Neuroprotective role of DPP-4 inhibitor Linagliptin against neurodegeneration, neuronal insulin resistance and neuroinflammation induced by intracerebroventricular streptozotocin in rat model of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is an age-related, multifactorial progressive neurodegenerative disorder manifested by cognitive impairment and neuronal death in the brain hippocampus, yet the precise neuropathology of AD is still unclear. Continuous failure of various clinical trial studies demands the utmost need to explore more therapeutic targets against AD. Type 2 Diabetes Mellitus and neuronal insulin resistance due to serine phosphorylation of Insulin Receptor Substrate-1 at 307 exhibits correlation with AD. Dipeptidyl Peptidase-4 inhibitors (DPP-4i) have also indicated therapeutic effects in AD by increasing the level of Glucagon-like peptide-1 in the brain after crossing Blood Brain Barrier. The present study is hypothesized to examine Linagliptin, a DPP-4i in intracerebroventricular streptozotocin induced neurodegeneration, and neuroinflammation and hippocampal insulin resistance in rat model of AD. Following infusion on 1st and 3rd day, animals were treated orally with Linagliptin (0.513mg/kg, 3mg/kg, and 5mg/kg) and donepezil (5 mg/kg) as a standard for 8 weeks. Neurobehavioral, biochemical and histopathological analysis was done at the end of treatment. Dose-dependently Linagliptin significantly reversed behavioral alterations done through locomotor activity (LA) and morris water maze (MWM) test. Moreover, Linagliptin augmented hippocampal GLP-1 and Akt-ser473 level and mitigated soluble Aβ (1–42), IRS-1 (s307), GSK-3β, TNF-α, IL-1β, IL-6, AchE and oxidative/nitrosative stress level. Histopathological analysis also exhibited neuroprotective and anti-amylodogenic effect in Hematoxylin & eosin and Congo red staining respectively. The findings of our study concludes remarkable dose-dependent therapeutic potential of Linagliptin against neuronal insulin resistance via IRS-1 and AD-related complication. Thus, demonstrates unique molecular mechanism that underlie AD.

Introduction

Alzheimer's disease is an irreversible multifactorial neurodegenerative disease that leads to the deterioration of brain functions (Hussain et al., 2018) and accounts for around 80% of dementia globally in elder patients (Fargo, 2014). There are several molecular mechanisms underlie AD yet the ideal neuropathology of AD is still unknown (Ayutyanont et al., 2013). It is manifested by neuropathological hallmarks that include extracellular deposition of amyloid-beta (Aβ) senile plaques, intracellular deposition of hyper phosphorylated tau protein neurofibrillary tangles, neuronal and synaptic death in the hippocampus (Zameer et al., 2019). CA1/CA3 region and cerebral cortex region of the hippocampus are markedly responsible for neuronal death in the brain (Padurariu et al., 2012). Currently approved medications including acetylcholinesterase (AchE) inhibitors like donepezil, rivastigmine and N-methyl-D-aspartate (NMDA) receptor antagonist like memantine are supposed as the golden standard for the symptomatic relief of AD (Zameer et al., 2019) and unfortunately failed to counteract the disease progression. Therefore, it is an important need to develop more effective, therapeutic and safer drugs which may reverse the disease condition.

Type 2 Diabetes Mellitus (T2DM) and neuronal insulin resistance (decreased insulin receptor sensitivity) due to serine phosphorylation of Insulin Receptor Substrate-1 (IRS-1) at position 307 have confirmed their correlation with AD (Siddiqui et al., 2021; Craft and Watson, 2004). Numerous research evidence supports

the ameliorative effect of DPP-4 inhibitors (DPP-4i) in different experimental models of brain disease (Kosaraju, et al., 2017; Kosaraju et al., 2013; Badawi et al., 2017; Nassar et al., 2015) by increasing the level of Glucagon-like peptide-1 (GLP-1) in the circulation that crosses Blood Brain Barrier (BBB) (Kastin et al., 2002) where binds with GLP-1 receptor (GLP-1R) and mitigates neurodegeneration and neuronal resistance (Siddiqui et al., 2021; Hamilton and Holscher, 2009; Katsurada and Yada, 2016; Holscher, 2010). Therefore, pharmaceuticals for the treatment of T2DM could also be effective in ameliorating hippocampal insulin resistance and AD-related complications.

Moreover, Rohnert et al first reported that DPP-4i showed neuroprotection against stroke via intracerebral administration of sitagliptin in the rat (Rohnert et al., 2012). 4 weeks per-oral pre-treatment followed by 3 weeks post-stroke treatment with Linagliptin have also indicated neuroprotection by reducing brain damage after stroke in both normal and T2D/obese mice (Darsalia et al., 2013). However, Darsalia et al have also reported that chronic administration of Linagliptin against stroke mitigates neurodegeneration which could be due to augmented proliferation of neural stem cells (Darsalia et al., 2014) and was not mediated by the GLP-1R (Darsalia et al., 2016). Nonetheless, more research is required to understand the exact cellular and molecular mechanisms of DPP-4i against neurodegeneration in the brain.

During the Insulin Signalling pathway (ISP), the binding of insulin to its receptor instigates downstream IRS-1/PI3K/AKT/GSK-3β pathway which results in a neuroprotective effect in the brain (Blazquez et al., 2014). Downregulation of this pathway due to deposition of Aβ oligomers/plaques in the hippocampus results in serine phosphorylation of IRS-1 at position 307 (IRS-1 ser307) leading to neuronal insulin resistance and neurodegeneration (Siddiqui et al., 2021). IRS-1 ser307 due to the release of neurotoxic cytokines was also found to be a key feature for brain insulin resistance (Siddiqui et al., 2021). The high density of IRS-1 ser307 level in the hippocampus has also been identified during an autopsy of the brain of AD patients (Caruso et al., 2014).

Streptozotocin (STZ) is a beta cytotoxic glucosamine-nitrosourea drug that has been cited to induce neurodegeneration and many pathological aspects of AD in subdiabetogenic doses. The ICV-STZ model is a streptozotocin induced model used for AD with insulin resistance in the brain as AD is associated with reduced insulin level and impairment of insulin receptor (IR) signaling in the brain (Rajasekar et al., 2016). Impaired cholinergic neurotransmission, Aβ (1–42) senile plaques, intracellular deposition of neurofibrillary tangles, cognitive dysfunctions, neuroinflammation and oxidative stress are the altered cellular conditions that have been triggered by intracerebroventricular streptozotocin (ICV-STZ) (Mishra et al., 2018; Javed et al., 2015). Considering all this evidence, the pathologies resembling AD were recapitulated in rats by administration of a subdiabetogenic ICV dose of STZ. This is our first study to examine the therapeutic effect of Linagliptin on neuronal insulin resistance via IRS-1 s307 and AD-related complications in ICV-STZ induced AD model in Albino Wistar rats (Fig. 1).

Linagliptin, a DPP-4 inhibitor exhibits therapeutic effects against peripheral insulin resistance (Neumiller, 2012) and thus holds a potential curative approach for mitigating neuronal insulin resistance and Alzheimer's disease-like complications. However, it does not cross the BBB but it inhibits DPP-4

peripherally that augments GLP-1 level in the circulation, which does cross BBB to provide consolidation of memory and synaptic plasticity (Siddiqui et al., 2021; Shannon, 2013). This is our first study to evaluate the effect of Linagliptin on AD-related pathologies by modulating neuronal insulin resistance via IRS-1 s307 and to determine whether the neuroprotective effect is associated with brain insulin resistance in ICV-STZ induced AD model in Albino Wistar rats.

Materials And Methods

Animals

After approval of the study from the Institutional Animal Ethics Committee (IAEC) [Reg. No & Date of Reg: 173/GO/Re/S/2000/CPCSEA, 28th Jan 2000], Jamia Hamdard (Delhi; India), 63 male albino Wistar rats (250-300g) were procured from the Central Animal House Facility (Jamia Hamdard, New Delhi, India). All the experiments following the acclimatization of animals for 2–3 days under laboratory conditions were conducted in compliance with Committee for the Purpose and Supervision on Experimentation on Animals (CPCSEA). Animals were housed in polypropylene cage under controlled laboratory conditions (12 hr light/night cycle) of temperature (25 ± 2 °C) and humidity (55-65 %) and had free access to the pellet diet & water *ad libitium*.

Drugs and chemicals

All the drug solutions were freshly prepared before conducting the experiment. Linagliptin was procured from Cayman Chemicals Company, USA. Donepezil was purchased from Cipla Pharmaceutical Company, India. STZ for rats was purchased from Sigma Aldrich, USA. Aβ (1-42) Elisa kit was procured from Elabsciences Pvt. Ltd. India. GLP-1 Elisa kit, IRS1-s307 Elisa kit, Akt-ser473 and Gsk-3β Elisa kit were obtained from Genxbio, India. TNF-α, IL-1β and IL-6 Elisa kits were purchased from Krishgen Biosystems, India.

