Original Article

Orally administered chebulinic acid negated neurobehavioral deficits in intracerebroventricular streptozotocin and Amyloid-β-induced experimental model of dementia

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ABSTRACT

Background: With the constant failure of the clinical trials and continuous exploration of a therapeutic target against Alzheimer's disease (AD) is the utmost need. Chebulinic acid (ChA) has been reported to possess neuroprotective potential in various neurodegenerative models such as anxiety and depression. In the current study, the ChA was challenged on the progression of AD induced by intracerebroventricular (ICV)-streptozotocin (STZ)-and Aβ induced neurotoxicity to determine its therapeutic potential in experimental dementia. **Material and Methods:** STZ and Aβ were infused bilaterally (3 mg/kg/icv) on day 1st and 3rd after surgery. ChA (25, 50 and 100 mg/kg/p.o) was administered from 7th day onwards up to 21st day following 1st ICV-STZ and Aβ infusion. Cognitive impairment was evaluated by actophotometer, Morris water maze (MWM) and object recognition task (ORT) in rats whereas biochemical and neurochemical, using hipoocampal brain regions on day 22nd. **Results:** Ventricular administration of STZ and Aβ in rats found to significantly shorten the latency time on the MWM and ORT which was associated with significant alterations in hippocampal biochemistry, including elevation in oxidative stress and compromised antioxidant defense (reduced glutathione). **Conclusion:** ChA treatment significantly prevented the ICV-STZ and Aβ induced memory compromised antioxidant defense and cholinergic deficits in rats. These results clearly pointed to the pivotal role of ChA in ICV-STZ and Aβ induced neurotoxicity and its association may be a promising alternative to be investigated in the treatment of AD-like dementia.

Keywords: Alzheimer's dementia, chebulinic acid, hippocampus, neuroprotection, streptozotocin

INTRODUCTION

A disease of unknown etiology, Alzheimer's disease (AD) is the most common type of dementia without care[1] and most AD cases are sporadic where age represents the greatest risk factor.[2] According to the World Alzheimer Report 2016, there are currently about 46.8 million people suffering with AD worldwide.[3] The aging of world population will further compound this problem and lead to a steep increase in the number of AD patients.[4] Causes of AD are not yet fully understood but advances in brain imaging have allowed researchers to see the development and accumulation of extracellular amyloid beta (Aβ) plaques and intraneuronal neurofibrillary tangles, in discrete regions of the basal forebrain, association cortices; including hippocampus (part of temporal lobe of the brain responsible for processing of memory), as well as shrinkage in brain structure and change in its function.[5] These affects worsens with age and consequently leads to atrophy (shrinking) of certain parts of the brain, oxidative stress, neuroinflammation, mitochondrial

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dysfunction, cholinergic deficits and gliosis along with dystrophic neurites, loss of neurons and synapses, accompanied by psychological and pathophysiological complications such as anxiety, depression, concentration problems, and motor disturbances.[6,7] Numerous *in vivo* studies have demonstrated that insulin resistance, oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, and neuroinflammation are also among the major pathophysiological features of AD.[7,8]

The intracerebroventricular (ICV) injection of streptozotocin (STZ) and Aβ results in a well-established rat model showing many aspects of SAD including oxidative stress, cholinergic deficits, accumulation of β-amyloid, and tau proteins as well as memory and learning impairment.[9,10] At present, the disorder is not curable; because available therapies help to maintain neuronal function, they do not provide a significant impact on the reversal of the disease process.[11,12] Based on these facts, the employment of natural products with infinitesimally, noticeable side effects constitute substitutes for treating these neurodegeneration. The plant *Terminalia chebula* also known as Haritaki has an esteemed origin in Indian mythology. *T. chebula* is a deciduous tree growing up to 30-m (98 ft) tall, with a trunk up to 1-m (3 ft 3 in) in diameter. The fruits are drupe-like, long, broad, and blackish, with five longitudinal ridges and are hard and yellowish-green in color.[13] The plant contains diverse chemical constituents such as ellagic acid, gallic acid, ellagitannins, and gallotannins[14] and has been known for a long time to show pharmacological effects such as cytoprotective,[15] antidiabetic,[16] antioxidant,[17,18] and antiarthiritic.[19] It has been reported that *T. chebula* also possess acetyl cholinesterase inhibitory[20] and neuroprotective potential,[21,22] etc. Chebulinic acid (ChA) is an ellagitannin found in the fruits of *T. chebula*. It has the molecular formula of $C_{41}H_{32}O_{27}$ and molecular weight of 956.67658 (g/mol).[13] Other studies have shown that ChA has reported to possess unique biochemical and pharmacological properties such as antidiabetic,[16] antimutagenic,[23] anti-apoptotic, antioxidant,[12,24] anti-inflammatory,[19] ischemic reperfusion injury,[25] acetylcholinesterase (AChE) inhibitory, and free-radical scavenging activity,[20,26] and cardio and hepatoprotective effects.[18] Moreover, ChA has been reported to show anxiolytic, antidepressant,[13] and protective potential in glutamate induced cell death experimental animals. In linked with this, we recently demonstrate the use of a ChA as an efficacious therapeutic agent that inhibits ICV STZ-induced memory deficit and ameliorates AD symptoms along with acetyl cholinesterase inhibitory activity.[27]Therefore, the objective of this present study is to evaluate the antidementia potentials of ChA in laboratory rats using universally accepted experimental models: ICV-STZ and Aβ model. Herewith, the current study may prove the use of ChA as therapeutic approach in amelioration and/or delaying the detrimental effects of AD.

