EVALUATION OF HUPERZINE AND RESVERATROL IN RAT MODELS OF COGNITIVE IMPAIRMENT

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ABSTRACT

The present study, designed to investigate the role of huperzine and resveratrol in stress andintracerebroventricular injection of streptozotocin (*i.c.v.* STZ) induced changes of cognitivefunction in rat. The cognitive impairment was induced by the application of chronic swimmingstress *i.e.*, 15 minutes / day, for 25 consecutive days. The pre-treatment of huperzine (20 and 40mg/kg); resveratrol (20 and 40 mg/kg); thalidomide (25) and piracetam (300 mg/kg) wereadministered by oral gavage (*p.o.*) method for 10 consecutive days from day of 15th to 25th day.The changes of stress and *i.c.v.* STZ induced cognitive dysfunction were assessed by Morriswater maze (MWM) test from the day of 21st to 25th day. Furthermore, the stress and *i.c.v.* STZinduced biochemical changes *i.e.*, acetylcholinesterase activity (indicator of neurotransmitterchanges),TBARS(lipidperoxidationprocess)and

reducedglutathione(endogenousantioxidantchanges) effects were assessed in the brain samples of rat. The pre-treatment of huperzine andresveratrolfoundtopossessthesignificant(p<0.05) neuroprotectiveeffectinstressand*i.c.v.* STZinducedcognitiveimpairmentsalongwithattenuationofbrainbiochemicalalterations inadose dependent manner. The neuroprotective action is similar to that of piracetam pre-treatedgroup. In addition, thalidomide treatment group also shown to produce the ameliorative effectagainst stress and *i.c.v.* STZ events. It may be its potential anti-inflammatory action viainhibition of tumor necrosis factor-alpha (TNF-α) actions. Hence, the huperzine and resveratrolmay serve as future candidate for the management of cognitive impairment against neuronalmetabolic damage and stressful conditions due to its potential anti-oxidative, anti-lipidperoxidative andacetylcholinesteraseinhibitoryactions.

Keywords: Acetyl cholinesterase, Intracerebroventricular injection, Piracetam, Reducedglutathione,Thalidomide,Thiobarbituric acidreactivesubstance.

INTRODUCTION

Thebrainisamajor organinour body.Itregulatesandcontrolsthevarioussystemsofthebodysuchascardiovascular,gastrointestinal, neuromuscular, respiratorysystemandsoonviacentralas well as peripheral neuronal network (Browning, 2015; Salman, 2016). Whereas, the brainitselfhasspecific functionssuchaslearning,memory,forgetfulness,thinkingand motivation.

These functions are also called as psychological and cognitive functions (Alderson-Day andFernyhough, 2015; Hishikawa *et al.*, 2017). Cognition (learning and memory) is a main processof the brain. The brain is the only organ that is produces the memory function also called themindfunctions.Thisfunctionbasedonthinking,feeling,wanting,perceiving,learning,curiosityand behavior. Memory is opposite to the forgetful function. The forgetfulness is a god gift forthe human mankind. Because, brain should be forgetting the unwanted things. This is essentialforthe healthyhuman beingsimilarlikesleep(ElHaj *et al.*,2015;Ryckman*et al.*,2017).

Whereas, if brain forgot basic needs of daily activity leads to lack of capacity to receive newinformation, inability of thinking, calculation, recognition and recalling. All these symptoms areknown as cognitive impairment (Guendouzi and Savage, 2017). Memory is fundamental actionofbrainprocess.

Withoutmemoryfunctionweareunabletodoanythingbutsimplereflexesandstereotypicactionarenot affected (Dickersonand Eichenbaum,2010;Takao*etal.*,2008).

Learning is defined as; it is a process of brain which is acquires the new information fromsurroundings of environment it may be visual, auditory, smell including taste and dermal feeling*i.e.*, touch, heat & cold (Morris, 2017; Sanchis-Mora *et al.*, 2017). In another term, memory is aprocess of renewal the stored message, which may be sensations, feelings, impressions and ideas(McCarroll, 2017). The both learning and memory are too complex phenomenon and it iscoordinated function with different areas of brain such as diencephalon (a subcortical region; ithas thalamus and hypothalamus). Furthermore, it has integrated neuronal network connectionzonebetweenthethalamus,hippocampus,amygdalaandstriatum.Threemajor regions*i.e.*,hippocampus,amygdaleandstriatumareplayinganimportantroleinthefunctioningofdiffer enttypes of memory (de Quervain *et al.*, 2017; Goldfarb and Phelps, 2017). The memoryimpairmentsareidentifiedwith**5'A'**principles *i.e.*,

Aphasia: Difficult to find the word, reduced speech output, impaired comprehension orrepetition,dyslexiaanddysgraphia.

Anomia: Difficulttorecallthenamesofeverydayobjects.Itisaformofaphasia.

Apraxia: Difficulttoperform amanual taskintheabsenceof significantsensoryormotordeficits.

Amnesia: Difficulttoreferthefacts,informationandexperiences,knownaslossofmemory.

Agnosia: Difficult to interpret the sensations and hence to recognize things due to the braindamage (AminandSchindler,2017;Vos*etal.*,2015).

The pathogenesis of cognitive impairment is too complex. The variety of cognitive impairmentdisorders has been identified in human such as autism also called autism spectrum disorders (duetosocial, communication and language problems); amnesia and dementia (Bhattacharya *et al.*,2017). In society, the memory dysfunction is familiarized with Alzheimer's disease (AD). AD isone of the types of dementia. The memory impairment is raised with above disease progress(Audrain *et al.,* 2016). The etiology of memory impairments is due to the involvement of severalfactors such as genetic and metabolic changes for autism; shock and sudden mild injury of brainforamnesia; andage,alcohol,smoking,diabetes,neurovasculardisease,metal toxicity fordementia(Choi,*etal.*,2017;Ogoh,2017).InADisageisaprimarykeyfactorinthepathogenesisofmemoryimpairments.Theneurologicaldamageandalterationofneuronalfunction is generally occurring due to the various factors such as ischemia, ischemic-reperfusionand neurotoxin *i.e.*, protein, amino acid, drug and chemicals (Kimura *et al.,* 2017; Sikazwe *et al.*,2017).

Effectofstressinmemoryimpairment

Stressfulcondition,theinterferenceinbrainfunctionishigherincludingmemoryfunction.Inthisconditi on,bodyreactsandreleasesthemultiplestresshormones*i.e.*,glucocorticoids,cortisol and hydrocortisone (de Quervain *et al.*, 2017; Drexler and Wolf, 2017). The abundantrelease of stress hormones are frequently making the memory impairment; paradoxically veryfewconditions,itcanenhancethememoryfunction(Dinse*etal.*,2017;Wolf, 2017).Inaddition,these hormones are specifically damage the hippocampus, prefrontal cortex and amygdale regionof the brain (Drexler and Wolf, 2017). Cortisol is playing a hallmark for the diagnosis of stressconditions(Chaby*etal.*,2017).Furthermore,thehealthybrainhippocampusabletoregulatesthe production of cortisol via negative feedback mechanism due to its sensitivity of stresshormone associated receptors (Ebner and Singewald, 2017). In other edge, abundant cortisolimpairsthehippocampalabilityfortherecalland retention memories(Dinse*et al.*,2017). Therefore, the stress hormones are playing a key role in memory function as well as memorydysfunction(Karisetty*etal.*,2017;Wolf,2017).

Thefollowingdiagramisrevealedthechronic

stress associated development of neurological disorders such as anxiety, depression;gastrointestinaldisorders(pepticulcer);neurodegeneration;ageing;diabetes;cardiovascular diseaseincludingcognitivedysfunction(**Figure1**).

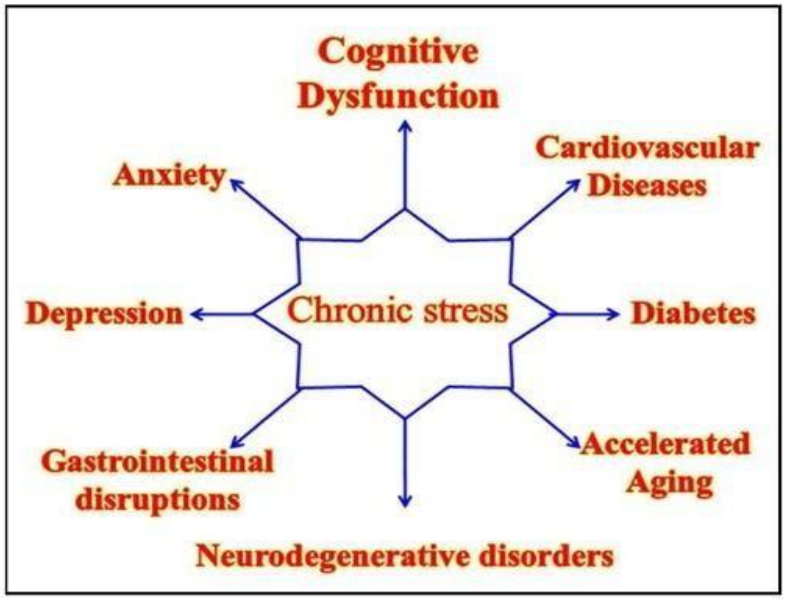

Figure1. Thisillustrationexpressedthealterationneurovascular;cardiovascularandgastrointestinal changes with chronic stress. In addition, the stress hormones are hampering theenergyaccessiblebyhippocampusduetoglucosedivertingactionofstresshormonetosurroundingmuscles (Kuhlmann*etal.*,2005). Moreover, the acute andchronic stresses aredocumented to produce the changes of in various cellular and molecular levels in different brainareas; which leads to induce the neuronal inflammatory process and neuronal death (Ménard *etal.*,2017;Serrats*etal.*,2017;Wolf,2017).

Effectofchemicalsinmemoryimpairment

Other than stress events, some of the chemicals are also induce the neuronal damage via multiplepathophysiological pathways (Akinyemi *et al.*,2017; Hritcu *et al.*,2017). Such chemicals arecysteine,homocysteine,sodiumnitrite,scopolamine,alcohol,aluminumtrichloride,lipopolysaccharide including streptozotocin (STZ) (Dam *et al.*,2017; Farooqui and Farooqui,2017; Ghosh *et al.*,2017; Goel *et al.*,2017). STZ is one of the naturally occurring chemical and itisobtainedfrom*Streptomycesachromogenes*.Earlier,itisusedasananti-tumourantibiotic.A

methyl group and a glucosamine are sandwiched between a nitrosourea moiety. And it also usedin the condition of insulinomas (tumor of the pancreatic β-cells) associated excessive insulinsecretion (Islam *et al.*, 2017). Clinically, it reduces the tumor size and symptoms at the dose of500 mg / m^2 / day; intravenous (*i.v.*) injection, for 5 days. And, repeated every 4 to 6 weeks (Dhir*et al.*, 2017; Romiti *et al.*, 2017). The molecular mechanism of STZ action is inhibiting thedeoxyribonucleic acid (DNA) synthesis in bacterial and mammalian cells. This action is due tocytosine moiety specific interaction leads to degrade the bacterial DNA (Islam *et al.*, 2017). Inaddition,itinhibitsthecell divisionsinmitosisprocess.In mammals,STZ iscausingthepotential toxicity for insulin-producing pancreatic beta cells. Instead of using STZ for treatingcertain cancers of the Islets of Langerhans; it is used as experimental tool for the induction oftype 1 and type 2 (dose dependent) diabetes mellitus in laboratory animal especially rodentspecies *i.e.*, rat and mice. Recently, the cerebroventricular injection either unilateral or bilateralinjection of STZ known to produce the neuronal damage especially in the hippocampal regionswithgenerationofoxidativestress,kinasesinhibition,phosphatasesactivationviainsulindysfunction (Genrikhs *et al.*, 2017; Kumar, 2017; Liu *et al.*,2014; Shah and Singh, 2006; Suchal*et al.*, 2017). In addition, the insulin dysfunction causes thememory impairments via inductionof inflammatory and apoptotic proteins expression and it also affects the tau protein pathology(Genrikhs *etal.*, 2017;Laurett*ietal.*,2017;Lu*etal.*, 2017).

Therapeuticapproachesforcognitivedysfunction

Treatmentformemoryproblemsdependsupontheunderlyingcausesandseverityofthecondition. The treatments start with lifestyle changes, medication, and other therapies. Lifestylechangesareeffective approachinthetreatmentoftheacute stageofmemorydisorders.

MATERIALS&METHODS

Experimentalanimals

Wistar rat (180 ± 200 g) were employed in the present study. Animals were maintained withstandard laboratory diet and allow to free access of water *ad libitum*. And 12 hours naturallight/dark cycle were maintained. The Institutional Animal Ethical Committee fully approved theexperimentalprotocol.Thecare oftheanimalswascarriedoutaccordingtothe guidelinesofthe

CPCSEA, Ministry of Environment and Forest, Government of India, (Reg. No.155/PO/Re/S/1999/CPCSEA;dated11/09/2015;IAECProposalNo.:206/2016).

DrugsandChemicals

The list of chemicals are used in this research work tabulated in **table**

1.**Table1.**Listofchemicals.

Sl. No	Name of the chemicals	Company details
1.	Donepezil	Wokhardt Ltd, Baddi, India.
2.	Folin-Ciocalteu's Phenol	Merck limited, Mumbai, India.
3.	Acetylthiocholine	Merck limited, Mumbai, India.
4.	Streptozotocin	Sigma Aldrich, USA
5.	5, 5, dithiobis (2-nitro benzoic acid) (DTNB)	Loba Chem, Mumbai, India.
6.	Reduced glutathione (GSH)	Loba Chem, Mumbai, India.
7.	Bovine serum albumin (BSA)	Loba Chem, Mumbai, India.
8.	Thiobarbituric acid	Loba Chem, Mumbai, India.
9.	Huperzine	I herb, United States.
10.	Resveratrol	Swanson Ultra, Mumbai.
11.	Thalidomide	United Biotech, New Delhi.

Inductionofcognitiveimpairmentinrat

All experimental animals were acclimatized for the laboratory condition for a period of one weekprior to the initiation of experiment. Animals were randomized to different groups based on thestratified body weight. Thereafter, experimental animals were employed for the swimming stress(Ahmadian-Attari*etal.*,2015)andsinglebilateralintracerebroventricularinjectionofstreptozotocin(STZ, 3mg/kg;*i.c.v.*)forinductionofcognitiveimpairmentinrat(Grieb, 2016).

Assessmentofcognitive functionby usingMorrisWaterMaze(MWM)test

Morris Water Maze test was employed to assess learning and memory of rats as describedmethodofMorris(1984)withslightmodification.TheMWMprocedurebasedonaprinciple,

where the animals were placed in a large pool of water, as animals dislike swimming, theirtendencytoescapefromthewaterbeingaccomplishedbyfindinganescapeplatform.Aenormous circular pool of 150 cm in diameter and 45 cm in height and filled to a depth of 30 cmwith water that was 28°C in temperature made up MWM. The water was made opaque withnontoxic white colored dye. The tank was divided into four equal quadrants with a help of twothreads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 cm^2),paintedinwhitewasplaced1cmbelowsurfaceof waterinsidethetargetquadrantTheplatform's position remained constant throughout the training. Each animal had four consecutivetrials with a 5-minute break on each day. The site of the drop was changed for each trial, and therat was gently placed in the water of the pool between quadrants, facing the pool wall, and given60 seconds to locate submerged platform. It was then permitted to remain on the platform for anadditional 15 seconds. If it failed to find the platform within 60 sec, it was guided gently onto theplatform and allowed to remain there for 15 sec. Escape latency time (ELT) to locate the hiddenplatform in watermaze was noted as an index of acquisition orlearning.Animal was subjectedto four acquisition trials daily for four consecutive days. On fifth day, the platform was removedand each rat was allowed to explore in the pool for 60 sec. All four quadrants' average time spentthere was reported. The average amount of time the animal spent in the target quadrant lookingfor the hidden platform was recorded as a retrieval (memory) index. The cognitive function *i.e.*,learning and memory process assessed by acquisition and retrieval trials respectively. The trailsdetailedprocedures areas below.