Three different treatment groups rats received three different doses of Linagliptin (0.513mg/kg, 3mg/kg, 5mg/kg) respectively. The dose of Linagliptin (0.513 mg/kg) was calculated from the corresponding human dose (5mg) as recommended by FDA for the treatment of diabetes. 3mg/kg and 5mg/kg doses were based on the prior evidence available (Kabel et al, 2018; Kosaraju et al., 2017). Linagliptin and reference standard donepezil (5mg/kg) (Zameer et al., 2019) were dissolved in water according to their respective doses prior to oral administration to treatment rats using oral gavage. STZ (3mg/kg) in 5µl 0.9% saline will be administered bilaterally as the ICV route. 2.5µl into the left ventricle and 2.5µl into the right ventricle of the brain on days 1 and 3 in rats (Zameer et al., 2019).

Treatment schedule

Rats were randomly and equally divided into 9 groups, comprising of 7 rats in each group. Group I (Control): Oral administration of water for 8 weeks; Group II (Sham): Intracerebroventricular (ICV) infusion of citrate buffer 5µl bilaterally on 1st and 3rd day; Group III (ICV-STZ): ICV infusion of STZ solution

bilaterally in the hippocampus on 1st and 3rd day to induce AD; Group IV,V and VI (ICV-STZ + Linagliptin): ICV-STZ solution was infused bilaterally in the hippocampus on 1st and 3rd day in 3 different groups and then 3 doses of Linagliptin (0.5mg/kg, 3mg/kg and 5 mg/kg) was administered in each group respectively for the period of 8 weeks; Group VII (Linagliptin per se): Oral administration of Linagliptin (5mg/kg) for 8 weeks. Group VIII (ICV-STZ + Donepezil): ICV-STZ solution was administered bilaterally in the hippocampus on the 1st and 3rd day then Donepezil (5mg/kg) was administered for the period of 8 weeks; Group IX (Donepezil per se): Rats with oral administration of Donepezil for 8 weeks. At the end of the treatment, all the rats were assessed for neurobehavioral, histopathological and biochemical parameters. (Fig.2).

Surgical procedure

Animals were anaesthetized with the admixture of ketamine hydrochloride (100mg/kg) and xylazine (5mg/kg) i.p. on the first day of our experiment. Following anaesthesia, rats were placed on stereotaxic apparatus with proper fitting of the head skull. A sagittal cut was made in the skull to recognize Bregma and lambda. Taking Bregma as the center point, reciprocal openings were bored into the rat skull bilaterally at the directions; - 3.6 mm anterior-posterior to the Bregma, 2.4 mm two-sided to sagittal stitch and 2.8 mm dorsoventral. Using 10 µl of Hamilton syringe (26-gauge), a prepared solution of 3mg/kg of STZ was gradually infused into each opening of parallel ventricles. The syringe was left in the opening for 120s (post-infusion period) to permit legitimate implantation of the toxicity into the hippocampus (Wei et al., 2015). Following this, the etched skull was stitched properly and antiseptic powder was sprinkled. Rats were then housed in the cages for induction of AD. The sham group went through the same procedure yet was infused with only citrate buffer (5µl) bilaterally.

Behavioural assessment

Locomotor activity

The locomotor activity (LA) of all experimental rats was performed to determine motor impairment in the video path analyzer. Responses were recorded between 8:30 a.m. to 1:30 p.m. under controlled laboratory conditions in the open field comprised of an acrylic box (40.6 x 40.6 x 40.6 cm3), connected with the two photo beams (16 beams/dimensions; 2.5 cm between beams) of a computer interface (TrueScan 2.0 version, Coulbourn Instruments, Allentown, Pennsylvania, USA) that counted beam breaks (100 ms sampling rate). LA of each rat in each group was recorded digitally, through disruptions of IR beams caused by movements of rats placed in the activity meter and counted for 20 min after 30 min acclimatization. The box was cleaned with 70 % alcohol after each recording to prevent olfactory clues from rat odours. Five responses of LA like move time (s), rest time (s), horizontal activity (cm), mean velocity (cm/s), and total movement (#) were recorded (Akhtar et al., 2014).

Morris water maze test

The Morris water maze (MWM) test was conducted to survey the memory impairment in rats. Time in both acquisition and probe trials was recorded using Any-maze software (ANY MAZE, Video tracking Software, US). A dark-colored water tank of stature (50 cm) and width (130 cm), topped off with water up to 30 cm in height at a temperature of 26 ± 2 °C. The water tank was virtually divided into 4 quadrants named north, east, south and west. During the acquisition trial, a black rectangular escape platform of 8 × 6 cm^2 area was submerged underwater in one quadrant (south-west) and stayed fixed. Rats were trained for 4 sequential trials for 4 consecutive days from each quadrant to explore the hidden platform (target) within the maximum time of the 60s and were allowed to sit onto the platform for 30s. If failed to find the platform, were physically coordinated to the platform. The escape latency period (time taken by each rat to allocate the hidden platform) was noted for 4 days.

A probe trial was conducted on the 5^{th} day to assess the reference memory. The platform was removed from the quadrant (south-west). Rats were allowed to assign the hidden platform quadrant for the 60s. The % dwell time (time spent by each rat to explore the hidden platform quadrant) and platform crossings (frequency to cross the target quadrant) was recorded in all the experimental groups (Kaundal et al., 2017).

Biochemical assessment

Brain homogenate preparation

At the end of the experiment, each rat was anaesthetized with light ether, forfeited and perfused with phosphate buffer saline (PBS). Brains were removed and rinsed with chilled saline. Hippocampus was isolated from the whole brain and were homogenized with chilled phosphate buffer (0.1 M; pH 7.4) at 800 X g at 4 °C for 5 min, 10 times of tissue volume. Supernatants obtained were centrifuged at 10,000g at 4 °C for 10 min. thus, the supernatant was collected and stored at −80 °C to estimate biochemical parameters.

Estimation of hippocampal GLP-1, soluble Aβ (1-42), IRS-1 (s307), Akt- (ser473), GSK-3β level

GLP-1, soluble Aβ (1–42), IRS-1 (s307), Akt- (ser473) and GSK-3β levels in the hippocampus were measured using the ELISA kits according to the instructions provided with the corresponding kits in all the groups.

Determination of acetylcholinesterase (AChE) level

AChE level was measured utilising Ellman's method. 0.2 ml of acetylthiocholine iodide (75 mM), 0.1 ml of buffered Ellman's reagent DTNB (5,5-dithio-bis-(2-nitrobenzoic acid, 10 mM in 15 mM NaHCO3) and 3 ml of PBS (25 mM, pH 7.4) admixture was incubated for 10 min at room temperature. Following this, 0.2 ml of supernatant prepared was added. The optical density was recorded at 412 nm within 5 min. Change in absorbance was recorded at 30s interval using Perkin Elmer Lambda 20 spectrophotometers. Results of

all groups were expressed in nanomoles of acetylthiocholine iodide hydrolyzed per min per mg of protein (Ellman et al., 1961).

Oxidative and Nitrosative stress biomarkers

Determination of lipid peroxidation

Free radicle damage due to ICV-STZ in the hippocampus was measured as an index of lipid peroxidation, expressed in terms of TBARS level. MDA level was evaluated by using Will's method. A reaction admixture cocktail containing 0.5 ml of tris-HCL and 0.5 ml supernatant was incubated for 2 h at 37 °C. After this, 1 ml of trichloroacetic acid (TCA, 10%) was added and centrifuged at 1000×g for 10 min. 1 ml of the supernatant obtained was mixed with 1 ml of thiobarbituric acid (TBA 0.67%). Mixture tubes were then placed in boiling water for 10 min. After cooling, distilled water (1ml) was added to it. Absorbance was recorded at 532 nm. TBARS level in all experimental groups was expressed as nmol MDA/mg protein (Sachdeva and Chopra, 2015, Wills, 1966).

Estimation of Catalase (CAT)

CAT level was measured by using the Calibrne method (1985). Briefly, a reaction cocktail (0.05 ml supernatant + 1 ml of hydrogen peroxide (H_2O_2 0.019 M)), 1.95 ml phosphate buffer (0.5 M, pH 7.0) were mixed to make up the final volume of 3 ml. Absorbance was recorded at 240 nm. Change in absorbance was measured at the 30s interval using Perkin Elmer Lambda 20 spectrophotometers. CAT activity was expressed in terms of nmol H_2O_2/min/ mg protein in all groups (Sachdeva and Chopra, Ayyub et al., 2017).

Estimation of nitrite level

The level of nitrite was determined by using the Griess reagent. Accordingly, a reaction mixture containing an equal volume of supernatant and Griess reagent (0.1% N-(-naphthyl ethylenediamine dihydrochloride, 1% sulfanilamide and 5% phosphoric acid) was incubated in dark at room temperature for 10 min. Absorbance was measured at 540 nm with a Perkin Elmer Lambda 20 spectrophotometer. Nitrite level in all groups was expressed in micromoles per mg protein using the sodium nitrite standard curve (Husain et al., 2017).

Estimation of pro-inflammatory cytokines TNF-α, IL-6 and IL-β

Hippocampal neuroinflammatory cytokines (TNF-α, IL-6 and IL-1β) levels in all the experimental groups were measured by using their commercially available Elisa kits in accordance to the instructions provided by the respective manufacturer.