MATERIALS AND METHODS

Animals

Adult male Wistar rats, weighing 250–280 g were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar and kept in Central Animal House of ISF College of Pharmacy, Moga, Punjab (India). The animals were housed in polyacrylic cages in a well-controlled atmosphere (room temperature 22±2°C and relative humidity of 60%) with 12 h light/dark cycle (lights sturned on at 7 AM). The animals were maintained on a commercial diet in the form of dry pellets and water *ad labium*. All the behavioral parameters were assessed between 9:00 and 17:00 h. The protocol of the study was approved by the Institutional Animal Ethics Committee and was carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines for the use and care of experimental animals. All the experiments for a given treatment were performed using age-matched animals in an effort to avoid variability between experimental groups.

Drugs and chemicals

Amyloid beta ($_{1–42}$) peptides for rat, Aβ ($_{1–42}$) ELISA kit (Genxbio Health Sciences (P) Ltd. New Delhi, India), Streptozotocin (STZ), and acetylthiocholine iodide (AChI) 5, 5'-dithiobis (2-nitrobenzoic acid) and ChA were purchased from Sigma–Aldrich, USA. STZ was diluted in citrate buffer (pH 4.4) and ChA was always prepared afresh by dissolving in 1% carboxymethylcellulose. Interleukin-1 beta (IL-1 β), IL-6, and tumor necrosis factor-alpha Elisa Kits were purchased from Krishgen Biosystem, India. Unless stated, all other chemicals and biochemical reagents of highest analytical grade were used for the study. Solutions of the drugs and chemicals were freshly prepared before use.

ICV infusion of stz and Aβ

Rats were anaesthetized with thiopentone sodium (35 mg/kg, i.p) and xylazine (5mg/kg, i.p). The head was placed in position in the stereotaxic apparatus (Stoelting Co. USA, Model no: 53311). Briefly, a midline sagittal incision was made in the scalp. Two holes were drilled through the skull and the infusion cannula was placed into the lateral cerebral ventricles (coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to sagittal suture; and 3.6 mm ventral from the surface of the brain).[28] Following cannulae placement animals were injected with gentamicin (5 mg/kg) and were placed in individual cages. Animals were observed for 1 week and special care was taken by administering sweetened milk daily, during the resting phase for recovery [Figure 1]. STZ was dissolved in citrate buffer (pH 4.4) just before administration and slowly injected (1 μl/min) (infusion pump QSI 53311) through the cannula using Hamilton microsyringe in a volume of 10 μl into each lateral cerebral ventricle (bilateral ICV) on days 1 and 3 as described previously[29] and in the group of Aβ rats was infused ICV with either artificial cerebrospinal fluid (ACSF; in mmol/L: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 1.7 $CaCl_2$, and 2.2 dextrose) or $Aβ_{1–42}$ oligomers (3 nmol /3μL) dissolved in ACSF, according to the methods described by Maurice.

Experimental groups

Animals were divided into five groups and each group comprised ten animals. The treatment schedule and the interval for estimation of various parameters are presented in Figure 1. Group 1: Served as sham control; Group 2: Rats were infused with ICV-STZ (3 mg/kg/10 μl) with infusion rate 1 μl/min into each cerebral ventricle

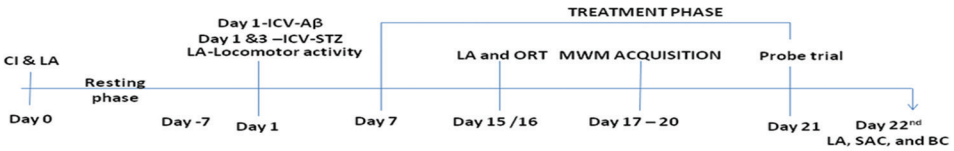

Figure 1: Experiment procedure and treatment schedule. CI: Cannula implantation; LA: Locomotor activity; SAC: Sacrifice; BC: Biochemoical analysis; MWM: Morris water maze; ORT: Objective recognition task