Acquisition trial: Trial of acquisition: Every rat underwent four trials each day. A restperiod of 5 min was allowed in between each trial. Four trials per day were repeated for fourconsecutive days. Q4 was kept as the target quadrant in all acquisition trials, and the startingposition for each day's four acquisition trials was altered as detailed below. During acquisitiontrials, the mean escape latency time (ELT), which was computed for each day, was utilized as anacquisitionindex.

Day-1	Q_1	Q_2	Q_3	Q_4
Day-2	Q_2	Q_3	Q_4	Q_1

Day-3	Q_3	Q_4	Q_1	Q_2
Day-4	Q_4	Q_1	Q_2	Q_3

Trial of retrieval: On the fifth day, the platform was taken away. Rat was put in a watermaze and given 120 seconds to explore it. Four of these trials wereconducted on each rat,starting in a different quadrant for each trial. The average amount of time spent in each of thethree quadrants—Q1, Q2, and Q3—was noted, and the amount of time spentin thefourthquadrant—the target quadrant—while looking for the missing platform—provided a retrievalindex. The experimenter maintained the same posture throughout. Care was taken to ensure thatthe water maze's position in relation to other lab items would not interfere with any strong visualcues throughout the length of the trial. All of the trials were finished in a single day, from 9:00 to18:00. ThedimensionofMWMisillustratedin**figure 2**.

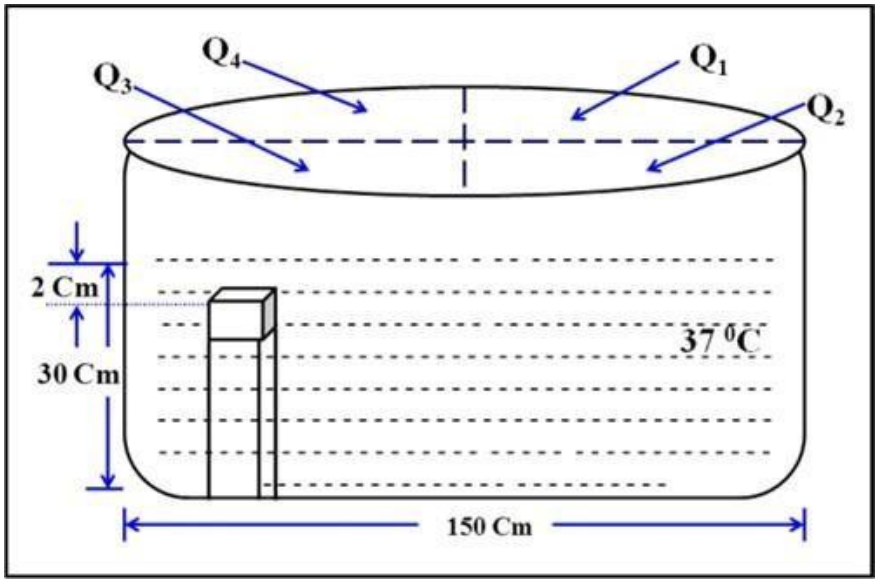

Figure2. ThetypicaltypeofMorriswatermazetankfor assessmentoflearningandmemoryfunction.

Experimentaldesign

This research work consists of two setups of experimental protocol. One is experimental protocolno I. It explores the role of huperzine and resveratrol in swimming stress induced cognitiveimpairmentinrat.SecondoneisexperimentalprotocolnoII.Itexplorestheroleofhuperzine

andresveratroli*n.c.v.*STZinducedcognitiveimpairmentinrat.Thedetailsofexperimentalprotocolasfollows.

Experimental Protocol no I: Role of huperzine and resveratrol in swimming stress inducedcognitiveimpairmentinrat

Eight groups were employed in the present study and each group was comprised of 6 Wistar rats(n=6). The duration of stress is 15 minutes per day for 25 consecutive days. The drug wasadministered after 1 hour of the stress events. The drug administration started from 16th day for10 consecutive days. On 21st day animal placed on MWM test apparatus with platform (locatedin target quadrant) for acquisition trail; and, it is continued up to 24th day. The retrieval trial wasperformed on 25th day. The both acquisition and retrieval trials were performed after one hour ofthedrugtreatment orafter2 hoursofthestressevents.Thedrug wasadministeredafter1 hourofthe stress events.

Group1:Normal control

Rats were exposed to Morris water maze for four-day acquisition (learning) trial (*i.e.,* 21, 22, 23and24thday);andonedayretrieval(memory)trialwasperformedon25thday.

Group2:Swimmingstresscontrolgroup

Ratswere employed to force swimminginwaterpoolintheextendedtimeduration*i.e.,*15minutes for 25 consecutive days as described method of (Ahmadian-Attari *et al.,* 2015) withslightmodification.Theacquisition(learning) trialteststartedfromday21thdayto24thday;andnext day (*i.e.,* 25th day), the animals were employed for retrieval (memory) trial test. Asdescribedingroup1.

Group 3:Huperzine (20mg/kg)treated group

Huperzine (20 mg/kg; *p.o.*) was administered to the swimming stress employed rats, startingfromday1.The acquisitiontrialandretrievaltrialtestswereperformedasdescribedingroup1.

Group 4:Huperzine (40mg/kg)treated group

Huperzine (40 mg/kg; *p.o.*) was administered to the swimming stress employed rats, startingfromday1.Theacquisitiontrialandretrievaltrialtestswereperformedasdescribedingroup1.

Group5: Resveratrol(20mg/kg)treatedgroup

Resveratrol(20mg/kg;*p.o.*)wasadministeredtotheswimmingstressemployedrats,startingfromday1. The acquisitiontrialandretrievaltrialtestswere performedasdescribedingroup1.

Group6: Resveratrol(40mg/kg)treatedgroup

Resveratrol(40mg/kg;*p.o.*)wasadministeredtotheswimmingstressemployedrats,startingfromday1. The acquisitiontrialandretrievaltrialtestswere performedas describedingroup1.

Group 7:Thalidomide(25mg/kg)treatedgroup

Thalidomide(25mg/kg;*p.o.*)wasadministeredtotheswimmingstressemployedrats,startingfromday1 .The acquisitiontrialandretrievaltrialtestswere performedasdescribedingroup 1.

Group8:Piracetam(300mg/kg)treatedgroup

Piracetam (300 mg/kg; *p.o.*) was administered to the swimming stress employed rats, startingfrom day 1. The acquisition trial and retrieval trial tests were performed as described in group 1.Piracetamwas suspendedin0.5%w/vofcarboxymethylcellulose.

Experimental Protocol no II: Role of huperzine and resveratrol in i.c.v. STZ induced cognitiveimpairmentinrat

Eight groups were employed in the present study and each group was comprised of 6 Wistar rats(n=6). The drug administration started from 0^{th} day for 10 consecutive days. On 6^{th} day animalplaced on MWM test apparatus with platform (located in target quadrant) for acquisition trails;and,itis continued up to 9^{th} day. The retrieval trial was performed on 10^{th} day. The bothacquisitionandretrievaltrials were performedafterone hourofthedrugtreatment.

Group1:Normal control

Rats were exposed to Morris water maze for four-day acquisition (learning) trial (*i.e.,* day 6, 7, 8andday9);andonedayretrieval(memory)trialon10th day.

Group 2:STZ(3mg/kg;i.c.v.)treatedgroup

Rats were involved the single bilateral intracerebroventricular injection of streptozotocin (STZ, 3mg/kg;*i.c.v.*)forinductionofcognitiveimpairmentinrat(Grieb,2016)on9thdayafteracquisition (learning) trial. The next day (*i.e.,* day 10), the animals were employed for retrieval(memory)trialtest.Asdescribedingroup1.Streptozotocinwasdissolvedinartificialcerebrospinal fluid (CSF); it consists of 147 µM of sodium chloride (NaCl; MW: 58.44); 2.9 µMofpotassiumchloride(KCl;MW:74.55);1.6µMofmagnesiumchloride(MgCl2;MW:95.21); 1.7 µMofcalciumchloride (CaCl2;MW: 110.98);and2.2µMofdextrose(MW:180.16).

Group 3:Huperzine (20mg/kg)treated group

Huperzine (20 mg/kg; *p.o.*) was administered for 10 consecutive days. On 9th day, STZ (*3 mg/kg;i.c.v.*)was administered aftercompletion of drug treatmentas well as MWM trails.After24hours of *i.c.v.* STZ administration the retrieval trial tests were performed *i.e.,* on 10th day. Theacquisitiontrialandretrievaltrialtestswere performedasdescribedingroup1.

Group 4:Huperzine (40mg/kg)treated group

Huperzine (40 mg/kg; *p.o.*) was administered for 10 consecutive days. On 9th day, STZ (3 mg/kg;*i.c.v.*)was administered aftercompletion of drug treatmentas well as MWM trails.After24hours of *i.c.v.* STZ administration the retrieval trial tests were performed *i.e.,* on 10th day. Theacquisitiontrialandretrievaltrialtestswere performedasdescribedingroup1.

Group5: Resveratrol(20mg/kg)treatedgroup

Resveratrol (20 mg/kg; *p.o.*) was administered for 10 consecutive days. On 9th day, STZ (*3mg/kg; i.c.v.*) was administered after completion of drug treatment as well as MWM trails. After24 hours of *i.c.v.* STZ administration the retrieval trial tests were performed *i.e.,* on 10th day. Theacquisitiontrialandretrievaltrialtestswere performedasdescribedingroup1.

Group6: Resveratrol(40mg/kg)treatedgroup

Resveratrol (40 mg/kg; *p.o.*) was administered for 10 consecutive days. On 9th day, STZ (3mg/kg; *i.c.v.*) was administered after completion of drug treatment as well as MWM trails. After24 hours of *i.c.v.* STZ administration the retrieval trial tests were performed *i.e.*, on 10th day. Theacquisitiontrialandretrievaltrialtestswereperformedas describedingroup1.

Group 7:Thalidomide(25mg/kg)treatedgroup

Thalidomide (25 mg/kg; *p.o.*) was administered for 10 consecutive days. On 9th day, STZ (3mg/kg; *i.c.v.*) was administered after completion of drug treatment as well as MWM trails. After24 hours of *i.c.v.* STZ administration the retrieval trial tests were performed *i.e.*, on 10th day. Theacquisitiontrialandretrievaltrialtestswereperformedasdescribedingroup1.

Group8:Piracetam(300mg/kg)treatedgroup

Piracetam (300 mg/kg; *p.o.*) was administered for 10 consecutive days. On 9th day, STZ (3mg/kg; *i.c.v.*) was administered after completion of drug treatment as well as MWM trails. After24 hours of *i.c.v.* STZ administration the retrieval trial tests were performed *i.e.*, on 10th day. Theacquisitiontrialandretrievaltrialtestswereperformedasdescribedingroup1.

Tissuesamplecollection

On 10th day, after completion of acquisition trial and retrieval trial tests; the animals weresacrificedandbrainsamplewerecollected.Thebrainhomogenatesarepreparedwithphosphatebuffer saline (pH 7.4, 10 % w/v) using Telfon homogenizer at 3500 rpm for 15 minutes. Theclear supernatants were used for the estimation of acetylcholinesterase (AchE) activity,thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) level and tissuetotalproteincontents.

Estimation ofbrainacetylcholinesterase(AChE)activity

TheEllmanetal. (1961)approachwasusedtoquantifytheAChE activitythroughouttheentirebrain. This was calculated using the yellow color that was produced when thiocholine anddithiobisnitrobenzoateionsreacted.Aspectrophotometerwasusedtodetermine the rateof

thiocholine synthesis from acetylthiocholine iodide in the presence of brain cholinesterase.Pipetting 0.5 ml of the brain homogenate's clear supernatant liquid into a 25 ml volumetric flaskallowedfordilutionusingnewlygeneratedDTNB(5,5'–dithiobis(2-nitrobenzoicacid)solution(10 milligrams of DTNB are dissolved in 100 ml of pH 8.0 Sorenson phosphate buffer.). Fromthe volumetric flask, two 4 ml portions were pipette out into two test tubes. Into one of the testtubes, 2 drops of donepezil solution were added. 1 ml of substrate solution (75 mg ofacetylthiocholineiodideper50 mlofdistilled water)waspipetteout intobothofthetesttubes.

Thetestsample'schangeinabsorbanceperminutewas measuredspectrophotometricallyat420nm using a DU 640B Spectrophotometer from Beckman Coulter Inc. in California as the blankand the test tube containing donepezil as the reference. The following formula was used todetermine AChEactivity:

$$R= (\delta O.D.XV)/(EXP)$$

WhereR=rateof enzymeactivityin‘n’moleof acetylthiocholineiodidehydrolyzed/minute/mgprotein

δ O.D. =changeinabsorbance/minuteV

=Volumeofassay

E=Extinctioncoefficient=(13600/M/cm)P=

Proteincontent(mg)

Preparationofreagents

Preparationof Sorensonphosphatebuffer(pH8.0)

Bycombining2.65mlof0.2Mmonobasic sodium phosphate with47.35 mlof0.2Mdibasicsodiumphosphate,Sorensonphosphatebufferwasfreshlymade.

Preparationof 0.2Mdibasicsodiumphosphate

0.2Mdibasic sodiumphosphate waspreparedbydissolving 28.39gofdibasicsodiumphosphateindistilledwaterandvolume wasmade upto1literwithdistilledwater.

Preparationof0.2Mmonobasicsodiumphosphate

0.2Mmonobasicsodiumphosphate waspreparedbydissolving 23.99 gofmonobasicsodiumphosphateindistilledwaterand volume wasmadeupto1literwithdistilledwater.

Preparationof5,5'–dithiobis(2-nitro benzoicacid)(DTNB)

10mgofDTNBwasdissolvedin100mlofSorensonphosphatebuffer.

Preparationof acetylthiocholineiodidesolution

75mgofacetylthiocholineiodidewasdissolvedin50mlofdistilledwater.

Estimationofthiobarbituricacidreactivesubstances(TBARS)

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index oflipid peroxidation in brain was performed according to the method of Ohkawa *et al.* (1979).About 0.2 ml of supernatant of homogenate was pipetted out in a test tube, followed by additionof 0.2 ml of 8.1 % sodium dodecyl sulphate, 1.5 ml of 30 % acetic acid (pH 3.5), 1.5 ml of 0.8%of thiobarbituric acid and the volume was made up to 4 ml with distilled water. The test tubeswere incubated for 1 h at 95 ^{0}C, then cooled and added 1 ml of distilled water followed byaddition of 5ml of n-butanol-pyridinemixture(15:1 v/v).For10 minutes, thetubes werecentrifugedat4000g.Theabsorbanceofdevelopedpinkcolorwasmeasuredspectrophotometricall y (DU 640B spectrophotometer, Beckman Coulter Inc., CA, USA) at 532nm.Inordertocreateastandardcalibrationcurve,1,1,3,3-tetramethoxypropanewasdilutedto 1–10 nM.Ananomole wasusedto representtheTBARSvaluepermg ofprotein.

Preparationofreagents

Preparationof sodiumdodecylsulphatesolution

810 mgofsodiumdodecylsulphatewasdissolvedin10mlofdistilledwater.

Preparation of30%acetic acid solution

30 ml of acetic acid was diluted to 100 ml with distilled water and pH was adjusted to 3.5 withsaturatedsolutionofsodiumhydroxide usingpHmeter.

