment showed neuronal degeneration. The hippocampal tissue of this group was hypochromic indicating degeneration. But the histological examination of α asarone treated group III and Glabridin compound treated group IV showed only marginal degeneration. This histological finding was supported by the behavioural experiments of the study. All the groups of rats were for 5 minutes subjected to open field maze test (OPMT) which helps to assess emotional aspect of the individual[30]., elevated plus maze test (EPMT) for assessing anxiety level and eight arm radial maze test (EARMT) to assess memory and spatial learning ability[31]. Scores of these behavioural studies parameters were least in experimental control which subjected to only stress. The treated groups III and IV even after underwent stress conditions scored similar to control which did not subjected to stress. Even, grooming and rearing behaviour of the drug treated rats were comparable with the control. Expulsion of pellets was more in experimental control than other groups. The latency period, the time to complete the maze was also significantly increased in treated group. To some extent, the chemical study also supports the above results. The Corticosterone level was more in rats underwent only stress condition; whereas the drugs treatment decreased Corticosterone levels in the treated groups III and IV it was still higher than the normal control.

Thus the study clearly proved that the administration of α asarone of *Acorus Calamus Linn*(9mg/kg/day/p.o) and Glabridin of *Glycyrrhiza Glabra* (4mg/kg/day/p.o) from natural plants could significantly reduce the induced neuronal damage in the hippocampus region in chronic restrain stressed model rats. The cause for this neuronal damage particularly in hippocampus, apart from oxygen free radicals induced due to oxidative stress, was due to the steroid hormone, Corticosterone released during stress as the increased level of Corticosterone has got more affinity to glucocorticoid receptors within hippocampus causing damage to CA1 region[29]. Age related changed in hippocampus analysed in various studies reported that the damages were seen mainly in CA1 and CA3 regions[26,27]. CA1 is more vulnerable to high level of glucocorticoids leading to pathological aging like Alzheimer's disease[28]. Thus, the protective effect of α asarone of *Acorus Calamus Linn* and Glabridin of *Glycyrrhiza Glabra*from natural plants may be useful not only stress condition but also can extend to aging.

CONCLUSION

The present study concludes that the pretreatment of rats with Glabridin and α asarone before exposure to chronic restrain stress showed observable neuro-protection and improved cognition that could be due to the effect of antioxidant action of the compounds in the rat hippocampus. Hence, we conclude that the two natural compounds play a role in neuronal stress adaptation mechanism and have potential to prevent progression of neurodegenerative diseases. This study recommends that these two natural compounds could be an adjuvant therapy for treatment of neurodegenerative diseases. Further clinical evaluation and trials are necessary for therapeutic use.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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