(bilateral ICV). Groups 3, 4, and 5: Received ChA at doses of 25, 50, and 100 mg/kg, p.o., respectively. Group 6: Received ICV-Aβ (3 nmol/3μl) into each cerebral ventricle (bilateral ICV). Groups 7, 8, and 9: Received ChA at doses of 25, 50, and 100 mg/kg, p.o., respectively, starting from day 7 after the 1st dose of STZ infusion and continued once daily for a period of 21 days.

Behavioral assessment

Spontaneous locomotor activity

Each animal was tested for spontaneous locomotor activity on day 22nd following 1st ICV-STZ infusion. Each animal was observed over a period of 10 min in a square closed arena equipped with infrared light sensitive photocells using a digital photoactometer (INCO, India).[29]

Morris water maze (MWM) test

Spatial learning and memory of animals in MWM were tested by the method described.[27] MWM was performed in rats from 17th to 20th days of our experimental protocol which consisted of 4 days of acquisition, i.e., training trials and after 24 h, i.e., on the 21st-day probe trial was performed. In this procedure, a water tank of 180 cm diameter and 60 cm of height filled to a depth of 40 cm with water at 25±1°C was used. It was filled up to the depth of 30 cm with water maintained at a temperature of 26±2°C. Division of pool into hypothetical four quadrants and measurement of time in both acquisition and probe trial was done. During the acquisition phase, a rectangle shaped platform of 8 cm^2 ×6 cm^2 area was submerged underwater in the fourth quadrant placed at a fixed position. Animals were trained for four trials of the maximum time of 120 s each for 4 consecutive days. Time taken by each rat to find and climb the hidden platform was noted as escape latency period. After climbing the hidden platform, rats were allowed to stay there for 30 s. If a rat could not locate the platform, it was placed manually on the platform. During probe trials, the platform was removed and each rat was given 60 s for the searching of the hidden platform. Percentage time spent by the rat in the target quadrant in the search of the hidden platform and platform crossing frequency was noted.[29]

Object recognition task (ORT)]

Novel object recognition (NOR) test was performed for analyzing non-spatial and short-term memory in rats. We followed the protocol previously described by Giorgetti *et al.*, 2012, with minor modifications.[30] Our protocol consisted of habituation, familiarization, and retention phase. The ORT consisted of two trial periods (T1 and T2) separated by a 24-h intertrial period. Each rat was acclimated to without objects to the arena (80 cm × 60 cm × 40 cm for rats, day 15th) environment for 30–45 min before testing for acclimatization. After 24 h (day 16th), the familiarization phase was commenced, in which two identical objects (FO1 and FO2) were placed in two opposite and parallel corners of the box. Each rat was allowed to explore both the objects for 5 min. After 1 h of this phase, the animal's spontaneous behavior was assessed in the test phase where one of the objects was replaced by a novel object (F and N). Each rat was again allowed to explore both the objects for 5 min. A criterion of minimal level of object exploration was used to exclude animals with low levels of spontaneous exploration; thus, only animals having a minimal level of object exploration of ≥ 5 s during the retention trial T2 (novel+familiar ≥5 s) were included in the study. To avoid the presence of olfactory trails, the apparatus and the objects were cleaned thoroughly after each trial. Exploration was considered as directing the nose to the objects at a distance ≤2 cm to the objects and/or touching it with the nose. The times spent by rats in exploring two objects in T1 and T2 were recorded separately. A series of variables was then calculated: Total time spent in exploring two identical objects in T1. The discrimination between the familiar and the novel object during T2 was measured by comparing the time spent in exploring the familiar object with that spent in exploring the novel object.

Biochemical assessments

On 22nd day, after completion of behavioral analysis, rats were sacrificed under light ether anesthesia. The blood was completely removed from the brain tissues using perfusion technique with phosphate-buffered through the heart to avoid any interference with the homogenate readouts. The brain was carefully removed from the skull and rinsed with ice-cold isotonic saline. Hippocampal tissues were separated from the whole brain and then homogenized (10% [w/v]) in ice-cold phosphate-buffered (0.1 M; pH 7.4) at 10,000 g for 15 min at 4°C. Supernatants were separated and stored at −80 °C for performing biochemical estimations. Hippocampal protein was measured by the method of Lowry *et al*.[31] using bovine serum albumin (1 mg/ml) as a standard.