Preparationof0.8 %thiobarbituricacidsolution

400 mgofthiobarbituric acidwasdissolvedin50mlofwarmdistilledwater.

Preparation of 15:1 v/v n-butanol-pyridine

mixture90 ml of n-butanol was mixed with 6 ml of

pyridine.***Preparation of1nM1,1,3,3-tetramethoxy***

propane

0.82 ml of standard 1, 1, 3, 3-tetramethoxy propane was diluted to 5 ml with distilled water tomake 1 M solution. 1 ml of this dilution was further diluted to 10 ml with distilled water and thisdilutionprocesswasfurtherrepeatedforeight timestoget1nM1,1,3,3-tetramethoxypropane.

Estimation ofreduced glutathione(GSH)

Using the Beutler et al. (1963) approach, it was possible to measure the reduced glutathione(GSH) concentration in tissue. The supernatant of homogenate was mixed with trichloroaceticacid (10 % w/v) in 1:1 ratio. The tubes were centrifuged at 1000 g for 10 min at 4 ^{0}C. Theresulting supernatant (0.5 ml) was combined with 2 ml of disodium hydrogen phosphate (0.3 M).Then 0.25 ml of 0.001 M freshly prepared DTNB [5, 5`-dithiobis (2-nitrobenzoic acid) dissolvedin 1% w/v sodium citrate] was added and absorbance was noted spectrophotometrically (DU640B spectrophotometer, Beckman Coulter Inc., CA, USA) at412 nm.Results were expressedas micromoles of reduced glutathione per mg of protein using a standard curve that was plottedusingreducedglutathioneconcentrations of10-100M.

Preparationofreagents

Preparationof10%trichloroaceticacid

10 goftrichloroaceticacid wasdissolvedin100mlofdistilled water.

Preparationof0.3Mdisodiumhydrogenphosphate

4.26 gofanhydrousdisodiumhydrogenphosphate wasdissolvedin100mldistilled water.

Preparationof5,5`-dithiobis(2-nitrobenzoicacid) in1%sodiumcitrate

7.92mg of5,5`-dithiobis(2-nitrobenzoicacid)wasdissolvedin20mlof1%sodiumcitrate.

Preparationof100µMofreduced glutathione

6.14mg ofreducedglutathionewasdissolvedin200 mldistilledwater.

Estimationofbrain totalprotein

The brain total protein was determined by Lowry's method (1951) using bovine serum albumin(BSA) as a standard. After diluting 0.15 ml of tissue homogenate supernatant to 1 ml, Lowry'sreagent was applied. After carefully combining the ingredients, the mixture was left to stand atroom temperature for 15 minutes. The mixture was vigorously vortexed after 0.5 ml of Folin-Ciocalteu reagent was added, and it was left to sit at room temperature for 30 minutes. 0.2-2.4mg/ml of BSA was used to plot the standard curve. The protein content was determinedspectrophotometrically(DU640BSpectrophotometer,BeckmanCoulterInc., CA, USA)at750nm.Proteinconcentrationwas expressedasmg/mlofsupernatant.

Preparationofreagents

PreparationofLowry'sreagent

Lowry's reagent was freshly prepared by mixing 1% w/v copper sulphate solution,2% w/vsodium-potassiumtartrateand 2% w/vsodiumcarbonatein0.1Msodiumhydroxide,intheratioof1:1:98.

Preparationof 0.1Msodiumhydroxidesolution

0.1Msodiumhydroxidewasprepared bydissolving4 gofsodiumhydroxideindistilled waterandvolume wasmade upto1literwithdistilledwater.

Preparationof1%coppersulphate($CuSO_4$)solution

1% $CuSO_4$ was prepared by dissolving 1 g of copper sulphate in distilled water and volume wasmade upto100mlwithsame.

Preparationof2%sodiumpotassiumtartratesolution

2gofsodiumpotassiumtartratewasdissolvedindistilledwater and volumewasmadeupto100mlwithsame.

Preparationof 2%sodiumcarbonatesolution

2gofsodiumcarbonatewasdissolvedin0.1 Msodiumhydroxideand volumewasmadeupto100mlwith0.1M sodiumhydroxide.

RESULTS

STATISTICALANALYSIS

All the results were expressed as mean ± standard deviation (SD). The behavioral data werestatistically analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni posthoc test and data of tissue biomarker *i.e.,* TBARS, GSH and AChE activity levels were analyzedusingonewayANOVAfollowedbyTukey'sMultipleRangetestusingGraphpadprismVersion-5.0software.Ap< 0.05wasconsideredtobe statisticallysignificant.

Effect of huperzine and resveratrol in stress induced changes of ELT and TSTQ in Morriswatermaze(MWM)test

In MWM test, the Wistar rat resulted to significant decrease of day 24th ELT, when compared today 21st ELT. It shows the normal learning (acquisition) ability. In addition, day 25 assessment,resulted to significant increasing of TSTQ, when compared to the time spent value in otherquadrants. Itshows the normal retrieval (memory) capacity. However, the swimming stressresulted to produce the significant increase of day 24 ELT and decrease of day 25 TSTQ whencompared to normal control group. It reveals that, the swimming stress shown to produce theabnormal learning and memory process in rats. Our previous study and other research reportrevealed that, the oral administration of CMC (0.5 % w/v) does not produce the any therapeuticeffectin the alteration stress inducedlearning andmemory impairments. Therefore, in thisresearch design was omitted the CMCas avehicle control group.Administration of huperzine(40 mg/kg); resveratrol (20 and 40 mg/kg); and thalidomide (25 mg/kg) for 10 consecutive daysshown to significantly (P < 0.05) attenuate the stress induced changes of learning and memoryimpairments in a dose dependent manner. Whereas, the huperzine (20 mg/kg) treated group doesnot produce the significant improvements in stress induced cognitive impairments. Treatment ofreference control piracetam (300mg/kg)produced the similareffects toimprovethe stressinducedcognitiveimpairments;anditisstatisticallysignificantdifferencetohuperzine(20

mg/kg)andstresscontrolgroups.Inaddition,TNF-αsynthesisinhibitor*i.e.*,thalidomidetreatment group also shown to produce the ameliorative effect against stress induced ELT andTSTQchanges (**Table2andFigure3**).

Table 2. Effect of huperzine and resveratrol in stress induced changes of learning behavior byusingMWM apparatus.

Groups	**Day 21 ELT** (Sec)	**Day 24 ELT** (Sec)
Normal	57.6 ± 1.9	7.4 ± 0.9[a]
Stress	58.1 ± 1.3	48.5 ± 2.2[a,b]
Huperzine (20)	52.2 ± 0.9	26.2 ± 1.4[a,b]
Huperzine (40)	55.3 ± 1.2	19.3 ± 1.7[a,c]
Resveratrol (20)	54.0 ± 1.9	20.5 ± 2.3[a,c]
Resveratrol (40)	51.5 ± 2.1	14.3 ± 1.2[a,c]
Thalidomide (25)	54.8 ± 1.1	11.7 ± 1.5[a,c]
Piracetam (300)	51.4 ± 1.4	9.3 ± 0.7[a,c]

Datawereexpressedasmean±Standarddeviation(SD),n=6,two-wayANOVAfollowedbyBonferroniposthoctests.Here,ELTisescapinglatencytimeasindicatoroflearningbehavior.

[a]p< 0.01versusDay21ELTinrespective group.

[b]p<0.05versusDay24ELTin normalcontrolgroup.

[c]p<0.05versusDay24ELTin stress controlgroup.

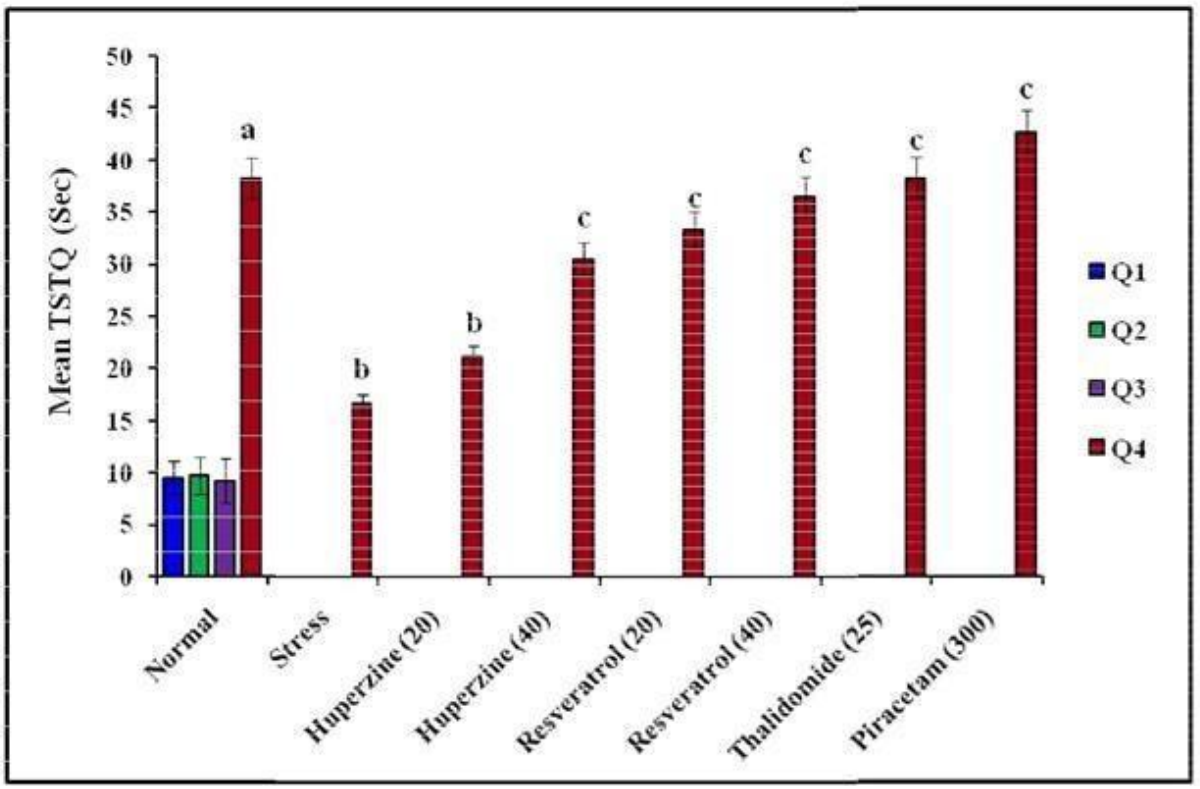

Figure 3. Effect of huperzine and resveratrol in stress induced changes of memory function byusingMWM apparatus.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digits in parentheses indicate that dose mg/kg. Here, TSTQ is timespentintargetquadrantasindicatorofmemoryfunction.

[a]p <0.05versusmeantimespentinQ_1quadrant ofnormalcontrolgroup.[b]p <

0.05 versus mean time spent in Q_4(TSTQ) in normal control group.[c]p

<0.05 versusmeantimespentinQ_4(TSTQ)instresscontrolgroup.

Effect of huperzine and resveratrol in stress induced changes of brain acetylcholinesterase(AChE)activitylevel

The swimming stress resulted to produce the significant increase of brain AChE activity level,when compared to normal control group. It shows that, swimming stress produces thepathologicalchangesincellularenvironmentalong withalterationneurotransmitteractioninrat

brain. The oral administration of huperzine (40 mg/kg) and resveratrol (20 and 40 mg/kg) for 10consecutive days; shown to produce the significant ($p < 0.05$) attenuation of stress inducedchanges of brain AChE activity levels in a dose dependent manner. Whereas, the huperzine (20mg/kg) treatedgroupdoesnotproducethesignificantimprovements instress inducedchangesofAChE activitylevels.Treatmentofreferencecontrolpiracetam(300mg/kg)producedthesimilareffects to improve the stress induced cognitive impairments; and it is statistically significantdifference to huperzine (20 mg/kg) and stress control groups. In addition, TNF-α synthesisinhibitor *i.e.*, thalidomide treatment group also shown to produce the ameliorative effect againststressinducedchangesofAChEactivitylevels.(**Figure4**).

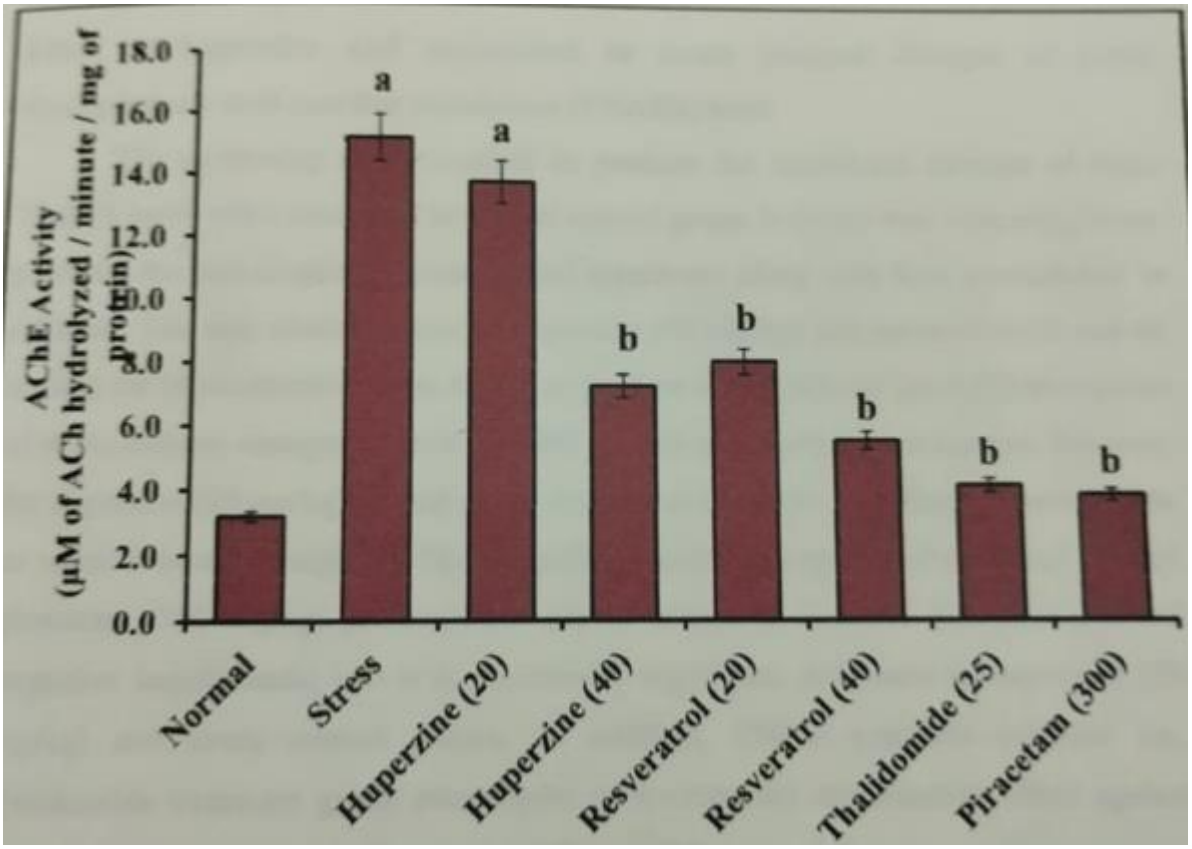

Figure4. EffectofhuperzineandresveratrolinstressinducedchangesofbrainAChEactivitylevel.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digitsin parenthesesindicate that dose mg/kg.Abbreviation*i.e.*,AChEisacetylcholinesterase;AChE,acetylthiocholine;andµM,micromole.

[a]p< 0.05versusnormalcontrolgroup.

[b]p < 0.05versusstresscontrolgroup.