Protein estimation

The total concentration of protein was estimated by Lowry's method using bovine serum albumin as a standard (Lowry et al., 1951).

Histopathological analysis

Hematoxylin and Eosin (H & E) staining

Whole brain stored in 10% formalin was fixed in paraffin. Using microtome, coronal section of thickness 5µm of CA1 region of hippocampus were cut. Silane-coated slides were prepared and then washed with xylene for deparaffinization. Slides were then rehydrated with ethanol. Following this, slides were counter-stained with Hematoxylin and eosin (H&E). Photomicrographs were taken at 40X magnification using Meiji fluorescent microscope and morphology of neurons was observed in each group (Thenmozhi et al., 2015; Zhang et al., 2005).

Congo red staining

Whole brain was stored in 10% neutral buffered formalin for 48 h. Brain sections were immersed in Congo red solution (0.2%) for 1 h. Following this, counter-stained with Hematoxylin and Eosin. Photomicrographs were captured at 40X magnification under a Moticam light microscope and deposition of Aβ plaques were observed in each experimental group (Hou et al., 2008; Remya et al., 2014).

Statistical Analysis

Data obtained from the behavioral and biochemical assessments in all the study groups were statistically analysed by using GraphPad Prism software version 5.01. Data was represented as mean ± SEM. Significance of difference between each experimental groups was determined by using two-way ANOVA (escape latency in MWM) and one-way ANOVA in all other parameters followed by post-hoc Tukey-Multiple Comparison test.

Results

Linagliptin attenuates ICV-STZ induced motor impairment in the locomotor activity

Locomotor activities such as move time (s), rest time (s), horizontal activity (cm), mean velocity (cm/s) and total movement were recorded in all the treatment groups. STZ infused toxic rats showed a significant (*** $P < 0.001$) decrease in move time ($df = 8, 54$ $F = 59.45$), horizontal activity ($df = 8, 54$ $F = 53.27$), mean velocity ($df = 8, 54$ $F = 65.67$), total movement ($df = 8, 54$ $F = 44.51$) and concurrently increase in rest time ($df = 8, 54$ $F = 93.04$) when compared to the control and sham groups. Linagliptin at 0.513mg/kg dose does not showed any significant ($p > 0.05$) difference when compared with STZ infused toxic rats. Whereas, Linagliptin (3mg/kg and 5mg/kg) and donepezil (DNP) significantly (**$p < 0.01$, ***$p < 0.001$) attenuated STZ infused motor impairment when compared with diseased rats (Table 1).

Linagliptin ameliorates ICV-STZ induced cognitive impairment in the Morris Water Maze (MWM) test

Acquisition trials: In MWM test, during acquisition trials of 4 days, STZ infused rats resulted in a significant increase in escape latency on successive days (except on Day 1, df = 8, 36 F = 0.3325, ***p < 0.001) as compared to control and sham groups which showed the memory impairment in rats. On the contrary, STZ induced increase in escape latency was found to be remarkably reduced by oral administration of Linagliptin at 3 and 5mg/kg doses (but not by 0.513mg/kg) and positive control DNP which is indicating the memory improving effect of Linagliptin (**p < 0.01, ***p < 0.001) (Fig. 3A).

Reference memory test or probe trial: During probe trial on day 5^{th}, reference memory was assessed in rats of all treatment groups. It was found that the rats infused with STZ spent significantly less time in target quadrant as indicated by reduced % dwell time as well as showed a decrease in platform crossing frequency as comparison to control and sham group (*p < 0.05, **p < 0.01, ***p < 0.001). After oral administration of Linagliptin at 3 and 5mg/kg doses and DNP at 5mg/kg for 8 weeks, the rats acquired significantly higher % dwell time (df = 8, 54 F = 42.33, *p < 0.05, ***p < 0.001) (Fig. 3B) and platform crossing frequency (df = 8, 54 F = 23.12 *p < 0.05, **p < 0.01, ***p < 0.001) (Fig. 3C) as compared to toxic rats. These outcomes depicts that Linagliptin at 3 and 5mg/kg dose but not at 0.513mg/kg dose has a potential to improve learning and memory in rats with AD-like pathological changes.

Linagliptin increases hippocampal GLP-1 level in STZ induced rats

DPP-4 inhibitors increases the level of GLP-1 in the circulation that crosses BBB to produce neuroprotective effect in brain. After depicting improvement in the neurobehavioral parameters, the effect of Linagliptin at different doses on GLP-1 level was examined. Results showed a significant decrease (df = 8, 45 F = 11.61 ***p < 0.001) in hippocampal GLP-1 level in STZ infused rats when compared with control and sham group respectively. Doses of Linagliptin (3 and 5 mg/kg) and DNP (5mg/kg) significantly augments the level of GLP-1 in brain of STZ infused rats (*p < 0.05, ***p < 0.001). Whereas, Linagliptin at the lower dose 0.513mg/kg did not produced any effect (p > 0.05) on GLP-1 level when compared to diseased rats (Fig. 4).

Linagliptin ameliorates the deposition of soluble Aβ (1–42) senile plaques in brain hippocampus

Deposition of Aβ (1–42) peptides results in the formation of senile plaque which is the pathological hallmark of AD. Level of soluble Aβ (1–42) in the rat hippocampus using the rat specific ELISA kit was examined. STZ rats indicated prominent elevation in the level of Aβ (1–42) in hippocampus (df = 8, 45 F = 52.45 ***p < 0.001). This elevation in Aβ (1–42) level was significant when compared to other groups. 3 and 5mg/kg dose of Linagliptin and 5mg/kg dose of DNP remarkably reversed the STZ induced augmentation in Aβ (1–42) in rat hippocampus (*p < 0.05, **p < 0.01, ***p < 0.001). Consistent to the trend, 0.513mg/kg dose of Linagliptin did not show any change in Aβ (1–42) level in rat hippocampus (p > 0.05) (Fig. 5).

Linagliptin dose-dependently decreases hippocampus IRS-1 (ser307) phosphorylation level

Serine phosphorylation of insulin receptor substrate-1 at 307 results in neuronal resistance which is also modulated by Aβ (1–42) plaques. In context to this notion, a significant increase in the level of phosphorylation of IRS-1 (ser307) was observed in STZ infused toxic group as compared to the control and sham groups (df = 8, 45 F = 99.08 ***p < 0.001). Linagliptin in a dose-dependent manner (3 and 5 mg/kg) and DNP (5mg/kg) markedly ameliorated the phosphorylation of IRS-1 (ser307) (***p < 0.001) whereas, insignificant change was exhibited in Linagliptin (0.513mg/kg) group as compared to toxic rats (Fig. 6).

Linagliptin attenuated AKT (ser473) phosphorylation in hippocampus in ICV-STZ induced rats

Aβ deposition in AD leads to down regulation of PI3K/Akt signalling pathway and up regulation of GSK-3β that results in hyper phosphorylation of Tau protein which sequentially results in the intracellular deposition of neurofibrillary tangles. Results showed a significant decrease (df = 8, 45 F = 35.48 ***p < 0.001) in hippocampal AKT (ser473) level in ICV-STZ infused rats when compared with control and sham group. Linagliptin (3 and 5 mg/kg) and DNP (5mg/kg) treatment significantly augments the level of AKT (ser473) level in brain of STZ infused rats (*p <0.05, ***p < 0.001). Linagliptin at the lower dose 0.513mg/kg did not produced any effect on AKT (ser473) level when compared to diseased group (Fig. 7).

Linagliptin prevented hippocampal GSK-3β stimulation in ICV-STZ induced AD in rats

Elevated GSK-3β level leads to the Tau phosphorylation, which ultimately leads to neurodegeneration. GSK-3β level was found to be significantly augmented (df = 8, 45 F = 48.80 ***p < 0.001) in rats infused with STZ when compared with control and sham group respectively. While Linagliptin dose dependently (except 0.513mg/kg) and DNP (5mg/kg) exhibited a significant (3mg/kg, **p < 0.01 and 5mg/kg, ***p < 0.001) decrease in the level of hippocampal GSK-3β level when compared with STZ toxic group (Fig. 8).

Linagliptin dose-dependently attenuated acetylcholinesterase (AChE) activity in hippocampus

It is well known that Acetylcholinesterase (AChE) enzyme is mainly liable for the degradation of ACh (Acetylcholine), a neurotransmitter in the brain, into acetate/choline resulting into cholinergic decline responsible for cognitive deficit in AD (Singh et al., 2013). A significant (df = 8, 45 F = 48.43 ***p < 0.001) increase in the AChE level in the STZ infused toxic group as compared to the control and sham groups. A trend was observed in the Linagliptin 0.513mg/kg group when compared with the STZ infused toxic group. Whereas, a significant decrease was observed in the treatment group with Linagliptin (3 and 5mg/kg) and DNP (5mg/kg) when compared with toxic group (*p < 0.05, ***p < 0.001) (Table 2).