AChE assay

The quantitative measurement of AChE activity in brain hippocampus was performed according to the method described by Ellman *et al*.[32]

Estimation of malondialdehyde (MDA)

The quantitative measurement of MDA end product of lipid peroxidation – in brain hippocampus homogenate was performed, according to the method of Wills.[33] The concentration of MDA was determined from a standard curve and expressed as nmole/mg protein.

Estimation of reduced glutathione (GSH)

Reduced GSH in brain hippocampus was estimated according to the method described by Ellman.[34] The concentration of GSH in the supernatant was determined from a standard curve and expressed as μMol/mg protein.

Estimation of nitrite

The accumulation of nitrite in the hippocampus supernatant, an indicator of the production of Nitrite (NO), was determined by a colorimetric assay using Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green *et al*.[35] The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as μMol/mg protein.

Statistical analysis

The results were analyzed using GraphPad Prism 6.01 (San Diego, CA, United States) and values were expressed as mean ± standard error mean (SEM). Escape latency period in MWM and total exploration time inT1, T2 on familiar and novel object, in NOR was measured using two-way analysis of variance (ANOVA). Catecholamines, GABA and glutamate, and pro-inflammatory were analyzed by repeated measure two-way ANOVA followed by Bonferroni's *post hoc* test for multiple comparisons and others behavior and biochemical parameters were analyzed by one-way ANOVA followed by Tukey's *post hoc* test. Values with $P < 0.05$ and $P < 0.001$ were considered to be statistically significant.

RESULTS

Effect of ChA on spontaneous locomotor activity in ICV-STZ and Aβ infused rats

The spontaneous locomotor activity on day 0, day 1, and day 22nd did not differ significantly among all the groups ($P > 0.001$) [Table 1], suggesting no effect whatsoever of ChA (25, 50, and 100 mg) or STZ on this parameter in the current study.

ChA attenuated ICV-STZ and Aβ-induced memory deficit during MWM task in rats

Repeated measure two-way ANOVA analysis indicated overall significant effect of treatment, time and a time × treatment interaction ($P < 0.001$). The latencies to reach the submerged platform decreased gradually in all the groups during 4 days of training in MWM task [Figure 2a], except those of the ICV STZ infused group of animals, days 17–20 ($P < 0.001$) as compared with those of sham control, indicating poorer learning abilities following STZ and Aβ administration. Chronic administration of ChA dose dependently attenuated STZ- and Aβ-induced acquisition deficit ($P < 0.001$). ChA treated rats showed improved learning abilities as compared to STZ and Aβ control rats.

During the probe trial, with the platform removed, STZ and Aβ infused rats failed to remember the precise location of the platform, spent less time in the target quadrant as compared with sham control animals ($P < 0.001$, Figure 2b). On the one hand, ChA treated rats were able to locate the target quadrant and % time spent in target quadrant was significantly higher to that of STZ and Aβ control rats indicating improved consolidation of memory ($P < 0.001$, Figure 2b).

ChA reverses ICV-STZ and Aβ-induced impairment in short term recognition memory task performance in rats

Non-spatial memory and short-term memory were assessed using NOR test. On day 15 following ICV-STZ and Aβ infusion, during the first test (T1), the non-significant difference was observed