Effect of huperzine and resveratrol in stress induced changes of brain thiobarbituric acidreactive substances (TBARS)level

The swimming stress resulted to produce the significant increase of brain TBARS level, whencompared to normal control group. It shows that, swimming stress produces the pathologicalchanges in cell membrane along with lipid peroxidation in rat brain. The oral administration ofhuperzine (40 mg/kg) and resveratrol (20 and 40 mg/kg) for 10 consecutive days; shown toproducethesignificant(p<0.05)attenuationofstressinducedchangesof brainTBARSlevelinadosedependentmanner. Whereas,thehuperzine(20mg/kg) treatedgroupdoesnotproducethesignificant improvements in stress induced changes of TBARS activity levels. Treatment ofreference control piracetam (300 mg/kg) produced the similar effects to improve the stressinduced cognitive impairments; and it is statistically significant difference to huperzine (20mg/kg) and stress control groups. In addition, TNF-α synthesis inhibitor *i.e.*, thalidomidetreatment group also shown to produce the ameliorative effect against stress induced changes ofTBARSlevel(**Figure5**).

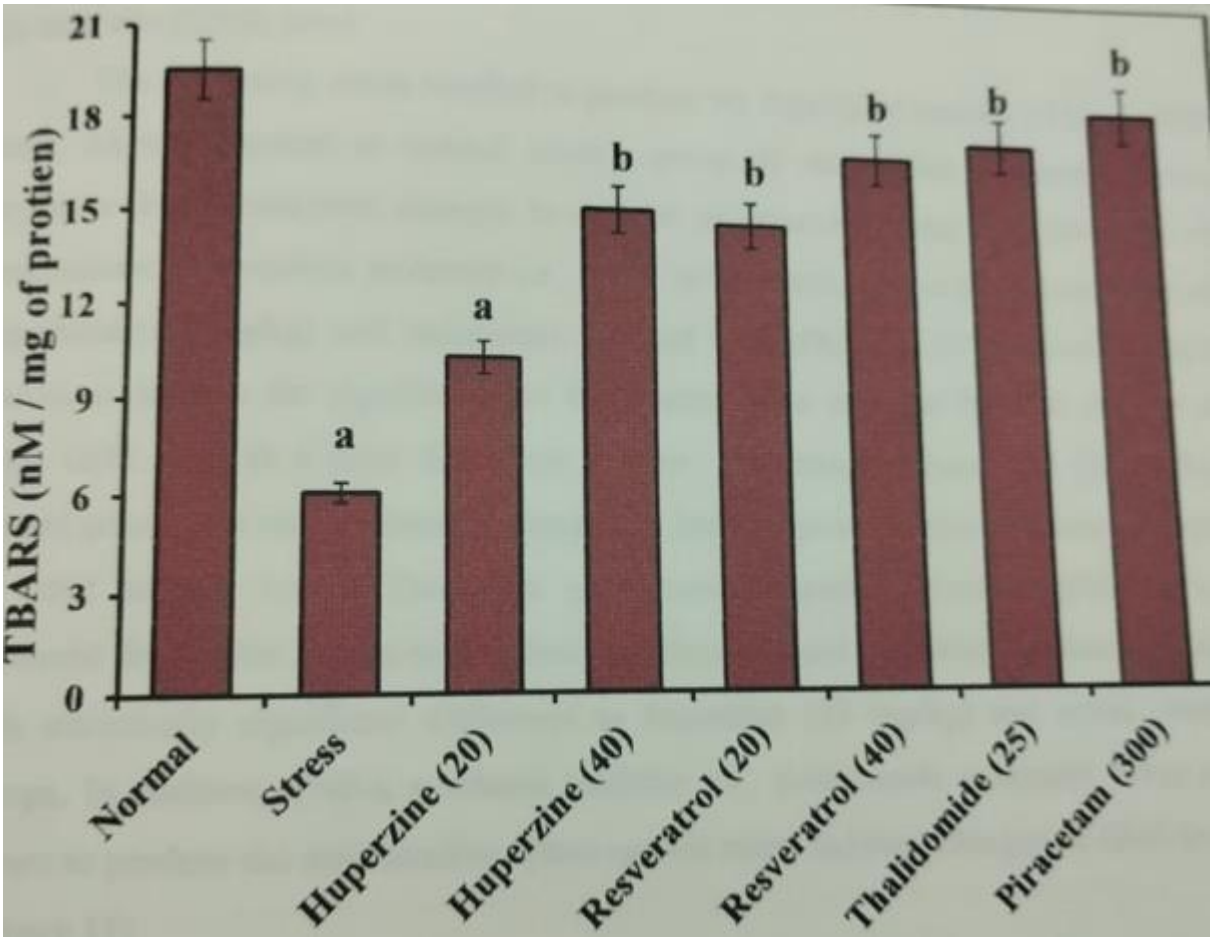

Figure 5. Effect of huperzine and resveratrol in stress induced changes of brain TBARS activitylevel.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digits in parentheses indicate that dose mg/kg. Abbreviation*i.e.*,TBARS,thiobarbituricacidreactivesubstances*i.e.*,malondialdehyde);andnM,nano mole.

[a]p< 0.05versusnormalcontrolgroup.

[b]p < 0.05versusstresscontrolgroup.

Effect of huperzine and resveratrol in stress induced changes of brain reduced glutathione(GSH)level

The swimming stress resulted to produce the significant increase of brain GSH level, whencompared to normal control group. It shows that, swimming stress produces the pathologicalchanges in cellular environment along with alteration of endogenous anti-oxidant molecule *i.e.*,GSH in rat brain. The oral administration of huperzine (40 mg/kg) and resveratrol (20 and 40mg/kg) for 10 consecutive days; shown to produce the significant ($p< 0.05$) attenuation of stressinduced changes of brain GSH level in a dose dependent manner. Whereas, the huperzine (20mg/kg) treatedgroupdoesnotproducethesignificantimprovementsinstress inducedchangesofGSH activity levels. Treatment of reference control piracetam (300 mg/kg) produced the similareffects to improve the stress induced cognitive impairments; and it is statistically significantdifference to huperzine (20 mg/kg) and stress control groups. In addition, TNF-α synthesisinhibitor *i.e.,* thalidomide treatment group also shown to produce the ameliorative effect againststressinducedchanges ofGSHlevels(**Figure6**).

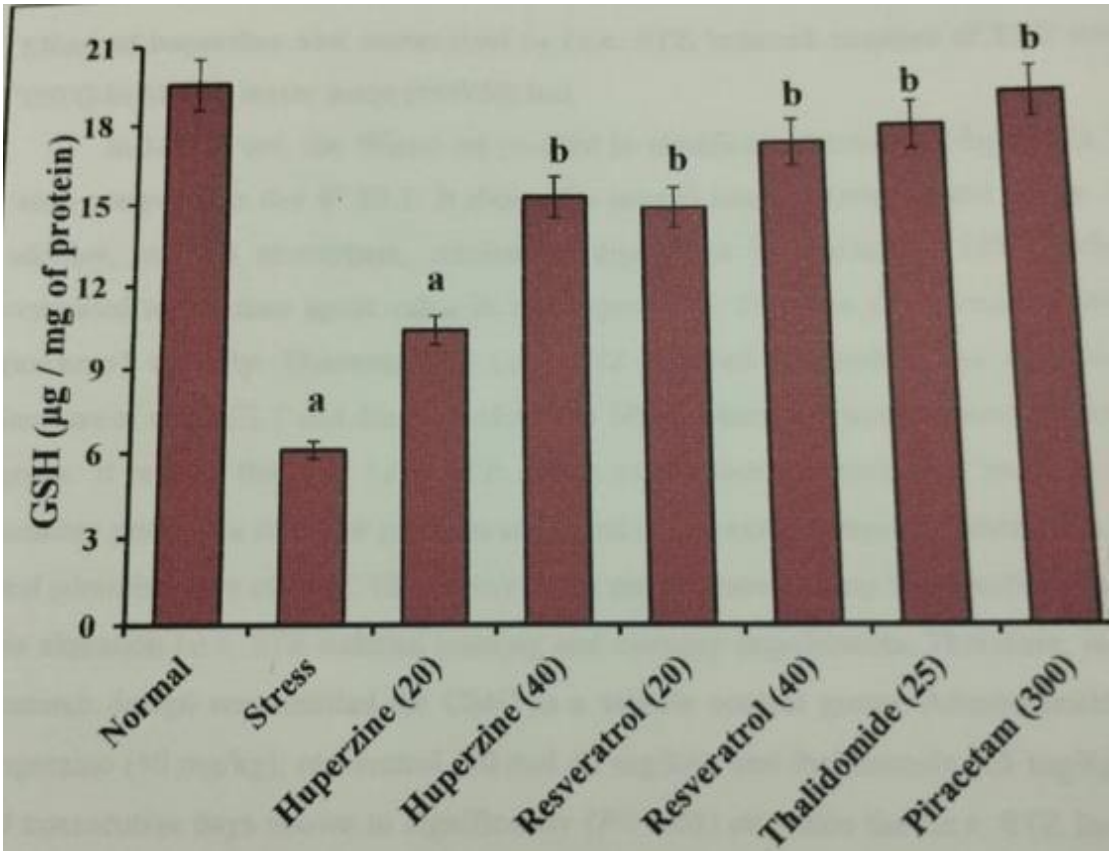

Figure 6. Effect of huperzine and resveratrol in stress induced changes of brain GSH activitylevel.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digits in parentheses indicate that dose mg/kg. Abbreviation *i.e.*, GSHisglutathione;andμg,microgram.

[a]p< 0.05versusnormalcontrolgroup.

[b]p< 0.05versusstresscontrolgroup.

Effect of huperzine and resveratrol in *i.c.v.* STZ induced changes of ELT and TSTQ inMorriswatermaze(MWM)test

In MWM test, the Wistar rat resulted to significant decrease of day 9th ELT, when compared today 6st ELT. It shows the normal learning (acquisition) ability. In addition, day 10 assessment,resulted to significant increasing of TSTQ, when compared to the time spent value in otherquadrants. It shows the normal retrieval (memory) capacity. However, the *i.c.v.* STZ resulted toproduce the significant increase of day 9 ELT and decrease of day 10 TSTQ when compared tonormalcontrolgroup.Itrevealsthat,the*i.c.v.*STZshowntoproducetheabnormallearning andmemory process in rats. Our previous study and other research report revealed that, the oraladministrationofCMC(0.5%w/v)doesnotproducetheanytherapeuticeffectinthealteration *i.c.v.* STZ induced learning and memory impairments. Therefore, in this research design wasomitted the CMC as a vehicle control group. Administration of huperzine (40 mg/kg); resveratrol(20 and 40 mg/kg); and thalidomide (25 mg/kg) for 10 consecutive days shown to significantly(P<0.05)attenuatethe*i.c.v.* STZinducedchanges oflearningandmemoryimpairmentsinadose dependent manner. Whereas, the huperzine (20 mg/kg) treated group does not produce thesignificant improvements in *i.c.v.* STZ induced cognitive impairments. Treatment of referencecontrol piracetam (300 mg/kg) produced the similar effects to improve the *i.c.v.* STZ inducedcognitive impairments;anditisstatisticallysignificantdifferencetohuperzine(20mg/kg)and *i.c.v.* STZ control groups. In addition, TNF-α synthesis inhibitor *i.e.,* thalidomide treatmentgroup also shown to produce the ameliorative effect against *i.c.v.* STZ induced ELT and TSTQchanges(**Table3andFigure7**).

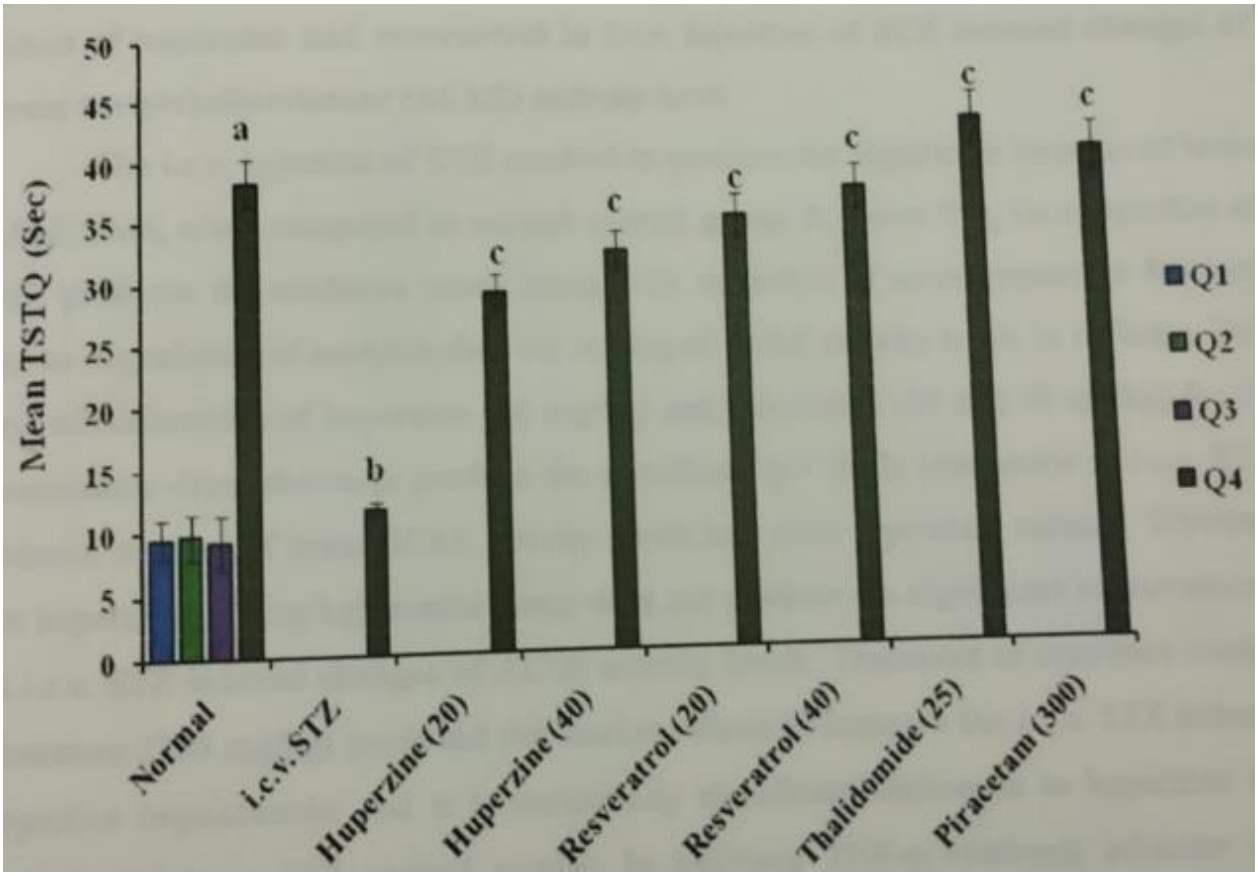

Table3. Effect of huperzine and resveratrol in *i.c.v.* STZ induced changes of learning behavior by using MWM apparatus.

Groups	Day 6 ELT (Sec)	Day 9 ELT (Sec)
Normal	57.6 ± 1.9	7.4 ± 0.9 [a]
i.c.v. STZ	61.7 ± 1.2	52.7 ± 1.7 [a,b]
Huperzine (20)	55.6 ± 1.4	21.6 ± 1.6 [a,b]
Huperzine (40)	58.1 ± 1.6	18.8 ± 1.3 [a,c]
Resveratrol (20)	56.3 ± 1.1	16.9 ± 1.7 [a,c]
Resveratrol (40)	57.2 ± 1.8	13.1 ± 1.8 [a,c]
Thalidomide (25)	52.9 ± 0.9	9.6 ± 1.9 [a,c]
Piracetam (300)	53.2 ± 1.6	10.2 ± 1.4 [a,c]

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroniposthoctests.Abbreviation*i.e.*,*i.c.v.*isintracerebroventricularregion;STZ,streptozotocinandELTisescapinglatencytime(asindicatoroflearningbehavior).