Dose-dependent effect of Linagliptin on hippocampal oxidative/nitrosative stress markers (TBARS, Nitrite, and CAT)

ICV-STZ infused rats showed dose-dependent significant difference on the level of oxidative and nitrosative stress markers. A significant (***p < 0.001) increase in the level of TBARS and Nitrite and decrease in CAT enzyme (anti-oxidant) was observed in the STZ infused toxic rats when compared with

control and sham groups. These alterations in TBARS, Nitrite and CAT enzyme level was significantly reversed by Linagliptin 3mg/kg and 5mg/kg (TBARS: df = 8, 45 F = 17.71 *p < 0.05, ***p < 0.001, Nitrite: df = 8, 45 F = 51.94 *p < 0.05, ***p < 0.001, CAT: df = 8, 45 F = 32.43 *p < 0.05, ***p < 0.001). 0.513mg/kg dose of Linagliptin showed insignificant changes in these stress markers (p > 0.05) (Table 2).

Linagliptin attenuates the level of hippocampal TNF-α, IL-6 and IL-1β

Neuroinflammatory cytokines (TNF-α, IL-6 and IL-1β) are also key factors for the development of AD. Cytokines level in rat hippocampus were estimated using specific ELISA kits. The ICV-infusion of STZ was found to elevate the levels of these cytokines. The differences were significant as compared to those of control and sham group respectively. Furthermore, these altered levels of TNF-α (df = 8, 45 F = 36.11 ***p < 0.001), IL-6 (df = 8, 45 F = 28.40 ***p < 0.001)and IL-1β (df = 8, 45 F = 36.15 ***p < 0.001) were markedly reversed after oral administration of Linagliptin (3 and 5mg/kg) and DNP (5mg/kg) for 8 weeks (*p < 0.05, **p < 0.01, ***p < 0.001). No significant changes were shown by lower dose of Linagliptin (0.513mg/kg) (Fig. 9 A, B and C).

Histopathological analysis

Histological analysis of rat's hippocampus was carried out by H&E and Congo red staining specific for morphology of neurons and deposition of Aβ respectively. In control and sham groups, H&E and Congo red staining exhibited intact neurons with prominent nuclei and no Aβ (1–42) deposition in the hippocampus region respectively.

However, STZ infused rat's exhibits toxic effect in both the staining. In H&E staining, a significant increase in the eosinophilic cytoplasm, pyknotic nuclei and extensive vacuolization was observed in rat hippocampus. Whereas, in Congo red staining, a significant augmentation in the Aβ (1–42) plaques deposition can be observed in STZ infused rats. Moreover, treatment with Linagliptin at 0.513mg/kg does not indicate any significant (p > 0.05) change in any of the histopathological staining when compared with toxic rats. In contrast to this notion, oral treatment with Linagliptin at 3mg/kg and 5mg/kg and DNP at 5mg/kg in H&E staining and Congo red staining showed significant reduction in eosinophilic stained neurons and mild neuronal toxicity and reduction in Aβ (1–42) plaques deposition respectively when compared to toxic infused group (Fig. 10, 11).

Discussion

Alzheimer's disease (AD) is the most devastating age-linked progressive neurodegenerative disorder, manifested by several behavioral, cognitive and neuronal loss. This neurodegenerative disorder causes severe dementia over a long period of time and failure of various clinical trial studies demands the utmost need to develop the therapeutic agent that should reverse the disease progression (Zameer et al., 2019). STZ administered Intracerebroventrically in subdiabetogenic dose in rats is a well-known non-transgenic model of AD.

In our present study, *in-vivo* experiment was conducted in which subdiabetogenic ICV dose of STZ was administered in rats to cause neurodegeneration due to development of pathologies including cognitive deficit, Aβ (1–42) deposition, GSK-3β phosphorylation, neuroinflammation, oxidative & nitrosative stress markers in hippocampus as compared to control and sham groups (Zameer et al., 2019). Linagliptin (0.513mg/kg, 3mg/kg and 5mg/kg) was investigated in the ICV-STZ induced AD in Albino Wistar rats to evaluate its neuroprotective role against hippocampal insulin resistance and AD-related complications. Moreover, an *in vitro* study reported that phosphorylation of IRS-1 at serine307 results in brain insulin resistance (Siddiqui et al., 2021; Bomfim et al., 2012; Bosco et al., 2011).

ICV-STZ infused rats exhibited an impaired motor and memory skills as assessed by behavioral paradigms viz Locomotor Activity (LA) and Morris Water Maze (MWM) test respectively. The effect of Linagliptin at different doses on motor and memory responses exhibited a dose-dependent significant changes in the photocell beam crossings in LA, acquisition and probe trial in MWM test when compared with toxic group. The observed outcomes are in line with the previous findings that acknowledged motor and memory impairment due to impairment in locomotor responses and MWM (Thome et al., 2018).

GLP-1 level in hippocampus by peripherally blocking the action of DPP-4 (enzyme that degrades GLP-1) have already proved its therapeutic benefits against various neurodegenerative disorders Kosaraju, et al., 2017; Kosaraju et al., 2013; Badawi et al., 2017; Nassar et al., 2015). ICV infusion of STZ have reduced the level of GLP-1 in rats as compared to control and sham. Treatment with Linagliptin for period of 8 weeks significantly and dose-dependently increases the hippocampal level of GLP-1 which mitigates the pathological hallmarks of AD. Prior evidence have reported that brain insulin resistance leads to neurodegeneration (Bosco et al., 2011) due to deposition of Aβ plaques (Siddiqui et al., 2021). Augmented GLP-1 level in hippocampus upregulates AKT/PI3K pathway whereas, downregulates GSK-3β and hyperphosphorylation of tau (Chen et al., 2012).

Deposition of Aβ in hippocampus is a key pathological hallmark of AD. Aβ (1–40) and Aβ (1–42) are the two major forms of Aβ senile plaques in the hippocampus. Aβ (1–42) is much more prone to aggregate and is toxic to neurons as compared to Aβ (1–40) (Jarrett and Lansbury, 1993; El-Agnaf et al., 2000). Therefore, in our present study treatment of ICV-STZ treated rats with Linagliptin for 8 weeks resulted in a dose-dependent and significant reduction of Aβ (1–42) level as compared to control and sham rats. Our finding is consistent with a prior evidence reported by Kosaraju et al., 2016 where Linagliptin was used for the treatment of AD in 3XTg-AD mouse model of the disease (Kosaraju et al., 2017). The proteolytic cleavage of APP by β and γ secretases in amyloidogenic pathway is the limiting step in neurons for the generation of Aβ plaques (Chou et al., 2010). The significant reduction in BACE 1 and γ secretase enzyme may indicate decreased deposition of plaques but no such literature is available depicting role of Linagliptin on these enzymes.

Numerous studies have reported the connection between AD and brain insulin resistance (Siddiqui et al., 2021; Bomfim et al., 2012; Bosco et al., 2011). This is the first study to evaluate that Linagliptin significantly and dose-dependently decreases IRS-1 S307 phosphorylation induced by i.c.v administration

of STZ. Prior evidence have demonstrated the profound action of Linagliptin in increasing insulin sensitivity in ICV-Aβ (1–42) infused rat model of AD (Siddiqui et al., 2021). The effect of Linagliptin for 8 weeks on the level of IRS-1 (s307) followed by the ICV-STZ in hippocampus exhibited dose-dependent and significant reduction in the neuronal resistance by decreasing the phosphorylation of IRS-1 (s307). It is well reported that deposition of Aβ oligomers results in Jun amino-terminal kinases − 1 (JNK-1) activation leading to neuronal insulin resistance (Talbot, 2014). Therefore, decrease in Aβ (1–42) and pro-inflammatory cytokines level can be the probable reason behind the neuroprotection. Moreover, effect of Linagliptin on IRS-1 (s612) and (s632/s635) through activation of IKK (NF-κB) and Extracellular signal-regulated kinase (ERK) respectively is not yet available.

PI3K/Akt and Glycogen Synthase Kinase- 3β (GSK-3β) enzyme shows connection with Aβ (Maqbool et al., 2016). Zhang et al. have reported that Vildagliptin treatment against cognitive deficits in a STZ-induced type 2 diabetic rat model, is mediated via enhancing Akt/BDNF/nerve growth factor expression levels (Zhang et al., 2016). Accumulation of Aβ leads to downregulation of PI3K/Akt and upregulation of GSK-3β that which eventually results in the intracellular deposition of neurofibrillary tangles (Hernandez et al., 2010). Experimental data exist revealed that augmentation in Akt protein or PI3K/Akt pathway results in the mitigation of GSK-3β level (Ma et al., 2009). Indeed even in our study, ICV-STZ rats exhibited significant decrease in Akt protein and increase GSK-3β level which was dose-dependently and significantly attenuated by Linagliptin for the 8 weeks period.