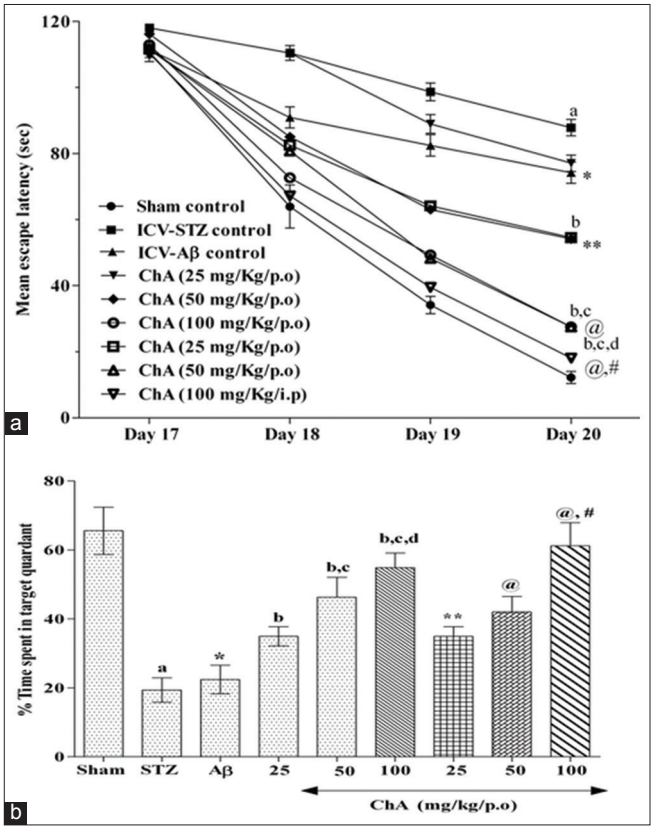

Figure 2: (a and b) Effect of chebulinic acid (ChA) alone (ChA, 25 mg, 50 mg, and 100 mg) on escape latency (spatial acquisition trial) and on reference memory (probe trial) in terms of time spent in Morris water maze task in intracerebroventricular-streptozotocin (ICV-STZ) and ICV-amyloid beta (Aβ) infused rats. Values are expressed as mean ± SEM, a $P < 0.001$ versus Sham, b $P < 0.001$ versus STZ, c $P < 0.001$ versus ChA (25mg/kg), d $P < 0.001$ versus ChA (50 mg/kg). *$P < 0.001$ versus Sham, **$P < 0.001$ versus Aβ, at $P < 0.001$ versus ChA (25 mg/kg), at, # $P < 0.001$ versus ChA (50 mg/kg)

Table 1: Effect of ChA on spontaneous locomotor activity in ICV- STZ and Aβ infused rats

Groups	Locomotor activity counts/10 min	Groups	Locomotor activity counts/10 min
Sham control	221.66±10.99	Sham control	221.66±10.99
ICV-STZ	205.66±11.77	ICV-Aβ	223.76±8.65
ChA+STZ (25 mg/kg/p.o)	207.83±12.45	ChA+Aβ (25 mg/kg/p.o)	217.83±9.45
ChA+STZ (50 mg/kg/p.o)	228.167±11.83	ChA+Aβ (50 mg/kg/p.o)	212.37±1.83
ChA+STZ (100 mg/kg/p.o)	202.167±9.43	ChA+Aβ (100 mg/kg/p.o)	222.67±6.43

ICV: Intracerebroventricular, STZ: Streptozotocin, ChA: Chebulinic acid, Aβ: Amyloid-beta

during familiarize phase in between all treatment groups ($P > 0.001$) [Figure 3a]. On the day second (16, T2), two-way ANOVA analysis indicated overall significant discrimination effect of treatment, when animals were exposed with familial (FO) and novel object (NO), STZ- and Aβ-infused rats were not able to discriminate them and spent equal time to explore the FO and NO. Whereas, treatment with ChA (25, 50, and 100 mg/kg p.o) significantly and dose dependently improved STZ- and Aβ-induced object discriminative ability in animals and the animals spent more time on, when exposed to FO and NO ($P < 0.001$, Figure 3b). ChA (100 mg/kg p.o) exhibits maximum effect among various doses tried [Figure 3a-c].