[a]*p*< 0.05versusDay6ELTinrespective group.

[b]*p* < 0.05 versus Day 9 ELT in normal control group. [c]*p* < 0.05versusDay9ELTin *i.c.v.*STZcontrolgroup.

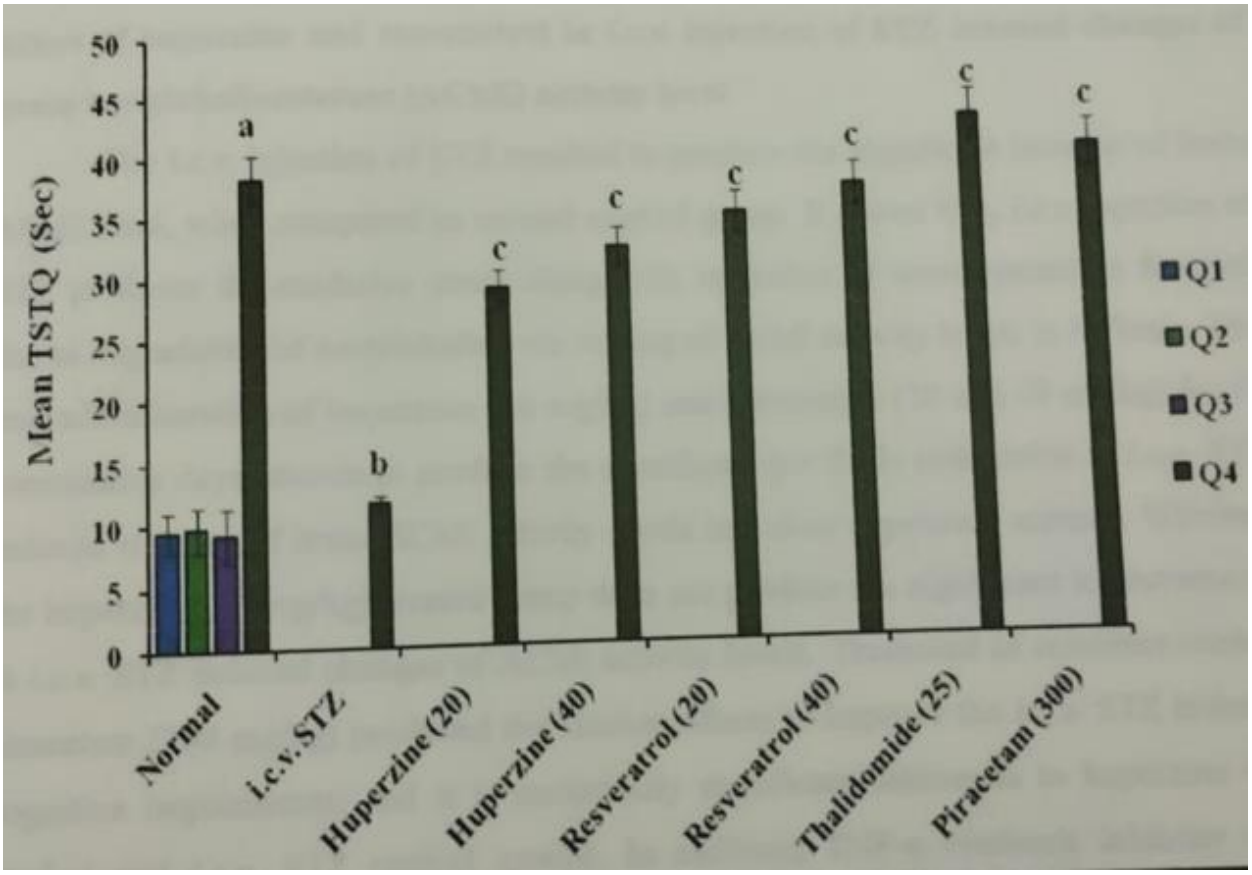

Figure8. Effectof huperzineandresveratrolin*i.c.v.*STZinducedchangesofmemoryfunctionbyusingMWM apparatus.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digits in parentheses indicate that dose mg/kg. Abbreviation *i.e.*, *i.c.v.*isintracerebroventricularregion;STZ,streptozotocin;andTSTQistimespentinQ_4(as indicator

ofmemoryretentionbehavior).Here,TSTQistimespentintargetquadrantasindicatorofmemoryimpro vementfunction.

[a]p<0.05versusmeantimespentinQ_1quadrantofnormalcontrolgroup.[b]p < 0.05 versus mean time spent in Q_4(TSTQ) in normal control group.[c]p<0.05versusmeantimespentinQ_4(TSTQ)in*i.c.v.*STZcontrolgroup.

Effect ofhuperzineandresveratrolin*i.c.v.*injection ofSTZinducedchangesof brainacetylcholinesterase(AChE)activitylevel

The *i.c.v.* injection of STZ resulted to produce the significant increase of brain AChE level, whencomparedtonormal control group. Itshows that,*i.c.v.* injection of STZ produces the oxidativestress alongwith reduction of neurotransmitterfunction due todegradation of acetylcholineviaraisingofAChEactivitylevelsinratbrain.Theoraladministration of huperzine(40mg/kg)andresveratrol(20 and40mg/kg)for10consecutivedays;showntoproducethe significant(p< 0.05)attenuation of *i.c.v.*STZ inducedchangesof brainAChEactivitylevelsin adosedependentmanner.Whereas,thehuperzine(20mg/kg)treatedgroupdoesnotproduce thesignificantimprovementsin*i.c.v.*STZinducedchangesofAChEactivitylevels.Treatmentofreferenc econtrolpiracetam(300mg/kg)producedthesimilareffectstoimprovethe*i.c.v.*STZinducedcognitiveim pairments;anditisstatisticallysignificantdifferencetohuperzine(20mg/kg)and *i.c.v.* STZ control groups. In addition, TNF-α synthesis inhibitor*i.e.,* thalidomide treatmentgroup also shown to produce the ameliorative effect against *i.c.v.* STZ induced changes of AChEactivitylevel(**Figure9**).

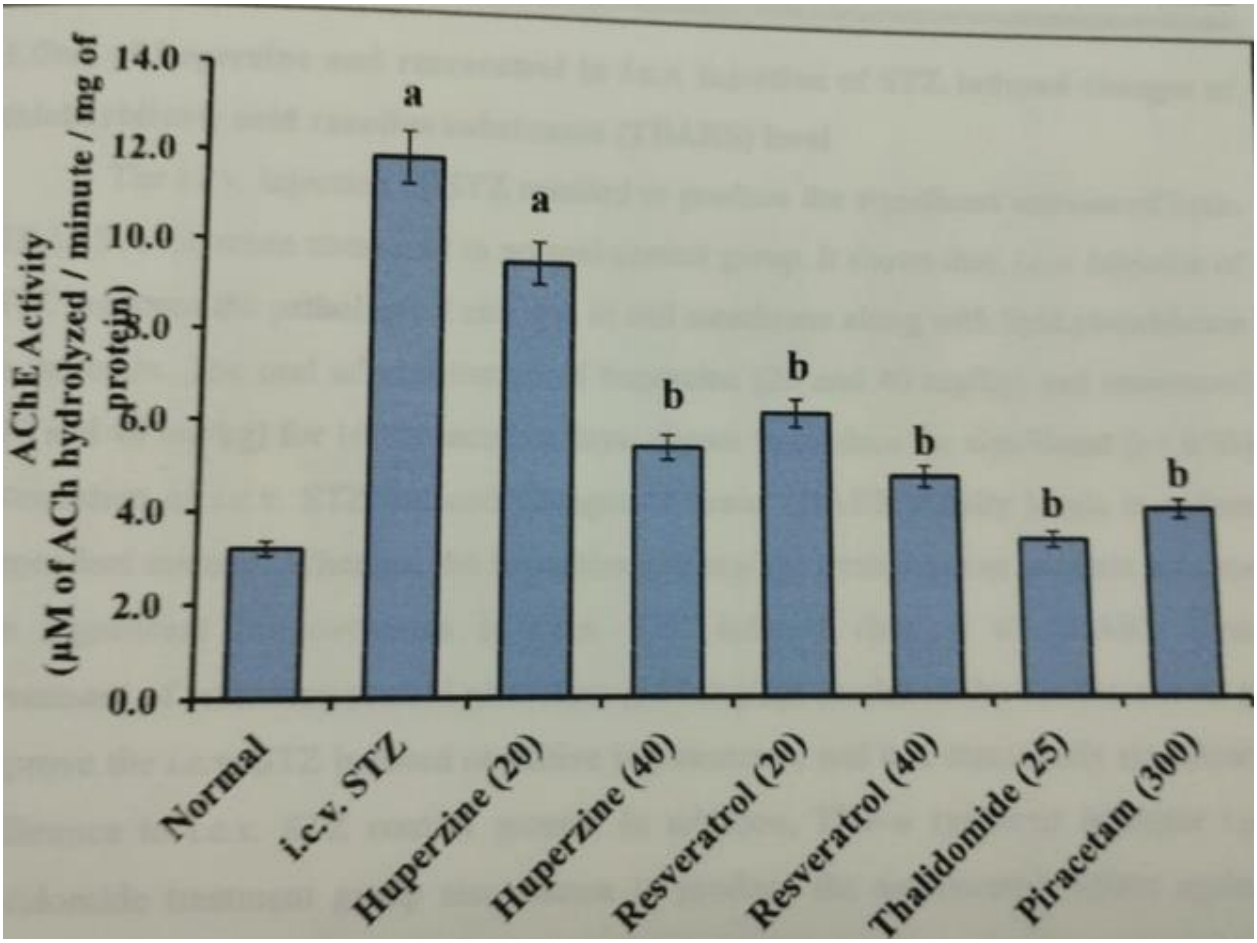

Figure 9. Effect of huperzine and resveratrol in *i.c.v.* STZ induced changes of brainAChEactivitylevel.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digits in parentheses indicate that dose mg/kg. Abbreviation *i.e.*, *i.c.v.*isintracerebroventricularregion;STZ,streptozotocin;AChEisacetylcholinesterase;ACh,acetylthiocholine;andµM,micromole.

[a]$p < 0.05$versusnormalcontrolgroup.

[b]$p < 0.05$versus *i.c.v.*STZcontrolgroup.

Effect of huperzine and resveratrol in *i.c.v.* injection of STZ induced changes ofthiobarbituric acidreactive substances (TBARS)level

The *i.c.v.* injection of STZ resulted to produce the significant increase of brain TBARS level,when compared to normal control group. It shows that, *i.c.v.* injection of STZ produces thepathological changesin cell membrane along with lipid peroxidation in rat brain.The oraladministrationofhuperzine(20and40mg/kg)andresveratrol (20and40mg/kg)for10consecutive days; shown to produce the significant ($p< 0.05$) attenuation of *i.c.v.* STZ inducedchanges of brain TBARS activity levels in a dose dependent manner. Whereas, the huperzine (20mg/kg)treated group does notproduce the significantimprovementsin*i.c.v.*STZinducedchanges of TBARS level. Treatment of reference control piracetam (300 mg/kg) produced thesimilar effects to improve the *i.c.v.* STZ induced cognitive impairments; and it is statisticallysignificant difference to *i.c.v.* STZ control groups. In addition, TNF-α synthesis inhibitor *i.e.*,thalidomide treatment group also shown to produce the ameliorative effect against *i.c.v.* STZinducedchangesofTBARSlevel(**Figure10**).

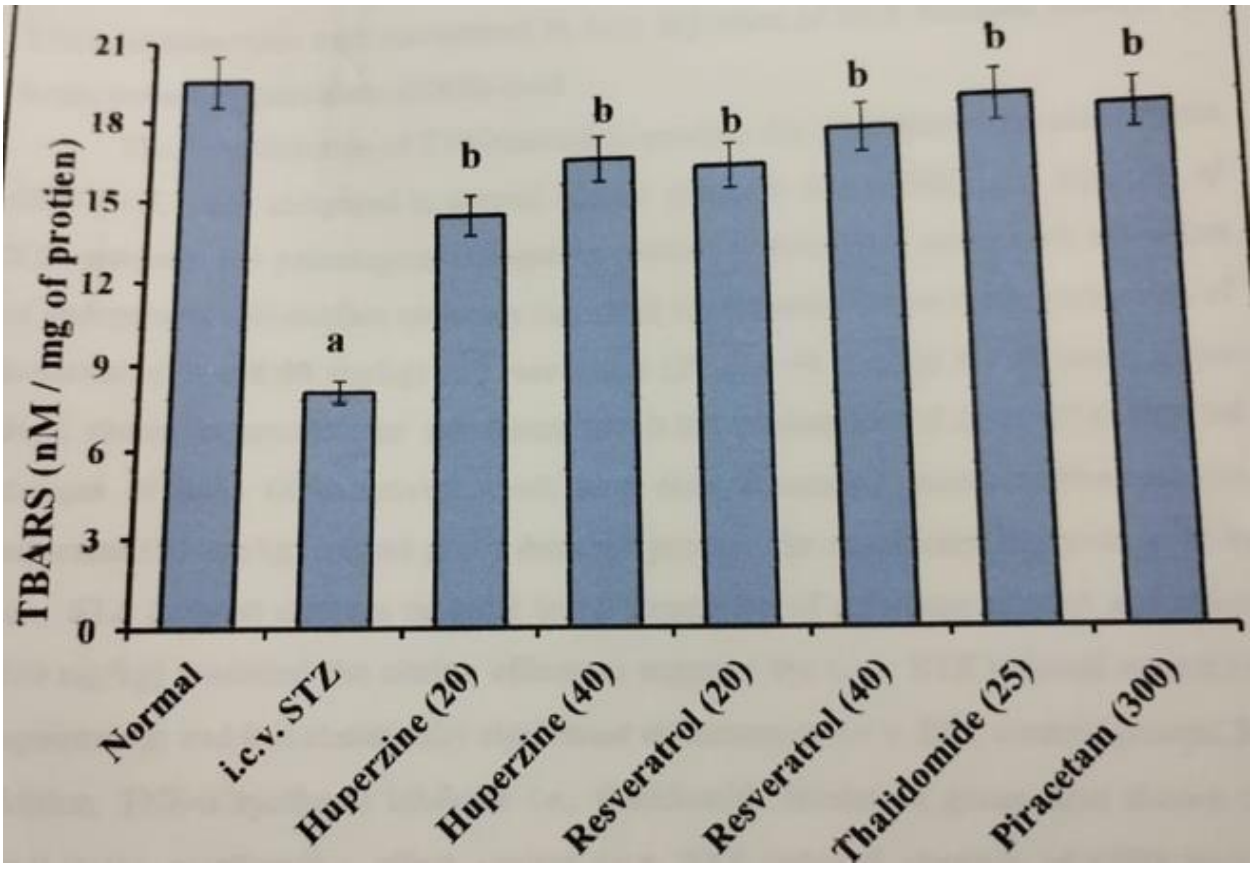

Figure 10. Effect of huperzine and resveratrol in *i.c.v.* STZ induced changes of brain TBARSlevel.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digits in parentheses indicate that dose mg/kg. Abbreviation *i.e.*, *i.c.v.*isintracerebroventricularregion;STZ,streptozotocin;TBARS,thiobarbituricacidreactivesubstances(*i.e.*, malondialdehyde);andnM,nanomole.

[a] $p < 0.05$versusnormalcontrolgroup.