The increased level of acetylcholinesterase (AChE) enzyme has been cited to decrease the level of acetylcholine (Ach), a neurotransmitter level in the hippocampus of AD brain by enhancing its hydrolysis (Stanciu et al., 2020). Deposition of Aβ (1–42) plaques exhibited impaired cholinergic neurotransmission by augmenting AChE level (Cui et al., 2012). Present research, ICV-STZ infusion depicted significant increase in hippocampal AChE level. Contrastly, Linagliptin showed dose-dependent and significant effect on AChE activity after 8 weeks of treatment. Our results were in support of finding of Kosaraju and co-workers summarizing the dose-dependent inhibitory effect of Linagliptin on AChE activity in hippocampus (Kosaraju et al., 2017).

Neuroinflammation responses have been evidenced to be linked to free radical formation and mitochondrial dysfunction in the development of AD (Wang et al., 2018a). Corresponding to the findings of Wang et al that STZ injection in brain increases oxidative stress we detected significant increase in level of TBARS, NO, and decrease in the level of CAT in hippocampus as compared to control and sham (Wang et al., 2018b). However, Linagliptin significantly and dose-dependently attenuated nitrite and TBARS level while, increases CAT level. Relevant to our finding it was additionally reported in previous studies that deposition of Aβ (1–42) plaques leads to the increase in oxidative/nitrosative stress markers (Pandey et al., 2018). Therefore, plausible reason behind Linagliptin mediated attenuation in oxidative/nitrosative stress can be due to decrease in Aβ (1–42) level.

Earlier evidences depicts that imbalance in the formation and clearance of Aβ peptides plaques in neurons releases pro-inflammatory cytokines (Song et al., 2014; Block et al., 2007). Results of our present

study showed significant increase in TNF-α, IL-6 and IL-1β cytokines level after ICV-STZ infusion in rats. Nonetheless, dose-dependent treatment with Linagliptin exhibited significant attenuation in the level of neurotoxic cytokines in hippocampus. Accumulation of Aβ (1–42) plaques incites over activation of microglial cells which leads to detrimental effect on release of pro-inflammatory cytokines causing brain damage (Puzzo et al., 2005).

Brain damage is correlated with infusion of ICV-STZ in rat's hippocampus. Histological examination by H & E and Congo red indicate neuronal injury characterised by high density of eosinophilic stained cytoplasm, pyknotic nuclei and neuronal shrinking (Asadbegi et al., 2017; Zhang et al., 2013) and deposition of Aβ (1–42), dipicting significant high density of red stains in STZ infused rat hippocampus respectively (Cioanca et al., 2013, Soheili et al., 2012). We have also analysed the histology of hippocampal sections by both H & E and Congo red staining photomicrographs. Linagliptin showed dose-dependent and significant protective effect against neuronal injury and plaque formation in ICV-STZ induced rats when compared with toxic group. These histological results were correspondence to the behavioural and biochemical assessments of our research.

Conclusion

From the outcomes of the present research, it could be concluded that Linagliptin for the period of 8 weeks mitigates cognitive impairment, hippocampal insulin resistance and AD-like complications in hippocampal neurons of rats which is attributable to the behavioural, biochemical and histological assessment in ICV-STZ rat model of AD. This research also present a perspective on the interaction between Aβ (1–42), Type 3 Diabetes Mellitus due to serine phosphorylation of IRS-1, neuroinflammation, AD-related complications and potential therapeutic role of Linagliptin. To the best of our knowledge, our study is the first to evaluate the ameliorative effect of Linagliptin at three different doses against subdiabetogenic dose of ICV-STZ induced neurodegeneration and AD related complications in rats and to determine whether the neuroprotective role is associated with neuronal insulin resistance, rendering Linagliptin a promising pharmacological intervention to prevent symptoms and slow down progression of AD. The ensemble of our research findings discussed gives new insight to the mode of action of Linagliptin on insulin signalling pathway that requires the need of further investigation in order to understand the exact cellular and molecular mechanism of action of DPP-4i against pathological events similar to AD.

Abbreviations

AD, Alzheimer's disease; T2DM, Type 2 Diabetes Mellitus; ICV, Intracerebroventricular; STZ, Streptozotocin; Aβ, Amyloid-beta; T3DM, Type 3 Diabetes Mellitus; IRS-1, Insulin Receptor Substrate-1; ISP, Insulin Signaling Pathway; DPP-4, Dipeptidyl peptidase-4; GLP-1, Glucagon-like peptide-1; GSK-3β, Glycogen synthase kinase-3 beta; p-tau, Phosphorylated tau; LA, Locomotor activity; MWM, Morris water maze; TNF-a, Tumor necrosis factor-a; IL-1β, Interleukin-1β; IL-6, Interleukin-6; BBB, Blood Brain Barrier; AchE, Acetylcholinesterase.

Declarations

Conflict of interest

Authors of our study declare no potential conflicts of interest in conducting research and publishing this article.

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References

1. Akhtar M, Imam SS, Ahmad MA, Najmi AK, Mujeeb M, Aqil M (2014) Neuroprotective study of Nigella sativa-loaded oral provesicular lipid formulation: in vitro and ex vivo study. Drug Deliv. 21:487–494.
2. Asadbegi M, Yaghmaei P, Salehi I, Komaki A, Ebrahim-Habibi A (2017) Investigation of thymol effect on learning and memory impairment induced by intrahippocampal injection of amyloid beta peptide in high fat diet-fed rats. Metab. Brain Dis. 32:827–839.
3. Ayutyanont N, Chen K, Fleisher AS, Langbaum J, Reschke C, Parks SA, Lee W, Liu X, Protas H, Bandy D (2013) Whole brain atrophy and sample size estimate via iterative principal component analysis for twelve-month Alzheimer's disease trials. Neurosci. Biomed. Eng. 1:40–47.
4. Ayyub M, Najmi AK, Akhtar M (2017) Protective effect of irbesartan an angiotensin (AT1) receptor antagonist in unpredictable chronic mild stress induced depression in mice. Drug Res. 67:59-64.
5. Badawi GA, Abd El Fattah MA, Zaki HF, El Sayed MI (2017) Sitagliptin and liraglutide reversed nigrostriatal degeneration of rodent brain in rotenone-induced Parkinson's disease. Inflammopharmacol. 25:369–382.
6. Blázquez E, Velázquez Hurtado-Carneiro V, Ruiz-Albusac JM (2014) Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. Front. Endocrinol. 5:161.
7. Block ML, Zecca L, Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat. Rev. Neurosci. 8(1):57-69.
8. Bomfim TR, Forny-Germano L, Sathler LB, Brito-Moreira J, Houzel JC, Decker H, Silverman MA, Kazi H, Melo HM, McClean PL, Holscher C, Arnold SE, Talbot K, Klein WL, Munoz DP, Ferreira ST, Felice

FGD (2012) An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease–associated Aβ oligomers. J. Clin. Invest. 122:1339–1353.

9. Bosco D, Fava A, Plastino M, Montalcini T, Pujia, A (2011) Possible implications of insulin resistance and glucose metabolism in Alzheimer's disease pathogenesis. J. Cell. Mol. Med. 15:1807–1821.
10. Caruso M, Ma D, Msallaty Z, Lewis M, Seyoum B, Al-Janabi W, Diamond M, Abou-Samra AB, Højlund K, Tagett R (2014) Increased interaction with insulin receptor substrate-1, a novel abnormality in insulin resistance and type 2 diabetes. Diabetes 63:1933–1947.
11. Chen S, Liu AR, An FM, Yao WB, Gao XD (2012) Amelioration of neurodegenerative changes in cellular and rat models of diabetes-related Alzheimer's disease by exendin-4. Age (Omaha) 34: 1211–1224.
12. Chow VW, Mattson MP, Wong PC, Gleichmann M (2010) An overview of APPprocessing enzymes and products. Neuromol. Med. 12(1):1–12.
13. Cioanca O, Hritcu L, Mihasan M, Hancianu M (2013) Cognitive-enhancing and antioxidant activities of inhaled coriander volatile oil in amyloid β (1–42) rat model of Alzheimer's disease. Physiol. Behav. 120:193–202.
14. Craft S, Watson GS (2004) Insulin and neurodegenerative disease: shared and specific mechanisms. Lancet Neurol. 3:169–178.
15. Cui H, Kong Y, Zhang H (2012) Oxidative stress, mitochondrial dysfunction, and aging. J. Signal Transduct. , 646354.
16. Darsalia V, Ortsater H, Olverling A (2013) The DPP-4 inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain: a comparison with glimepiride. Diabetes. 62:1289–96.
17. Darsalia V, Larsson M, Lietzau G, Nathanson D, Nystrom T, Klein T, Patrone C (2016) Gliptins-mediated neuroprotection against stroke requires chronic pre-treatment and is glucagon-like peptide-1 receptor independent. Diabetes Obes. Metabol. 18(5):537–541.
18. Darsalia V, Olverling A, Larsson M, Mansouri S, Nathanson D, Nystrom T, Klein T, Sjoholm A, Patrone C (2014) Linagliptin enhances neural stem cell proliferation after stroke in type 2 diabetic mice. Regul. Pept. 190–1:25–31
19. El-Agnaf OM, Mahil DS, Patel BP, Austen BM (2000) Oligomerization and toxicity of beta-amyloid-42 implicated in Alzheimer's disease. Biochem. Biophys. Res. Commun. 273:1003e1007.
20. Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.
21. Fargo K (2014) Alzheimer's Association Report: 2014 Alzheimers disease facts and figures. Alzheimer's Dement. 10:e47–e92.
22. Hamilton A, Holscher C (2009) Receptors for the incretin glucagon-like peptide-1 are expressed on neurons in the central nervous system. Neuroreport. 20:1161-1166.
23. Hernandez E, Barreda GD, Fuster-Matanzo A, Lucas JJ, Avila J (2010) GSK3: a possible link between beta amyloid peptide and tau protein. Exp. Neurol. 223:322–325.