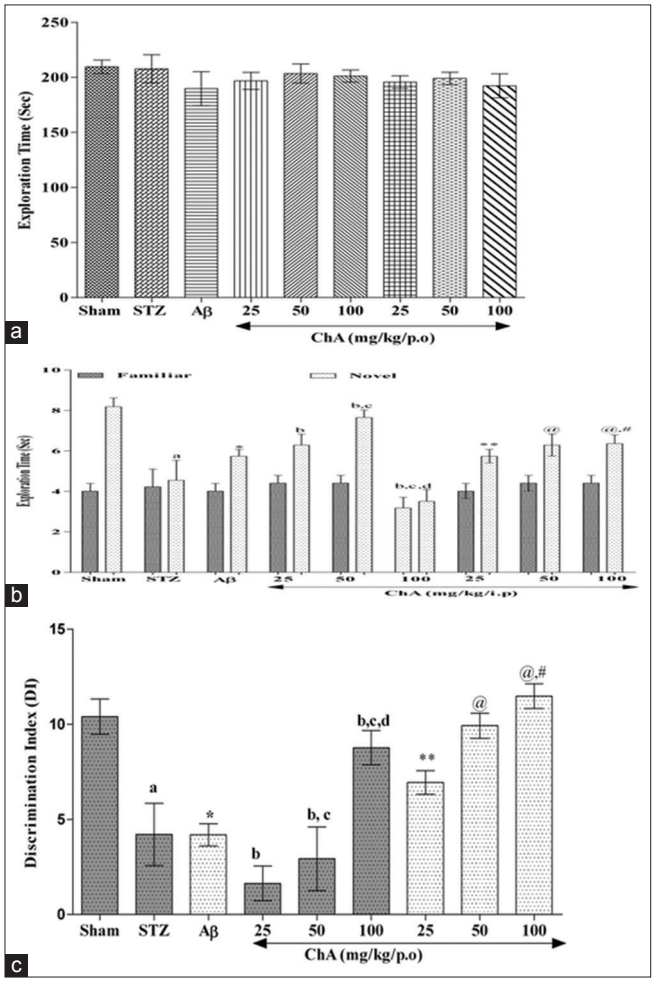

Figure 3: (a) Effect of chebulinic acid (ChA) on memory performance (acquisition phase, day 15th) in object recognition test in intracerebroventricular-streptozotocin (ICV-STZ) and amyloid beta (Aβ)-treated rats. Values are expressed as mean ± SEM, $P < 0.001$. (b) Effect of ChA alone (ChA, 25 mg, 50 mg, and 100 mg) on memory performance (Retention phase, day 16th) in object recognition test (ORT) in ICV-STZ and ICV-Aβ infused rats. Values are expressed as mean ± SEM, $^{a}P < 0.001$ versus Sham, $^{b}P < 0.001$ versus STZ, $^{c}P < 0.001$ versus ChA (25 mg/kg), $^{d}P < 0.001$ versus ChA (50 mg/kg). $*P < 0.001$ versus Sham, $**P < 0.001$ versus Aβ, $^{@}P < 0.001$ versus ChA (25 mg/kg), $^{@,\#}P < 0.001$ versus ChA (50 mg/kg). (c) Effect of ChA, (25, 5 mg, and 10 mg) alone on memory performance (discrimination index, day 16th) in ORT in ICV-STZ and ICV-Aβ infused rats. Values are expressed as mean ± SEM, $^{a}P < 0.001$ versus Sham, $^{b}P < 0.001$ versus STZ, $^{c}P < 0.001$ versus ChA (25 mg/kg), $^{d}P < 0.001$ versus ChA (50mg/kg). $*P < 0.001$ versus Sham, $**P < 0.001$ versus Aβ, $^{@}P < 0.001$ versus ChA (25mg/kg), $^{@,\#}P < 0.001$ versus ChA (50 mg/kg)

ChA ameliorated hippocampal AChE in ICV-STZ and Aβ-infused rats

According to the cholinergic hypothesis, the activity of AChE significantly increases during AD which leads to degradation of acetylcholine. Ventricular administration of STZ and Aβ produced significant increase in brain AChE activity when compared with that of sham control ($P < 0.001$) [Figure 4]. Oral administration of ChA (100 mg/kg) very significantly attenuated STZ- induced increase in AChE activity ($P < 0.001$). However, ChA (25 and 50 mg/kg/p.o) also showed a decrease in AChE activity in dose-dependent manner but with lesser significance when compared with ICV-STZ and Aβ infused rats.

ChA reversed ICV-STZ and Aβ-mediated increase in MDA and Nitrite level while a decrease in GSH activity in the hippocampus in rats

Increased brain nitrite expression and lipid peroxidation (Malondialdehyde level) and a decrease antioxidant (GSH level) enzyme which leads to nitrosative and oxidative stress, respectively, are an integral part of AD-affected brains. Systemic administration of ICV-STZ and (3 mg/kg) on day 1 and day 3 and Aβ (3 nmol /3μL) on day 1 following surgery significantly ($P < 0.001$) increased MDA, nitrite concentration and depleted GSH as compared to sham control group [Table 2]. Chronic administration of ChA at all three does attenuated STZ- and Aβ-induced elevation in MDA, nitrite levels, and restored levels of antioxidant enzyme GSH as compared to STZ- and Aβ-infused group.

DISCUSSION

Despite remarkable progress in understanding pathogenesis of dementia, the search for a cure against these diseases is troublesome and frustrating; for decades now, complications of the AD have made