[b] $p < 0.05$versus*i.c.v.*STZcontrolgroup.

Effect of huperzine and resveratrol in *i.c.v.* injection of STZ induced changes ofbrainreducedglutathione(GSH)level

The*i.c.v.*injectionofSTZresultedtoproducethesignificantincreaseofbrainGSHlevel,whencomparedtonormalcontrolgroup.Itshowsthat,*i.c.v.*injectionofSTZproducesthepathological changesincellularenvironmentalongwith alteration of endogenous anti-oxidantmolecule*i.e.*,GSHinratbrain.Theoraladministrationofhuperzine(20and40mg/kg)andresveratrol(20 and40mg/kg)for10consecutivedays;showntoproducethe significant($p < 0.05$)attenuationof*i.c.v.*STZinducedchangesofbrainGSHactivitylevelsinadosedependentmanner.Whereas,thehuperzine(20mg/kg)treatedgroupdoesnotproduce thesignificantimprovementsin*i.c.v.*STZinducedchangesofGSHlevel.Treatmentofreferencecontrolpiracetam(300mg/kg)producedthesimilareffectstoimprovethe*i.c.v.*STZinducedcognitiveimpairments;anditis statisticallysignificantdifferenceto*i.c.v.*STZ controlgroups. Inaddition,TNF-αsynthesisinhibitor*i.e.*,thalidomidetreatmentgroupalsoshowntoproducetheameliorativeeffectagainst*i.c.v.*STZinducedchangesofGSHlevels(**Figure 11**).

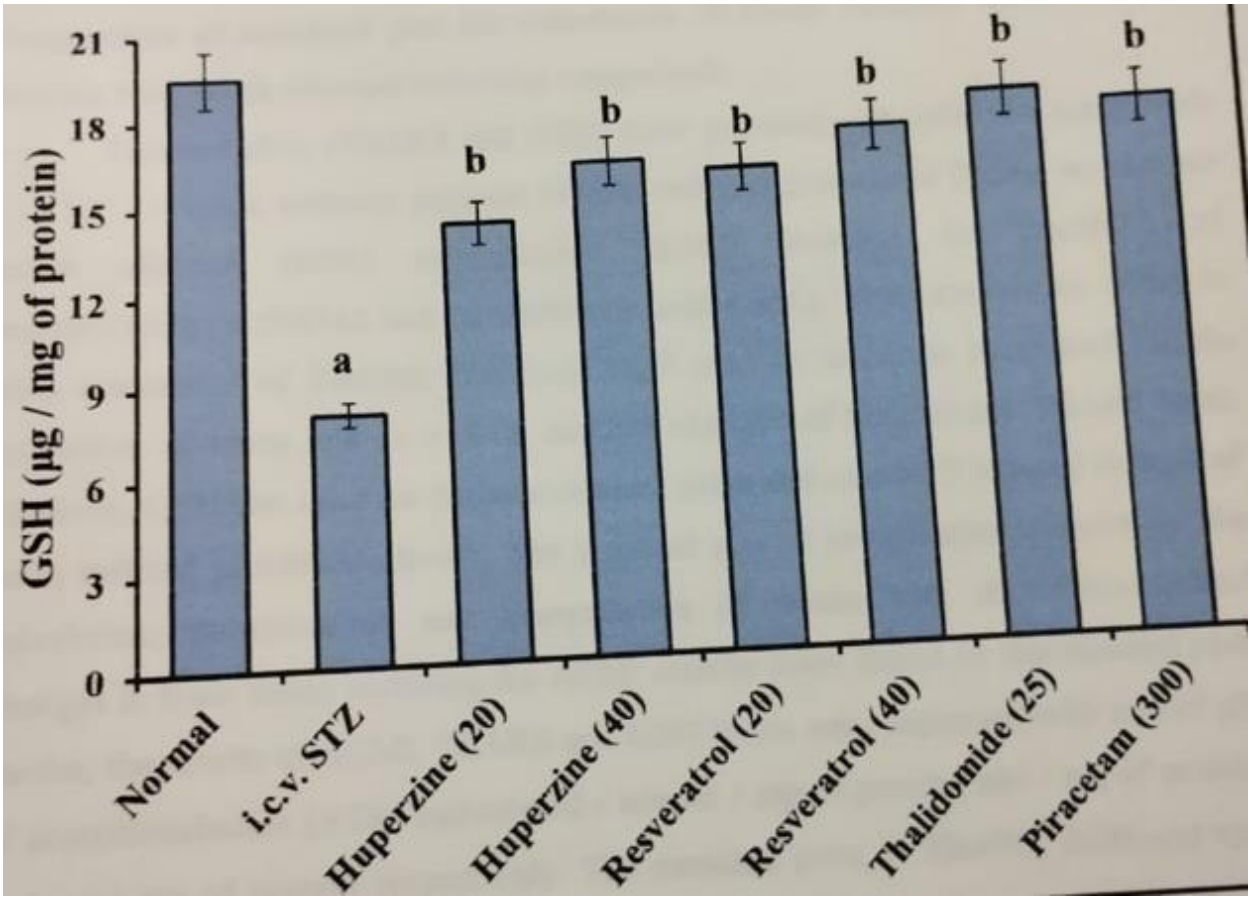

Figure11. Effectofhuperzineandresveratrolin*i.c.v.*STZinducedchangesofbrainGSHlevel.

Data were expressed as mean ± Standard deviation (SD), n = 6, two-way ANOVA followed byBonferroni post hoc tests. Digits in parentheses indicate that dose mg/kg. Abbreviation *i.e.*, *i.c.v.*isintracerebroventricularregion;STZ, streptozotocin;GSHisglutathione;andµg, microgram.

[a]*p*< 0.05versusnormalcontrolgroup.

[b]*p* <0.05versus*i.c.v.*STZcontrolgroup.

Preparation of standard plot for calculation of tissue TBARS; GSH and total protein levelswithrelevantreferencecompounds

Standard plots (TBARS and GSH) were prepared with reference compounds *i.e.,* 1,1',4,4'-tetramethoxy propane (TMP); reduced glutathione (GSH) and bovine serum albumin (BSA)respectively. TBARS indicates the reactivity of malondialdehyde (MDA) and thiobarbituric acid(TBA).

TMPmimicstheMDA*invitro*assessmentofTBARS;therefore,TMPusedasreference

compound for the estimation of stress and *i.c.v.* STZ induced changes of brain tissue TBARSlevel. Similarly, GSH also used for the estimation of stress and *i.c.v.* STZ induced changes ofbrain reduced glutathione levels. The standard plot of protein also prepared for the calculation;quantification and interpretation of results with above biochemical changes in brain tissueincluding the AChE activity level. Based on this standard plots results; the results of AChE,TBARSandGSHlevelswereexpressedwithunitofμMofacetylthiocholine(ACh)hydrolyzed /minute/mg ofprotein;nM/mg ofprotein;andμg /mg ofproteinrespectively.ThestandardplotsofTBARS,GSHandtotalproteinshowninthe**figure12-14**.

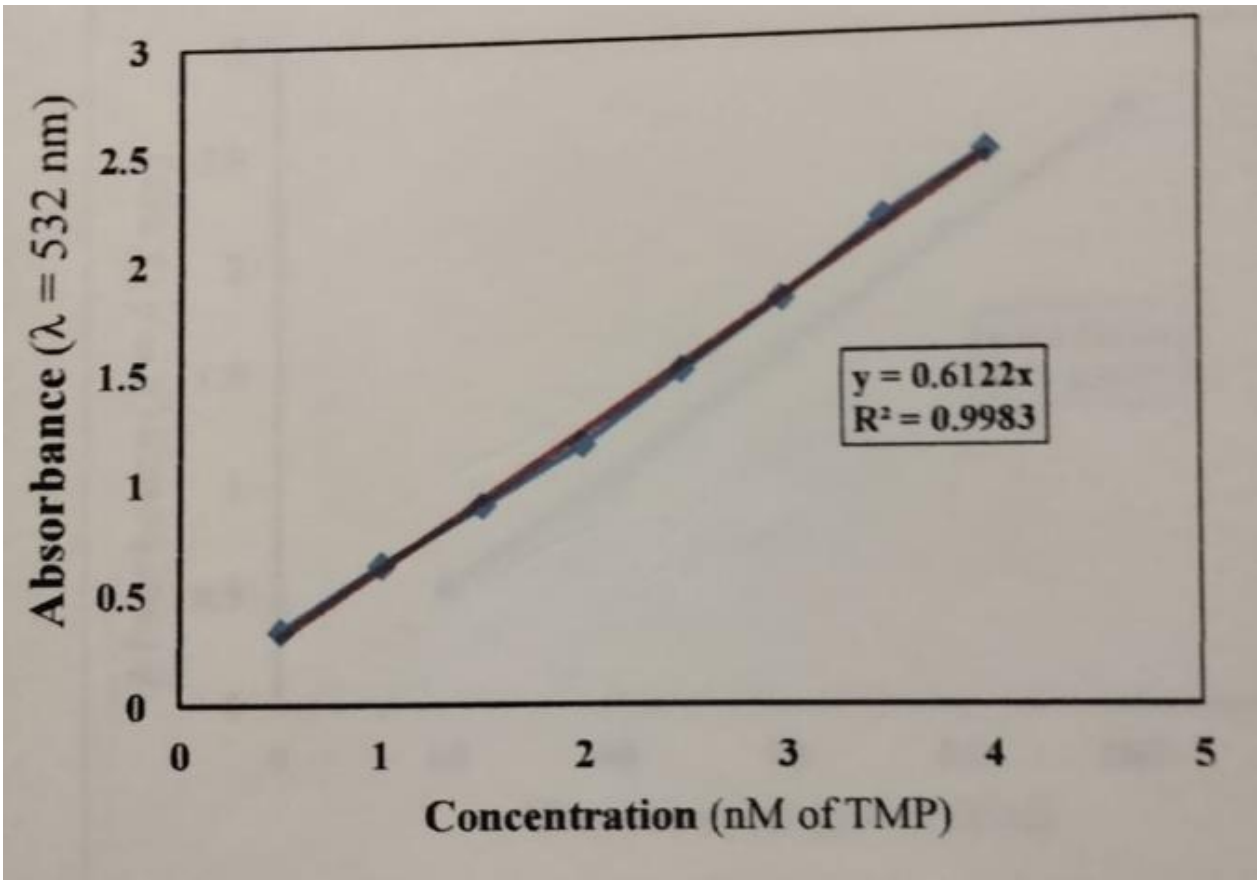

Figure12.StandardplotofTBARS.

Blue color line indicates the absorbance versus concentration of TMP. TMP, as a referencestandard for TBARS (*i.e.*, tissue MDA) estimation described method of Ohkawa *et al.* (1979).Pink color line indicates the linearity correction line (y = mx + C) and R^2 value 0.998 shownbelowthevalue1.Thisstandardplotisusedforfurtherestimationandcalculationofbrain

TBARS levels expressed as nM of TBARS per g of tissue. Followed by, it used to interpret thefinalresults withcorrelationoftissueproteinlevel.

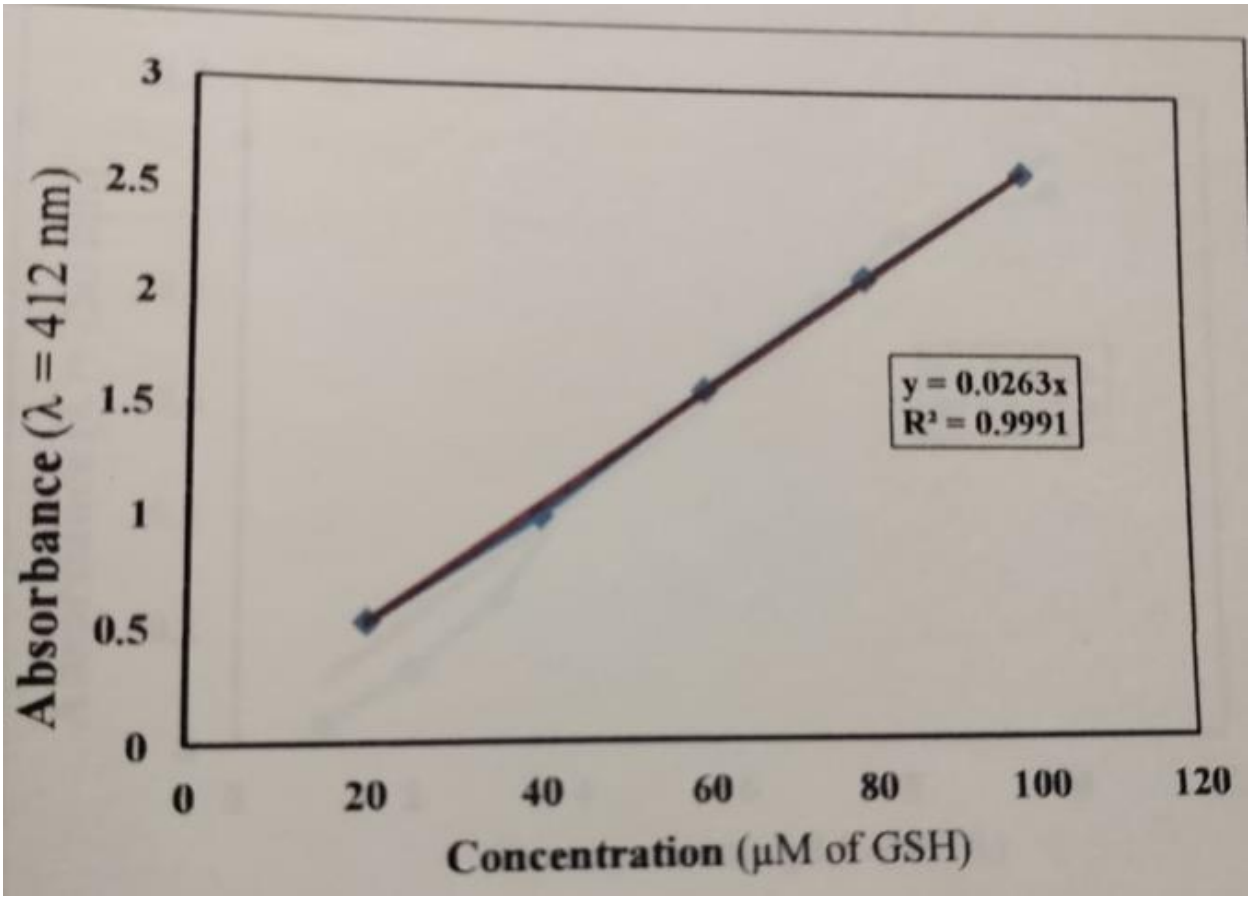

Figure13.Standard plotofGSH.

Bluecolorlineindicatestheabsorbanceversusconcentrationofreducedglutathioneandestimation of GSH performed as described method of Beutler *et al.* (1963). Pink color lineindicates the linearity correction line (Y= mx + C) and R^2 value 0.994 shown below the value 1.This standard plot is used for further estimation and calculation of brain reduced glutathionelevels with expression of μM of GSH per g of tissue. Followed by it used to interpret the finalresultswithcorrelationoftissue proteinlevel.