24. Holscher C (2010) Incretin analogues that have been developed to treat type 2 diabetes hold promise as a novel treatment strategy for Alzheimer's disease. Recent Pat CNS Drug Discov 5:109–117.
25. Hou DR, Wang Y, Zhou I, Chen K, Tian Y, Song Z, Bao J, Yang QD (2008) Altered angiotensin-converting enzyme and its effect on the brain in a rat model of Alzheimer's disease. Chin. Med. J. (Engl.) 121:2320-2323.
26. Husain I, Akhtar M, Abdin MZ, Islamuddin M, Shaharyar M, Najmi AK (2018) Rosuvastatin ameliorates cognitive impairment in rats fed with high-salt and cholesterol diet via inhibiting acetylcholinesterase activity and amyloid beta peptide aggregation. Hum. Exp. Toxicol. 37(4): 399–411.
27. Husain I, Akhtar M, Vohora D, Abdin MZ, Islamuddin M, Akhtar MJ, Najmi AK (2017) Rosuvastatin attenuates high-salt and cholesterol diet induced neuroinflammation and cognitive impairment via preventing nuclear factor kappaB pathway. Neurochem. Res. 42:2404–2416.
28. Jarrett JT, Lansbury PT (1993) Seeding "one_dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell. 73:1055-1058.
29. Javed H, Vaibhav K, Ahmed ME, Khan A, Tabassum R, Islam F, Safhi MM, Islam F (2015) Effect of hesperidin on neurobehavioral, neuroinflammation, oxidative stress and lipid alteration in intracerebroventricular streptozotocin induced cognitive impairment in mice. J. Neurol. Sci. 348 (1-2):51–59.
30. Kabel AM, Omar MS, Alhadhrami A, Alharthif SS, Alrobaianget MM (2018) Linagliptin potentiates the effect of L-dopa on the behavioural, biochemical and immunohistochemical changes in experimentally-induced Parkinsonism: role of toll-like receptor 4, TGF-β1, NF-κB and glucagon-like peptide 1. Physiol. Behav. 188:108–118.
31. Kastin AJ, Akerstrom V, Pan W (2002) Interactions of glucagon-like peptide-1 (GLP-1) with the blood brain barrier. J. Mol. Neurosci. 18:7-14.
32. Katsurada K, Yada T (2016) Neural effects of gut- and brain-derived glucagon-like peptide-1 and its receptor agonist. J Diabetes Investig. 7:64–69.
33. Kaundal M, Akhtar M, Deshmukh R (2017) Lupeol isolated from Betula alnoides ameliorates amyloid beta induced neuronal damage via targeting various pathological events and alteration in neurotransmitter levels in rat's brain. J. Neurol. Neurosci. 8:1-8.
34. Kosaraju J, Gali CC, Khatwal RB, Dubala A, Chinni S, Holsinger RMD, Rao MVSSTS, Kumar MNS, Duraiswamy B (2013) Saxagliptin: a dipeptidyl peptidase-4 inhibitor ameliorates streptozotocin induced Alzheimer's disease. Neuropharmacology 72:291-300.
35. Kosaraju J, Holsinger RMD, Guo L, Tam KY (2017) Linagliptin, a Dipeptidyl Peptidase-4 Inhibitor, Mitigates Cognitive Deficits and Pathology in the 3xTg-AD Mouse Model of Alzheimer's disease. Mol Neurobiol. 54:6074–6084.
36. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265-275.

37. Ma QL, Yang F, Rosario ER, Ubeda OJ, Beech W, Gant DJ, Chen PP, Hudspeth B, Chen C, Zhao Y, Vinters HV, Frautschy SA, Cole GM (2009) β-amyloid oligomers induce phosphorylation of tau and inactivation of insulin receptor substrate via c-Jun N-terminal kinase signaling: suppression by fatty acids and curcumin. J Neurosci. 29:9078–9089.
38. Maqbool M, Mobashir M, Hoda N (2016) Pivotal role of glycogen synthase kinase-3: a therapeutic target for Alzheimer's disease. Eur. J. Med. Chem. 107:63–81.
39. Mishra SK, Singh S, Shukla S, Shukla R (2018) Intracerebroventricular streptozotocin impairs adult neurogenesis and cognitive functions via regulating neuroinflammation and insulin signaling in adult rats. Neurochem. Int. 113:56–68.
40. Nassar NN, Al-Shorbagy MY, Arab HH, Abdallah DM (2015) Saxagliptin: a novel antiparkinsonian approach. Neuropharmacology 89:308–317.
41. Neumiller JJ (2012) Pharmacology, Efficacy, and Safety of Linagliptin for the Treatment of Type 2 Diabetes Mellitus. The Annals of Pharmacother. 46:358-367.
42. Padurariu M, Ciobica A, Mavroudis I, Fotiou D, Baloyannis S (2012) Hippocampal neuronal lossin the CA1 and CA3 areas of Alzheimer's disease patients. Psychiatr. Danub. 24:152-158.
43. Pandey A, Bani S, Dutt P, Kumar Satti N, Avtar Suri K, Nabi Qazi G (2018) Multifunctional neuroprotective effect of Withanone, a compound from Withania somnifera roots in alleviating cognitive dysfunction. Cytokine 102:211–221.
44. Puzzo D, Vitolo O, Trinchese F, Jacob JP, Palmeri A, Arancio A (2005) Amyloid-β peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element binding protein pathway during hippocampal synaptic plasticity. J. Neurosci. 25:6887–6897.
45. Remya C, Dileep KV, Tintu I, Variyar EJ, Sadasivan C (2014) Flavanone glycosides as acetylcholinesterase inhibitors: computational and experimental evidence. Indones. J. Pharm. Sci. Technol. 76:567–570.
46. Rohnert P, Schmidt W, Emmerlich P, Goihl A, Wrenger S, Bank U, Nordhoff K, Tager M, Ansorge S, Reinhold D, Striggow F (2012) Dipeptidyl peptidase IV, aminopeptidase N and DPIV/APN-like proteases in cerebral ischemia. J Neuroinflamm. 9:44.
47. Sachdeva AK, Chopra K (2015) Lycopene abrogates Abeta (1-42) mediated neuroinflammatory cascade in an experimental model of Alzheimer's disease. J. Nutr. Biochem. 26:736-744.
48. Shannon RP (2013) DPP-4 inhibition and neuroprotection: do mechanisms matter? Diabetes. 62: 1029–1031.
49. Siddiqui N, Ali J, Parvez S, Zameer S, Najmi AK, Akhtar M (2021) Linagliptin, a DPP-4 inhibitor, ameliorates Aβ (1-42) peptides induced neurodegeneration and brain insulin resistance (BIR) via insulin receptor substrate-1 (IRS-1) in rat model of Alzheimer's disease. Neuropharmacol. 195:1-14.
50. Singh B, Sharma B, Jaggi AS, Singh N (2013) Attenuating effect of lisinopril and telmisartan in intracerebroventricular streptozotocin induced experimental dementia of Alzheimer's disease type: possible involvement of PPAR-gamma agonistic property. J. Renin. Syst. 14(2):124-136.