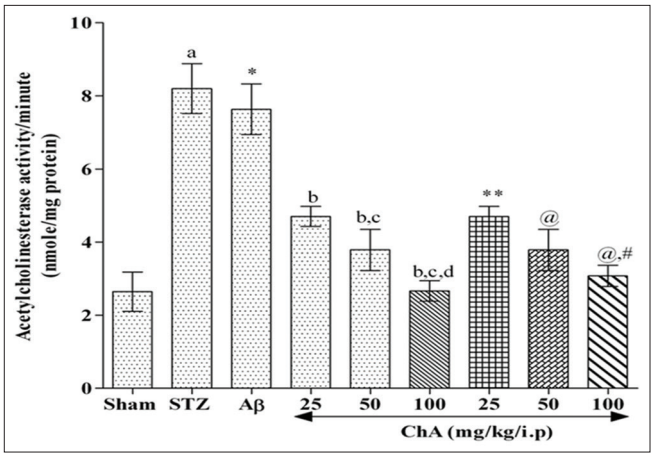

Figure 4: Effect of chebulinic acid (ChA) alone (ChA, 25, 50 mg, and 100 mg) on brain (hippocampus) acetylcholinesterase activity in intracerebroventricular-streptozotocin (ICV-STZ) and ICV-amyloid-beta infused rats. Values are expressed as mean ± SEM, $^{a}P < 0.001$ versus Sham, $^{b}P < 0.001$ versus STZ, $^{c}P < 0.001$ versus ChA (25 mg/kg), $^{d}P < 0.001$ versus ChA (50 mg/kg). $*P < 0.001$ versus Sham, $**P < 0.001$ versus Aβ, $^{@}P < 0.001$ versus ChA (25 mg/kg), $^{@,\#}P < 0.001$ versus ChA (50 mg/kg)

Table 2: Effect of ChA (25 mg, 50 mg, and 100 mg) alone on lipid peroxidation, nitrite, and glutathione levels in ICV-STZ and Aβ-infused rats

Groups	Biochemical parameters		
	MDA (nmole/mg protein)	Nitrite (μmole/mg protein)	GSH (μmole/mg protein)
Sham control	0.365±1.37	7.72±0.25	2.129±0.23
ICV-STZ control	1.933±2.44[a]	26.5±0.54[a]	0.029±0.08[a]
ICV-Aβ control	1.884±1.67*	13.92±1.42*	0.125±0.17*
STZ+ChA (25 mg/kg/p.o)	0.840±2.32[b]	11.85±0.33[b]	0.183±0.18[b]
STZ+ChA (50 mg/kg/p.o)	0.735±0.75[b,c]	9.84±0.34[b,c]	1.079±0.88[b,c]
STZ+ChA (100 mg/kg/p.o)	0.636±1.36[b,c,d]	8.44±0.41[b,c,d]	1.156±0.28[b,c,d]
Aβ+ChA (25 mg/kg/p.o)	1.656±1.82**	11.55±1.33**	0.156±1.18**
Aβ+ChA (50 mg/kg/p.o)	1.135±1.55[@]	9.41±0.34[@]	1.119±0.88[@]
Aβ+ChA (100 mg/kg/p.o)	0.562±1.06[@,#]	8.02±0.41[@,#]	1.178±0.28[@,#]

Values are expressed as mean±SEM, [a,*]$P<0.001$ versus Sham, [b]$P<0.001$ versus STZ, [c]$P<0.001$ versus ChA (25 mg/kg), [d]$P<0.001$ versus ChA (50 mg/kg). *$P<0.001$ versus Sham, **$P<0.001$ versus Aβ, [@]$P<0.001$ versus ChA (25mg/kg), [@,#]$P<0.001$ versus ChA (50 mg/kg). MDA: Malondialdehyde, GSH: Reduced glutathione, ICV: Intracerebroventricular, STZ: Streptozotocin, ChA: Chebulinic acid, Aβ: Amyloid beta

the development of its therapeutic intervention quite a challenging task.[27] Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects and lack of their curative effects. Thus, it is imperative to keep exploring different approaches that can be used to target the AD complications which could be translated into successful clinical trials. The herbs have been used as medicines from ancient times, as they are safer and have no side effects. The ultimate aim of this research is to find a solution for this disease using herbal compound. In present study, we demonstrate the therapeutic potential of ChA in ICV-STZ and Aβ-induced experimental sporadic AD. ICV-STZ and Aβ-induced model have been commonly used to explore the various behavioral, biochemical, and cellular alterations, implicated in pathogenesis of SAD. In the present study, bilateral ICV infusion of STZ and Aβ produced cognitive impairment, cholinergic deficiency, elevation in oxidative stress, and hippocampus neurochemical alterations in rats.[36] The observed changes are in line with earlier studies demonstrating similar behavioral and biochemical alterations following STZ and Aβ infusion in rats. ICV administration of STZ and Aβ has been reported to produce AD like symptoms in animals and non-human primates. The findings of the present study are in tune with earlier reports which also observed behavioral, biochemical, neurochemical, and histopathological changes in rats following ICV-STZ and Aβ infusion in rats. In the present study, the results of memory consolidation and NOR in ICV-STZ and Aβ infusion in rats, evaluated by MWM and OR paradigms shown poor learning and discriminative abilities. Although, the exact mechanism of ICV-STZ and Aβ-induced cognitive deficits is not clear. However, various mechanisms such as cellular energy failure, mitochondrial damage, oxidative stress, excitotoxicity, upregulation of inflammatory markers, degeneration of cholinergic neurons, and ultimately leading to cell death which are primarily linked with the deterioration of learning and memory abilities has been correlated in STZ- and Aβ-induced learning and memory impairment.[36]