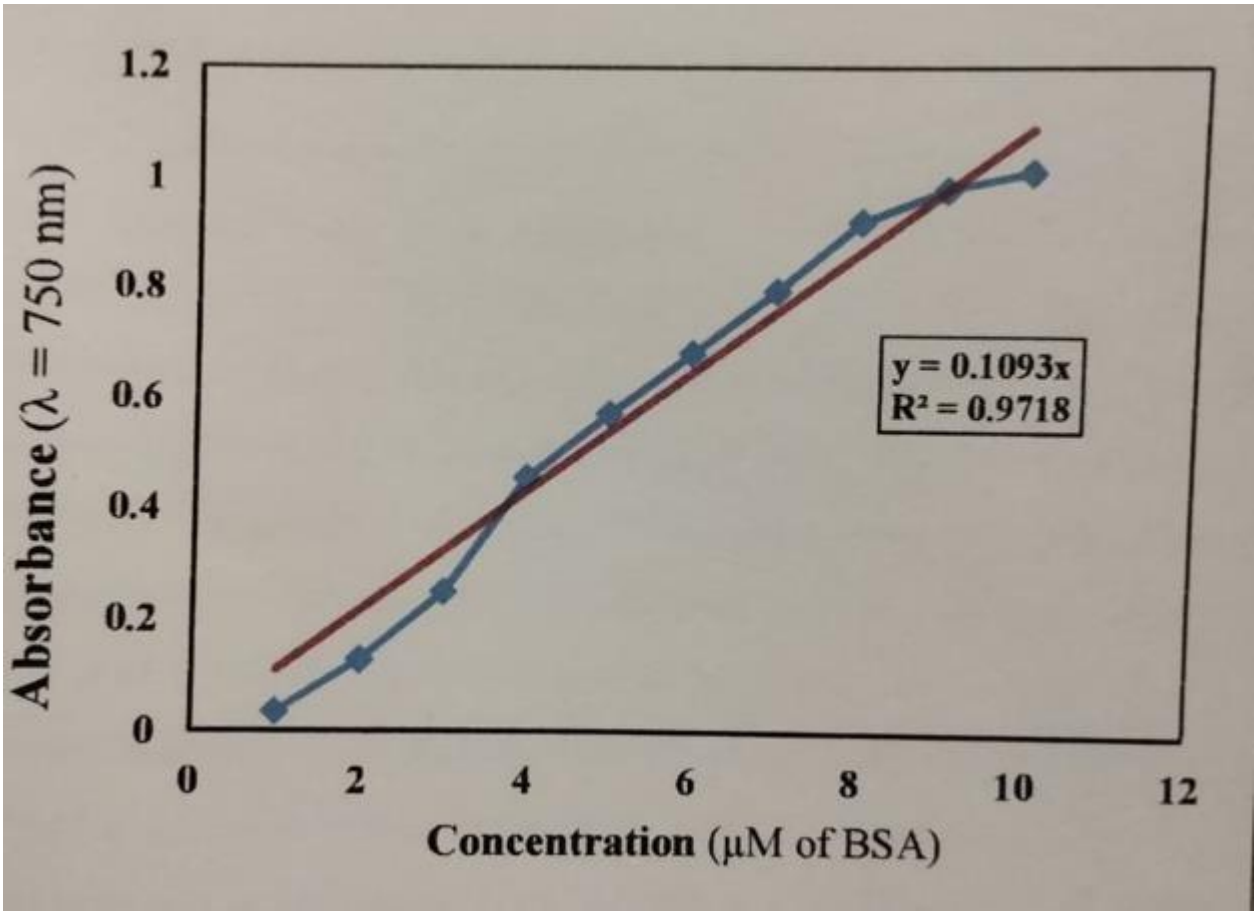

Figure14.Standard plot oftotalproteins.

Blue color line indicates the absorbance versus concentration of bovine serum albumin (BSA).BSA, as a standard for total protein estimation described in Lowry's *et al.* (1951) method. Pinkcolor line indicates the linearity correction line (Y= mx + C)and R^2 value 0.971 shown belowthe value 1. This standard plot is used for further estimation and calculation of brain total proteinlevelswithexpressionofmgofproteinpergramoftissue.

STATISTICALANALYSIS

The standard deviation (SD) was used to express all data as mean SD. The behavioral data werestatistically analyzed using two-way analysis of variance (ANOVA), followed by the Bonferronipost hoc test, and the data of tissue biomarker activity levels, such as TBARS, GSH, and AChE,wereanalyzedusingone-wayANOVA,followedbytheTukey'sMultipleRangetest,using

GraphPadPrismVersion-5.0software.Thethresholdforstatisticalsignificancewas apvalueof 0.05orlower.

DISCUSSION

MWM testwas employedin the presentstudy to assess the cognitive dysfunction. This testisone of the best methods for the evaluation of spatial learning and memory (Morris *et al.*,1984;ParleandSingh, 2007).Theresultsofthepresentinvestigationindicatethatswimmingstressand *i.c.v.*STZproduceda significant(p<0.05) raisethecognitiveimpairmentasindication ofincrease the time duration of ELT and TSTQ levels. In addition, the tissue biomarkers change *i.e.*,ariseinAChEandTBARS; anddecreaseinGSHlevelswhen comparedtonormal group.Whereas,thepre-treatmentofhuperzine(20and40mg/kg;*p.o.*)andresveratrol(20and40mg/kg)for10consecutivedayssignificantly(P<0.05)attenuatetheswimmingstressand*i.c.v.*STZinducedcognitiveimpairmentsandtissuebiomarkerchangesinrat.Similarly,theAChEinhibitor*i.e.*,piracetam(300mg/kg)andTNF-αsynthesisinhibitor*i.e.*,thalidomidetreatmentgroupsarealsoknowntoproducetheameliorativeeffectagainststressand*i.c.v.*STZinducedchangesofcognitivefunctionaswell asbiomarkerchanges.Theseresultsaresimilartothatofourpreviousstudiesfromourownlabandotherlaboratories(Gulati*etal.*,2015;KumarandSingh,2017).BilateraladministrationofSTZ(3mg/kg)via*i.c.v.*routeproducesthepotentialoxidativestress,freeradicalgenerationandexpressionofcytokines.Inadditio n,itenhancestheenergydemand;neurodegenerationandaccumulationoftauproteins.Clinicallyitisresemblingtosporadic Alzheimer'sdisease(Grieb,2016;Lu*etal.*,2017;Ponce-Lopez*etal.*,2017). The establishedconceptof cognitive dysfunctionis closely related tooxidative stress ofthebrain(Bhardwaj*etal.*,2016;Reeta*etal.*,2017a).Inaddition,reactiveoxygenandreactivenitrogenspeciesarealsocontributestoalterationofbrainlearningandmemoryfunctions(Cirmi*etal.*,2016;Libro*etal*.,2016).Inchronicstage,itisalsodocumentedtoproducetheneuronalapoptosis,neurodegenerationandneuronaldeath(Ghosh*etal.*,2017;Saxena*etal.*,2011;Song*etal.*,2014).Thesimilarpathophysiologicalmechanismalsoinvolvedinthestresscondition;andthealteration ofbiologicalpathway ismay beduetothechangesofneuroendocrinefunctionespeciallythechangesofhypothalamic-pituitary-adrenal(HPA)axisfunction(Choi*etal.*,2017;Grafe*etal.*,2017;Sanchís-Ollé*etal.*, 2017;Sela*etal.*,2017).

Inthis study,stress and*i.c.v.*injectionoftheSTZ(3mg/kg)hasproducedthecognitiveimpairmentalongwith raisein brain oxidativeandlipidperoxidation alongwith risein brainAChEactivity level (Anuradha*etal.*, 2010; Haleagrahara*etal.*,2009). Similarresults are alsoobservedindifferentstudy(Fatranska*etal.*,1987;KumarandSingh,2017;Mitra*etal.*,2009).Thebrai nAChEactivitylevelisalsooneofthegoldstandardbiomarkersforthebrainfunction.Thechangesofcholi nergicnervoussysteminvariouspartsofthebrainespeciallybraincortex,hippocampus,cerebellum,stria tum andthalamusareinvolvingthechangesofacetylcholinelevels(Saxena*etal.*,2011;Sela*etal.*,2017;Silver man*etal.*,2014).Thestressandneuroinflammatoryconditionofbraincellsexpressthevariousproteinsuc hasapoptosisrelatedproteins,transcriptome,proteome,hypoxia-inducible factor(HIF),neurotrophinsincludingreleaseendothelialderivedrelaxingfactor(EDRF)*i.e.*,ni tricoxide(NO)andincreasetheendothelialNOsynthase(eNOS)expression,therebyabundantnoproduc tionoccurinthebrain(Huang *et al.,* 2015; Plaza-Zabala *et al.,* 2017; Sabernia *et al.,* 2016; Wu *et al.,* 2015). In addition,itcausesthecerebrovascularinjuryinvariousregionsof thebrainandsubsequentlyitgeneratesthesuperoxide($^{\cdot}O^{2-}$)anion(SinghandPrakash,2017).Superoxideanionisakeymoleculetogeneratethereactivenitrogenspe ciesintheformofperoxynitrate($^{\cdot}ONOO-$)withreactivity ofnitricoxide(Ma*etal.*,2017;Matsubara*etal.*,2015;Olas,2017).Furthermore,thebothradicals*i.e.*,super oxideanion andperoxynitrateradicalsarewell documentedtoenhancethelipidperoxidationofneuronalmembrane(Abdel-Salam*etal.*,2017;Islam,2017).Thelipidperoxidationismostvulnerablereactioninthenervoussystem.B ecause,ithashighcontentofpolyunsaturated fatty acids (PUFA) and these unsaturated bonds of PUFA are easy targets to freeradicalsleadstoproducetheoxidativedamage(DeFranceschi*etal.*,2017;Naudí*etal.*,2017).The hippocampus (a crucial brainareaforcognition)region of brainis potentially damagedintransientischemicvasculardamageconditionviageneratingfreeradicalandoxidativestress(Bagatini *etal.*, 2017; Choi,*etal.*,2017). However, the neuronal synthesis of endogenous anti-oxidantmolecule*i.e.*,reducedglutathione(GSH)isalsodecreaseinthestressedandinflamedbraintissue(Samarghandian*etal.*,2017;Tian*etal.*,2017).Whichisscavengethefreeradicalsandpreventtheneuronal damage(NarkhedeandKulkarni,2017;Reeta*etal.*,2017b).Collectively,theneuronalpro-oxidantandanti-

oxidantbalancingeffectsareswitchovertopathologicalstatusleadstoneuroinflammation,vascularinjuryandoxidativestressassociatedcognitive dysfunction(Baluchnejadmojarad*etal.*,2017;Reis *etal.*,2017).

The many natural polyphenolic compounds including huperzine and resveratrol are known toproduce the neuroprotective effects (Omar *et al.,* 2017; Tao *et al.,* 2016; Zhao *et al.,* 2017). Inaddition, itis also documented that, these compounds are able to cross the blood brain barrierand directly scavenge the free radicals *i.e.,* reactive oxygen and nitrogen species (Pallas *et al.,*2014; Wang *et al.,* 2011); and chelate the biomolecule (Belguendouz *et al.,* 1997; Haviv *et al.,*2007).Inaddition,huperzineandresveratrolisnotedtoproducetheanti-oxidative,anti-inflammatory action by reduction free radical generation, scavenging free radicals including theprevention of lipid peroxidation (Muhammad *et al.,* 2017). The current results have also beenindicated that, huperzine and resveratrol possess the memory improving action in stress and *i.c.v.*STZ induced memory dysfunction in rat. In addition, the AChE inhibitor *i.e.,* piracetam is aconventional medicine for the management of memory disorders as a nootropic agent (Tripathi *etal.,*2017).Inaddition,ithasanti-oxidant,anti-lipidperoxidation,anti-inflammatory,anti-ischemic and reduction of hypoxic environment in the brain (Diaz-Gerevini *et al.,* 2016; Shi *etal.,* 2012). It also induces the synthesis and elevation of reduced glutathione in brain tissue(Gawlik *et al.,* 2017; Mao *et al.,* 2014). Therefore, ithas been taken up as a positive control inthe present study. In this study, it is shown to attenuate the stress and *i.c.v.* STZ induced learningand memory impairments along with regulation of neuronal biomarker changes. These results arealso line with other research laboratory report; their study revealed that, piracetam shown toproduce the ameliorative effect in lipopolysaccharide and postnatal propofol exposure in miceinduced memory impairment (Tripathi *et al.,* 2017; Wang *et al.,* 2016). The neuroinflammatoryprocesses are well documented in the stress and *i.c.v.* STZ treatment conditions in laboratoryanimals (Kumar *et al.,* 2015; Zhao *et al.,* 2017). Hence, the anti-inflammatory agents are knownto produce the neuroprotection as well as enhancing of neuronal function including cognitiveimprovements (Budni *et al.,* 2016; Li *et al.,* 2017). Similarly, the treatment of TNF-α synthesisinhibitor *i.e.,* thalidomide also protects the nervous system from stress and *i.c.v.* STZ inducedneuronal damage and cognitive impairment. Based on literature and data in hand, it may besuggested that huperzine and resveratrol are known to ameliorate the stress and *i.c.v.* injection ofSTZinducedlearningandmemorydysfunctionbyvirtueofitsmultiplemoleculareffectincludinganti-oxidative,anti-inflammatoryandanti-lipidperoxidativeactivities.Therefore,huperzine and resveratrol has beneficial effect in stress and neuroinflammatory conditions of thebrainforimprovementofcognitive dysfunction.

SUMMARYANDCONCLUSION

The present study has been designed to investigate the role of huperzine and resveratrol in stressand *i.c.v.* injection of STZ induced cognitive dysfunction in rat. The behavioral assessment *i.e.,*learning and memory were assessed by using MWM test method. The brain AChE activity levelwas estimated to correlate the function of memory with neurotransmitter action. It is primarymarkerbraincholinergicandmemoryfunctions.ThebrainTBARSasamarkeroflipidperoxidation and GSH as a marker of endogenous anti-oxidant molecule levels were estimated toassess the degree of oxidative stress. Piracetam is an AChE inhibitor and it is served as positivecontrol in the present study. In addition, the thalidomide is a TNF-α synthesis inhibitor and it isalso known to produce the ameliorative effect against stress and *i.c.v.* STZ induced changes ofcognitivefunctionalongwithtissuebiomarkerchanges.

On the basis of results obtained in the present study, the following salient findings may besummarized:

1. The stress and intracerebroventricular (*i.c.v.*) administration of single dose of STZ (3 mg/kg)produced a significant cognitive dysfunction, as an index of raise in ELT and decrease inTSTQlevels.Furthermore,thesegroup of animalshowsthesignificantrisingofbrainoxidative stress whichisindicatedby raisingTBARS; anddecreaseinGSHlevels.Inaddition,AChEactivitylevelisalsoincreasedinbraintissue.Itindicatesthat,theadministrationofSTZby*i.c.v.*injectionmethodispotentiallyimpairingthecognitive(learningandmemory)functioninrat.

2. Treatment with huperzine (20 and 40 mg/kg, *p.o.*; for 10 consecutive days) and resveratrol (20and 40 mg/kg, *p.o.*; for 10 consecutive days); thalidomide (25 mg/kg, *p.o.*; for 10 consecutivedays); and piracetam (300 mg/kg, *p.o.*; for 10 consecutive days) are significantly attenuatedthe stress and *i.c.v.* injection of STZ inducedimpairment of learning andmemory functionsas reflected by MWM test along with reduce the AChE activity levels. In addition, bothagents are also attenuating the stress and *i.c.v.* Injection of STZ induced oxidative biomarker*i.e.,* TBARSandGSHlevelchanges.

Hence,itmaybeconcludedthat,huperzineandresveratrolmayserveasnewerherbalcandidatetotreatsthe neurologicaldamageandoxidativestressassociatedmemory impairments.However,more extensive study isneeded before utilization in clinical trial.Further,itshouldbe study intheaspects of effectivenessof huperzineandresveratrol invariousconditionslikeischemic,hypoxicandneurological damageassociatedmemory impairmentwith suitableexplanation ofmolecularmechanisminrodentas wellasnon-rodentspecies.