51. Soheili M, Tavirani MR, Salami M (2012) Clearance of amyloid beta plaques from brain of Alzheimeric rats by lavandula angustifolia. Neurosci. Med. 3(04):362.
52. Song Y, Cui T, Xie N, Zhang X, Qian Z, Liu J (2014) Protocatechuic acid improves cognitive deficits and attenuates amyloid deposits, inflammatory response in aged A beta PP/PS1 double transgenic mice. Int. Immunopharmacol. 20(1):276–281.
53. Stanciu GD, Luca A, Rusu RN, Bild V, Chiriac SIB, Solcan C, Bild W, Ababei DC (2020) Alzheimer's disease Pharmacotherapy in Relation to Cholinergic System Involvement. Biomolecules 10:40.
54. Talbot K (2014) Brain insulin resistance in Alzheimer's disease and its potential treatment with GLP-1 analogs. Neurodegener Dis. Manag. 4:31–40.
55. Thenmozhi AJ, Raja TRW, Janakiraman U, Manivasagam T (2015) Neuroprotective effect of hesperidin on aluminium chloride induced Alzheimer's disease in Wistar rats. Neurochem Res. 40: 767–776.
56. Thome GR, Oliveira VA, Chitolina Schetinger MR, Saraiva RA, Souza D, Dorneles Rodrigues OE, Teixeira Rocha JB, Ineu RP, Pereira ME (2018) Selenothymidine protects against biochemical and behavioral alterations induced by ICV-STZ model of dementia in mice. Chem. Biol. Int. 294:135–143.
57. Wang D, Liu L, Li S, Wang C (2018a) Effects of paeoniflorin on neurobehavior, oxidative stress, brain insulin signaling, and synaptic alterations in intracerebroventricular streptozotocin-induced cognitive impairment in mice. Physiol. Behav. 191:12–20.
58. Wang D, Wang C, Liu L, Li S (2018b) Protective effects of evodiamine in experimental paradigm of Alzheimer's disease. Cogn. Neurodyn. 12(3):303–313.
59. Wei L, Lv S, Huang Q, Wei J, Zhang S, Huang R, Lu Z, Lin X (2015) Pratensein attenuates Aβ-induced cognitive deficits in rats: enhancement of synaptic plasticity and cholinergic function. Fitoterapia. 101:208–217.
60. Wills ED (1966) Mechanisms of lipid peroxide formation in animal tissues. Biochem. J. 99:667-676.
61. Zameer S, Kaundal M, Vohora D, Ali J, Najmi AK, Akhtar M (2019) Ameliorative effect of alendronate against intracerebroventricular streptozotocin induced alteration in neurobehavioral, neuroinflammation and biochemical parameters with emphasis on Aβ and BACE-1. Neurotoxicol. 70:122-134.
62. Zhang D, Shi N, Fang H, Ma L, Wu W, Zhang Y, Tian J, Tian L, Kang K, Chen S (2018) Vildagliptin, a DPP4 inhibitor, alleviates diabetes-associated cognitive deficits by decreasing the levels of apoptosis-related proteins in the rat hippocampus. Exp Ther Med. 15(6):5100–5106.
63. Zhang X, Dong F, Ren J, Driscoll MJ, Culver B (2005) High dietary fat induces NADPH oxidase-associated oxidative stress and inflammation in rat cerebral cortex. Exp. Neurol. 191:318–325.
64. Zhang YY, Fan YC, Wang M, Wang D, Li XH (2013) Atorvastatin attenuates the production of IL-1β, IL-6, and TNF-α in the hippocampus of an amyloid β1-42- induced rat model of Alzheimer's disease. Clin. Interv. Aging 8:103.

Tables

Table 1: Effect of Linagliptin on locomotor activity (LA) in ICV- STZ infused rat.

STZ infused toxic group showed significant changes in the locomotor activities as compared to control and sham group respectively. Linagliptin (3mg/kg and 5mg/kg) and DNP (5mg/kg) exhibited significant difference whereas, 0.513mg/kg dose exhibits insignificant ($p > 0.05$) change when compared with toxic infused rats. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil

Groups	Move time(s)	Rest time(s)	Horizontal activity(cm)	Mean velocity(cm/s)	Total movement(#)
Control	493.6 ± 21.55	141.2 ± 11.31	5121 ± 158.0	738.0 ± 27.17	665.5 ± 30.12
Sham	491.8 ± 29.17	142.3 ± 18.30	4705 ± 190.4	717.9 ± 36.48	653.6 ± 26.77
STZ	182.5 ± 15.41$^{\#\#\#\$\$\$}$	534.6 ± 21.64$^{\#\#\#\$\$\$}$	1135 ± 355.9$^{\#\#\#\$\$\$}$	246.7 ± 20.11$^{\#\#\#\$\$\$}$	223.1 ± 15.18$^{\#\#\#\$\$\$}$
STZ + Lina 0.513mg /kg	188.0 ± 27.70	532.7 ± 24.35	1089 ± 341.3	243.8 ± 19.60	212.4 ± 77.74
STZ + Lina 3mg/kg	219.0 ±26.95 *	433.1 ±29.17 **	1469 ± 251.9*	353.7 ± 19.49 *	346.1 ± 16.53**
STZ + Lina 5mg/kg	332.1 ±19.83***	328.9 ±18.24 **	4453 ± 320.4***	432.3 ± 23.79**	404.6 ± 38.61***
Lina Per se	511.0 ±11.19***	149.1 ±26.40***	4822 ± 280.7***	723.0 ± 30.25***	558.5 ± 31.69***
STZ + DNP 5mg/kg	517.0 ± 21.12***	151.6 ±26.20***	4964 ± 337.6***	751.1 ± 29.81***	534.0 ± 25.30***
DNP Per se	529.1 ±25.81 ***	154.2 ±13.23***	5045 ±275.0 ***	715.7 ± 28.29***	548.8 ± 31.16***

The values are expressed as mean ± SEM.

###$$$P < 0.001 vs control and sham group respectively

*p < 0.05 vs STZ infused toxic group

**p < 0.01 vs STZ infused toxic group

***p< 0.001 vs STZ infused toxic group

Table 2: Effect of Linagliptin on AChE, MDA, Nitrite and CAT level in ICV- STZ infused rat.

Toxic rats infused with STZ showed significant changes in AChE, MDA, Nitrite and CAT level as compared to control and sham group. 0.513mg/kg dose of Linagliptin does not exhibits significant (p > 0.05) changes as exhibited by 3 and 5 mg/kg dose of Linagliptin and 5mg/kg dose of DNP when compared with toxic group. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil

Groups	AChE (nmol/min/mg protein)	TBARS (nmol MDA/mg protein)	Nitrite (µmol/mg protein)	CAT (nmol H2O2/min/ mg protein)
Control	102.9 ± 5.39	1.18 ± 0.22	81.2 ± 3.21	14.33± 0.49
Sham	103.1 ± 5.65	1.22 ± 0.33	84.5 ± 3.19	13.45 ± 0.65
STZ	448.1 ± 34.62###$$$	5.01 ± 0.42###$$$	293.8 ± 20.46###$$$	6.52 ± 0.56###$$$
STZ + Lina 0.513mg/kg	446.6 ± 36.98	4.89 ± 0.33	298.2 ± 19.81	7.83 ± 0.56
STZ + Lina 3mg/kg	356.1 ± 27.01*	3.94 ± 0.37*	238.1 ± 19.13*	8.96 ± 0.55*
STZ + Lina 5mg/kg	228.1 ± 28.00***	2.85 ± 0.32**	193.6 ± 18.97**	10.20 ± 0.51***
Lina Per se	107.1 ± 6.50***	1.26 ± 0.30***	82.6 ± 6.60***	14.03 ± 0.78***
STZ + DNP 5mg/kg	105.7 ± 4.41***	1.16 ± 0.23***	87.6 ± 6.38***	14.83 ± 0.81***
DNP Per se	188.8 ± 4.50***	1.30 ± 0.19***	81.7 ± 6.42***	15.43 ± 0.66***

The values are expressed as mean ± SEM.