The present findings illustrated the ICV-STZ and Aβ-infused rats significantly prolonged escape latency in MWM task as compared to animals of sham control, suggesting ICV-STZ and Aβ impaired spatial learning and memory. Similar observations were also observed in novel ORT that defines spatial learning, origination of new memories, and recapitulations of stored memories.[37] In the present study, ICV-STZ and Aβ administrated rats were unable to discriminate between familial and novel objects in ORT. The changes in spontaneous locomotor activity have been suggested to modulate the learning and memory in ORT and MWM paradigms.[29,38] However, no significant difference in spontaneous locomotor activity was observed in any of the experimental group. ChA treated rats showed dose dependently significant improvement in acquisition and consolidation and were able to discriminate between familiar and novel objects suggesting improvement of learning and memory in STZ treated rats without affecting spontaneous locomotor activity in line with the previous reports.[27] The results of current study show that ChA displayed behavioral profile that is consistent with an antidepressant and anxiolytic actions[13] and neuroprotective potential glutamate induced excitotoxicity. In line with the previous observations, the behavioral disruption in STZ and Aβ treated rats observed in present study may be due to upregulation of AchE enzyme[27,29] further supporting the impairment of cholinergic system, namely, loss of memory function and coordination. It has been reported that *T. chebula* possess AchE inhibitory activity.[20] In the present study, administration of CA significantly attenuated the elevated levels of AchE whereas these effects were more profound when CA was given at higher doses. Evidently the improved memory performance was observed in MWM and novel ORT as well as improvement of AchE enzyme level in STZ- and Aβ-treated group by administration of ChA suggesting its potential role in cognitive performance.

It has been demonstrated that hippocampus is highly enriched in cholinergic axon terminals and ACh neurotransmitters known to play crucial role in encoding, storage, and expression of memory. A close relationship exists between impairment in behavioral function and disruption of neurotransmitters homeostasis in hippocampus in AD as well as in experimental animals.[39,40] Linked with this, these alterations in hippocampal AChE following ICV-STZ and Aβ infusion in rats in the present study. On the other hand, significant alterations in hippocampal cholinergic neurochemistry have also been reported to occur in AD[22,41] as well as following ICV-STZ and Aβ infusion in rats.[27] In the present study, ICV-STZ and Aβ treatment significantly increases AChE level. In the present study, treatment with ChA significantly restored the hippocampal AChE level implicated that

ChA may improve neurotransmitters homeostasis in hippocampus in STZ and Aβ treated animals and also in pathophysiology of AD.

Oxidative stress is initiated by reactive oxygen species (ROS), which are produced as a by-product of electron transport in mitochondria, play a key pathogenic role in disease progression and thought to be involved in STZ- and Aβ-induced cognitive deterioration.[42] The previous findings suggested that mitochondria consist of multiple electron carriers competent of producing ROS and widespread network of antioxidant defense mechanism. Any abnormality or internal insult to mitochondrial can cause an imbalance between generation of ROS and defense, leading to oxidative damage.[9] Interestingly, ROS not only cause damage to cellular structures which lead to neurotic cell death but also provoke cellular responses which are evident in vulnerable neurons in AD. Excess ROS causes cell injury by damaging lipids, proteins and DNA in cell.[43] In the present study, ICV administration of STZ and Aβ significantly increased MDA and nitrite concentration, depleted the levels of reduced GSH and SOD, signifying oxidative damage. Recent studies showed the antioxidant properties of different extracts of *T. chebula* fruits.[12] In an earlier report, a 70% methanol extract of *T. chebula* fruits was found to have good efficacy in radical scavenging abilities.[26] In another report, chloroform, ethanolic, n-butanolic, and organic aqueous extracts were investigated for anti-lipid peroxidation, anti-superoxide radical formation, and free-radical scavenging activities.[12] Thus, in the present study, chronic administration of ChA significantly attenuated oxidative damage, by attenuating MDA and nitrite level and improve antioxidant defense, demonstrating its anti-oxidative effect. Moreover, this antioxidant effect was enhanced when ChA is given at higher dose.

Therefore, we predict that, ChA dose dependently attenuated STZ- and Aβ-induced cognitive deterioration and other biochemical and neurochemical alterations in the present study. ChA produced neuroprotective action by reduction of nitro-oxidative stress and anti-cholinergic activities. The current study further provides a hope that these ChA could be used in the treatment and management of cognitive disorders such as AD. However, their exact mechanism at cellular and molecular level is still poorly understood and needs to be explored further.

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