REFERENCES

1. Abdel-Salam, O.M., Youness, E.R., Mohammed, N.A., Shaffie, N., Abouelfadl, D.M., Sleem,A.A., 2017. The effect of 2, 4-dinitrophenol on oxidative stress and neuronal damage in ratbrain inducedbysystemic rotenoneinjection.Reactive OxygenSpecies3(8).
2. Acsády,L.,Harris,K.D.,2017.Synaptic scalingin sleep.Science 355(6324),457-457.Ahmadian-Attari,M.M.,Dargahi,L.,Mosaddegh,M.,Kamalinejad,M.,Khallaghi,B.,Noorbala, F., Ahmadiani, A., 2015. Impairment of rat spatial learning and memory in a newmodel of cold water-induced chronichypothermia:ImplicationforAlzheimer's Disease.NeurotoxRes 28(2),95-107.
3. Akhondzadeh, S., Tajdar, H., Mohammadi, M.R., Mohammadi, M., Nouroozinejad, G.H.,Shabstari, O.L., Ghelichnia, H.A., 2008. A double-blind placebo-controlled trial of piracetamadded to risperidone in patients with autistic disorder. Child Psychiatry Hum Dev 39(3), 237-245.
4. Akinyemi, A.J., Okonkwo, P.K., Faboya, O.A., Onikanni, S.A., Fadaka, A., Olayide, I.,Akinyemi, E.O., Oboh, G., 2017. Curcumin improves episodic memory in cadmium inducedmemory impairmentthrough inhibition of acetylcholinesteraseandadenosinedeaminaseactivitiesinaratmodel.MetabolicBrainDisease32(1),87-95.
5. Alberini, C.M., 2011. The role of reconsolidation and the dynamic process of long-termmemoryformationandstorage.FrontBehavNeurosci5,12.
6. Alderson-Day, B., Fernyhough, C., 2015. Inner speech: Development, cognitive functions,phenomenology,andneurobiology.PsycholBull141(5),931-965.
7. Alizadeh,A.A.,Hamzeh-Mivehroud,M.,Farajzadeh,M.,Dastmalchi,S.,2017.IdentificationofnovelpeptidesagainstTNF-αusingphagedisplaytechniqueandinsilico

modeling of their modes of binding. European Journal of Pharmaceutical Sciences 96, 490-498.

8. Amin,H.P.,Schindler,J.L.,2017. Stroke syndromes,vascularneurology boardreview.Springer,pp.23-35.
9. Bae, G.Y., Olkkonen, M., Allred, S.R., Wilson, C., Flombaum, J.I., 2014. Stimulus-specificvariabilityincolorworkingmemorywithdelayedestimation.J Vis14(4).
10. Bagatini, P.B., Xavier, L.L., Bertoldi, K., Moysés, F., Lovatel, G., Neves, L.T., Barbosa, S.,Saur,L.,deSenna,P.N.,Souto,A.A.,2017.Anevaluationofaversivememoryandhippocampal oxidative status in streptozotocin-induced diabetic rats treated with resveratrol.Neuroscience Letters 636,184-189.
11. Bakhtiari, R., Shariat, B.S., Motazedian, F., Wu, Z., Zhang, J., Yang, H., Liu, Y., 2017.Complex transformation field created by geometrical gradient design of NiTi shape memoryalloy.FunctionalMaterialsLetters 10(01),1740011.
12. Balasubramanian, B.A., Cohen, D.J., Davis, M.M., Gunn, R., Dickinson, L.M., Miller, W.L.,Crabtree, B.F., Stange, K.C., 2015. Learning evaluation: blending quality improvement andimplementationresearch methodstostudyhealthcareinnovations.ImplementSci10,31.
13. Baluchnejadmojarad, T., Kiasalari, Z., Afshin-Majd, S., Ghasemi, Z., Roghani, M., 2017. S-allyl cysteine ameliorates cognitive deficits in streptozotocin-diabetic rats via suppression ofoxidative stress, inflammation, and acetylcholinesterase. European Journal of Pharmacology794,69-76.
14. Carey,J.L.,Nader,N.,Chai,P.R.,Carreiro,S.,Griswold,M.K.,Boyle,K.L.,2017.Drugsandmedical devices:adverseeventsandtheimpactonwomen'shealth.ClinicalTherapeutics.
15. Carsrud, A., Brännback, M., Elfving, J., Brandt, K., 2017. Motivations: The entrepreneurialmind andbehavior,revisitingthe entrepreneurialmind.Springer,pp.185-209.
16. Carter, M.J., Ste-Marie, D.M., 2016. An interpolated activity during the knowledge-of-resultsdelayintervaleliminatesthelearningadvantagesofself-controlledfeedbackschedules.PsycholRes.
17. Castillo-Hernández, J.C., Velázquez-Moyado, J.A., Reyes-Ramírez, A., Ramírez-López, E.,González-Andrade, M., Navarrete, A., 2017. Effect of N-benzylpiperazine, its metabolite N-Benzylethylenediamine,anditsdisubstitutedanalogueN,N'-Dibenzylpiperazineonthe

acquisition, formation, and consolidation of memory in mice. Pharmacology 99(5-6), 268-274.

18. de Oliveira, A.M., Radanovic, M., de Mello, P.C., Buchain, P.C., Vizzotto, A.D., Celestino,D.L., Stella, F., Piersol, C.V., Forlenza, O.V., 2015. Nonpharmacological interventions toreduce behavioral and psychological symptoms of dementia: a systematic review. BiomedResInt2015,218980.
19. de Quervain, D., Schwabe, L., Roozendaal, B., 2017. Stress, glucocorticoids and memory:implicationsfortreatingfear-relateddisorders.NatureReviewsNeuroscience18(1),7-19.
20. Dhir, M., Shrestha, R., Steel, J.L., Marsh, J.W., Tsung, A., Tublin, M.E., Amesur, N.B.,Orons, P.D., Santos, E., Geller, D.A., 2017. Initial treatment of unresectable neuroendocrinetumor liver metastases with trans arterial chemoembolization using streptozotocin: A 20-YearExperience.Annals ofSurgicalOncology24(2),450-459.
21. Ebner, K., Singewald, N., 2017. Individual differences in stress susceptibility and stressinhibitorymechanisms.CurrentOpinioninBehavioralSciences14,54-64.
22. El Haj, M., Gandolphe, M.C., Allain, P., Fasotti, L., Antoine, P., 2015. "Forget to whom youhave told this proverb": directed forgetting of destination memory in Alzheimer's disease.BehavNeurol2015,215971.
23. Ellman, G.L., Courtney, K.D., Andres, V., Jr., Feather-Stone, R.M., 1961. A new and rapidcolorimetric determinationofacetylcholinesterase activity.BiochemPharmacol7,88-95.
24. Endres, T., Carpenter, S., Martin, A., Renkl, A., 2017. Enhancing learning by retrieval:enrichingfree recallwithelaborative prompting.Learningand Instruction49,13-20.
25. English, M.C., Visser, T.A., 2014. Exploring the repetition paradox: the effects of learningcontextandmassedrepetitiononmemory.PsychonBullRev21(4),1026-1032.
26. Farooqui,A.A.,Farooqui,T.,2017.Garlicanditseffectsinneurologicaldisorders.neuroprotective effectsofphytochemicalsinneurologicaldisorders,113.
27. Gainotti, G., Quaranta, D., Vita, M.G., Marra, C., 2014. Neuropsychological predictors ofconversion from mild cognitive impairment to alzheimer's disease. J Alzheimers Dis 38(3),481-495.
28. Gawlik,M.,Gawlik,M.B.,Smaga,I.,Filip,M.,2017.Manganeseneurotoxicityandprotective effects of resveratrol and quercetin in preclinical research. Pharmacol Rep 69(2),322-330.

29. Genrikhs, E.E., Stelmashook, E.V., Golyshev, S.A., Aleksandrova, O.P., Isaev, N.K., 2017.Streptozotocincausesneurotoxiceffectinculturedcerebellargranuleneurons.BrainResearchBulletin.
30. Haleagrahara, N., Radhakrishnan, A., Lee, N., Kumar, P., 2009. Flavonoid quercetin protectsagainst swimming stress-induced changes in oxidative biomarkers in the hypothalamus ofrats.EurJ Pharmacol621(1-3),46-52.
31. Hartley,C.A.,Lee,F.S.,2015.Sensitiveperiodsinaffectivedevelopment:nonlinearmaturationofferlearning.Neuropsychopharmacology40(1),50-60.
32. Hashiguchi,M.,Ohta,Y.,Shimizu,M.,Maruyama,J.,Mochizuki,M.,2015.Meta-analysisof the efficacy and safety of ginkgo biloba extract for the treatment of dementia. J PharmHealthCareSci1,14.
33. Hasson,U.,Chen,J.,Honey,C.J.,2015.Hierarchicalprocessmemory:memory asanintegralcomponentofinformationprocessing.Trends CognSci19(6),304-313.
34. Islam, M., Rupeshkumar, M., Reddy, K.B., 2017. Streptozotocin is more convenient thanalloxanfortheinductionoftype2diabetes.InternationalJournalofPharmacologicalResearch7(1),06-11.
35. Islam, M.T., 2017. Oxidative stress and mitochondrial dysfunction-linked neurodegenerativedisorders.NeurologicalResearch39(1),73-82.
36. Jung, I.H., Jang, S.E., Joh, E.H., Chung, J., Han, M.J., Kim, D.H., 2012. Lancemaside Aisolatedfromcodonopsislanceolataanditsmetaboliteechinocysticacidamelioratescopolamine-inducedmemoryandlearningdeficitsinmice.Phytomedicine20(1), 84-88.
37. Karakaya, T., Fusser, F., Schroder, J., Pantel, J., 2013. Pharmacological treatment of mildcognitive impairment as a prodromal syndrome of alzheimer s disease. Curr Neuropharmacol11(1),102-108.
38. Karisetty, B.C., Maitra, S., Wahul, A.B., Musalamadugu, A., Khandelwal, N., Guntupalli, S.,Garikapati,R.,Jhansyrani,T.,Kumar,A.,Chakravarty,S.,2017.Differential effectofchronic stress on mouse hippocampal memory and affective behavior: role of major ovarianhormones.Behavioralbrainresearch318,36-44.
39. Lauretti, E., Li, J., Di Meco, A., Praticò, D., 2017. Glucose deficit triggers tau pathology andsynapticdysfunction inatauopathymousemodel.TranslationalPsychiatry7(1),e1020.

40. Law, B.Y., Chan, E.A., 2015. The experience of learning to speak up: a narrative inquiry onnewlygraduatedregisterednurses.J ClinNurs 24(13-14),1837-1848.
41. Leboe, J.P., Ansons, T.L., 2006. On misattributing good remembering to a happy past: Aninvestigationintothecognitive rootsofnostalgia.Emotion6(4),596-610.
42. LePort, A.K., Stark, S.M., McGaugh, J.L., Stark, C.E., 2016. A cognitive assessment ofhighlysuperiorautobiographicalmemory.Memory,1-13.
43. Ma, M.W., Wang, J., Zhang, Q., Wang, R., Dhandapani, K.M., Vadlamudi, R.K., Brann,D.W., 2017. NADPH oxidase in brain injury and neurodegenerative disorders. MolecularNeurodegeneration12(1),7.
44. Mao, X.Y., Cao, D.F., Li, X., Yin, J.Y., Wang, Z.B., Zhang, Y., Mao, C.X., Zhou, H.H., Liu,Z.Q., 2014. Huperzine A ameliorates cognitive deficits in streptozotocin-induced diabeticrats.IntJ MolSci15(5),7667-7683.
45. Martino, M.V., Guandalini, L., Mannelli, L.D.C., Menicatti, M., Bartolucci, G., Dei, S.,Manetti, D., Teodori, E., Ghelardini, C., Romanelli, M.N., 2017. Piperazines as nootropicagents:newderivativesofthepotentcognition-enhancerDM235carryinghydrophilicsubstituents.Bioorganic&MedicinalChemistry25(6),1795-1803.
46. Nicoll, R.A., 2017. A brief history of long-term potentiation. Neuron 93(2), 281-290. Nobili,F., Arnaldi, D., Morbelli, S., 2016. Is dopamine transporter invariably impaired at the time ofdiagnosisindementia withlewybodies?EurJNuclMedMolImaging.
47. Norman, G.R., Monteiro, S.D., Sherbino, J., Ilgen, J.S., Schmidt, H.G., Mamede, S., 2017.The causes of errors in clinical reasoning: cognitive biases, knowledge deficits, and dualprocessthinking.AcademicMedicine 92(1),23-30.
48. Oberauer,K.,Lin,H.-Y.,2017.Aninterferencemodelofvisualworkingmemory.PsychologicalReview124(1),21.
49. Oga-Baldwin, W.Q., Nakata, Y., 2017. Engagement, gender, and motivation: a predictivemodelforjapaneseyounglanguagelearners.System65,151-163.
50. Ogoh, S., 2017. Relationship between cognitive function and regulation of cerebral bloodflow.TheJournalofPhysiologicalSciences,1-7.
51. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues bythiobarbituric acidreaction.AnalBiochem95(2),351-358.

52. Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues bythiobarbituric acidreaction.Analyticalbiochemistry95(2),351-358.
53. Pearson, B., Raskevicius, J., Bays, P.M., Pertzov, Y., Husain, M., 2014. Working memoryretrievalas adecisionprocess.J Vis14(2).
54. Pellicano, E., Kenny, L., Brede, J., Klaric, E., Lichwa, H., McMillin, R., 2017. Executivefunctionpredictsschoolreadinessinautisticandtypicalpreschoolchildren.CognitiveDevelopment43,1-13.
55. Peng, P., Fuchs, D., 2017. A randomized control trial of working memory training with andwithoutstrategyinstruction:effectsonyoungchildren'sworkingmemoryandcomprehension.Journaloflearningdisabilities 50(1),62-80.
56. Quak, M., London, R.E., Talsma, D., 2015. A multisensory perspective of working memory.FrontHumNeurosci9,197.
57. Rafii,M.S., Aisen, P.S., 2015. Advances inalzheimer's disease drug development. BMCMed13,62.
58. Rahimi, R., Irannejad, S., Noroozian, M., 2017. Avicenna's pharmacological approach tomemoryenhancement.NeurologicalSciences,1-11.
59. Redondo, R.L., Kim, J., Arons, A.L., Ramirez, S., Liu, X., Tonegawa, S., 2014. Bidirectionalswitch of the valence associated with a hippocampal contextual memory engram. Nature513(7518),426-430.
60. Rojas-Hortelano, E., Concha, L., de Lafuente, V., 2014. The parietal cortices participate inencoding, short-term memory, and decision-making related to tactile shape. J Neurophysiol112(8),1894-1902.
61. Romiti, A., Falcone, R., Roberto, M., Marchetti, P., 2017. Tackling pancreatic cancer withmetronomic chemotherapy.CancerLetters.
62. Salman, I.M., 2016. Major autonomic neuroregulatory pathways underlying short- and long-termcontrolofcardiovascularfunction.CurrHypertensRep18(3),18.
63. Salmazo-Silva, H., Parente, M.A.d.M.P., Rocha, M.S., Baradel, R.R., Cravo, A.M., Sato, J.R.,Godinho,F.,Carthery-Goulart,M.T.,2017.Lexical-retrievalandsemanticmemoryinParkinson's disease: the question of noun and verb dissociation. Brain and Language 165, 10-20.

64. Wang,B.,2014.Effectof time delay on recognition memory for pictures: themodulatoryrole ofemotion.PLoSOne 9(6),e100238.
65. Wang,Y.,Wei,Y.,Oguntayo,S., Jensen,N., Doctor,B.P.,Nambiar,M.P.,2011. [+]-Huperzine A protects against soman toxicity in guinea pigs. Neurochem Res 36(12), 2381-2390.
66.66.