###$$$ P < 0.001 vs. control and sham groups respectively

*p < 0.05 vs. STZ infused toxic group

**p < 0.01 vs. STZ infused toxic group

***p < 0.001 vs. STZ infused toxic group

Figures

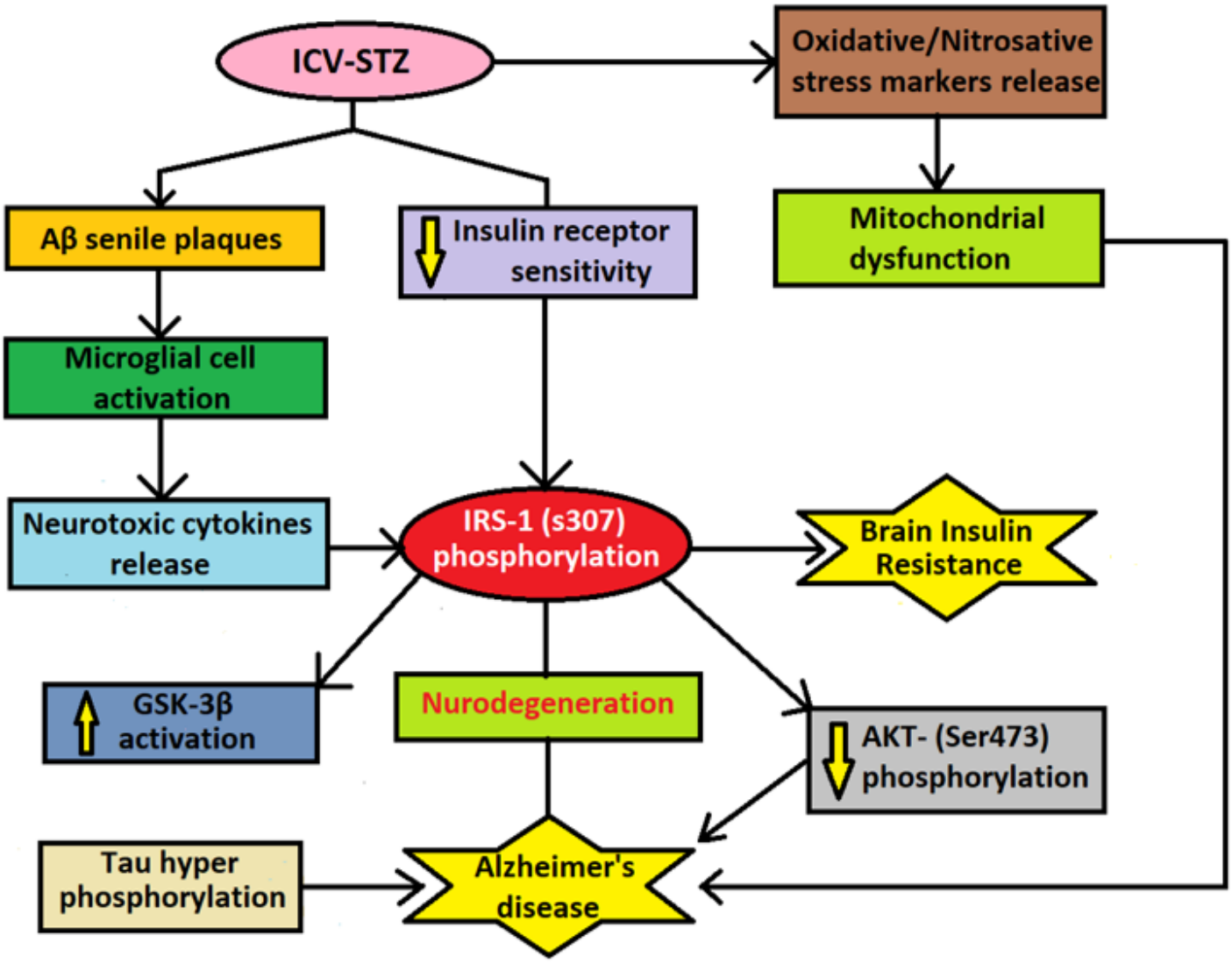

Figure 1

Flowchart representation of the study rationale

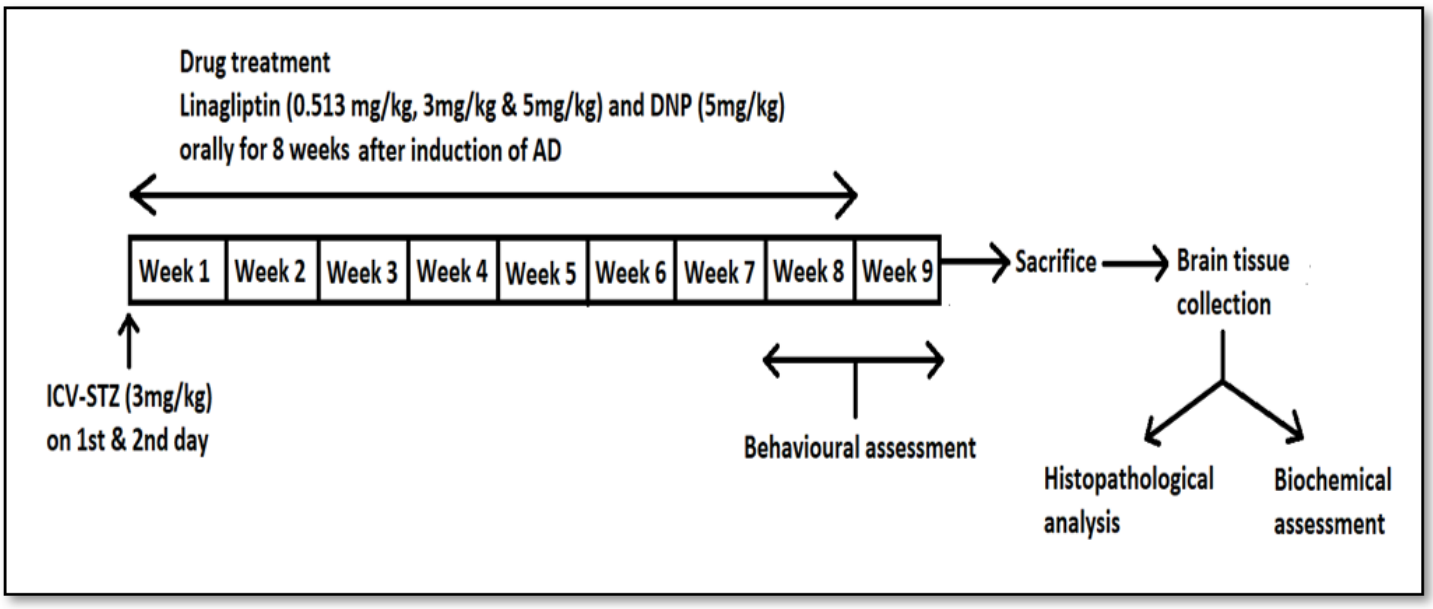

Figure 2

Diagrammatic representation of experimental procedure

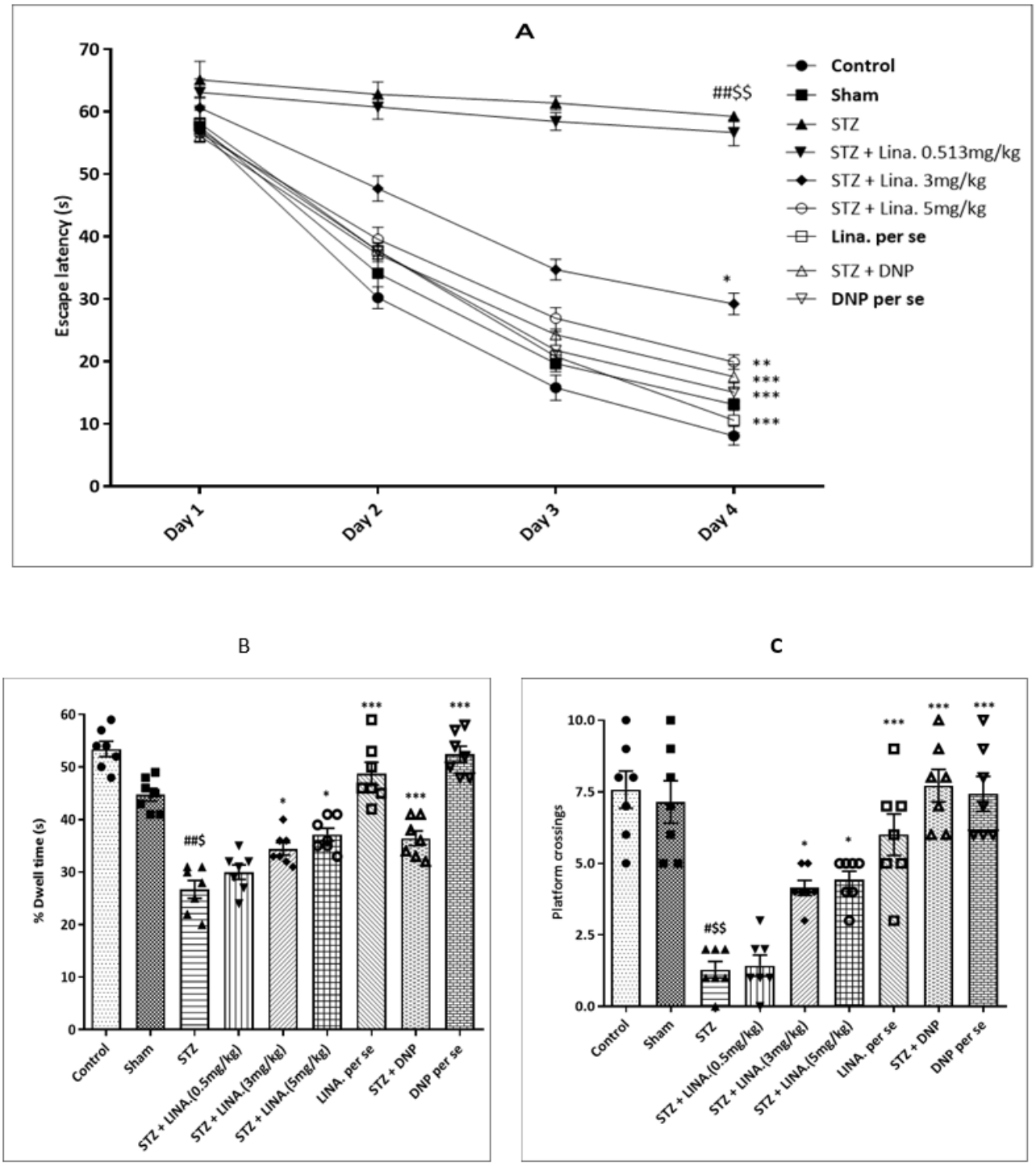

Figure 3

Morris water maze (MWM) test in ICV-STZ induced AD in rats. (A) Escape Latencies during acquisition phase for 4 consecutive days. **(B)** % Dwell time during probe trial. **(C)** Platform crossings during probe trial. The values are expressed as mean ± SEM. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ when compared to control and $^{\$\$\$}p < 0.001$ when compared to sham. *p < 0.05; **p < 0.01; ***p < 0.001 when compared to

STZ infused toxic group. STZ + Lina 0.513mg/kg group did not show any significant change ($p > 0.05$) in all the three parameters. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

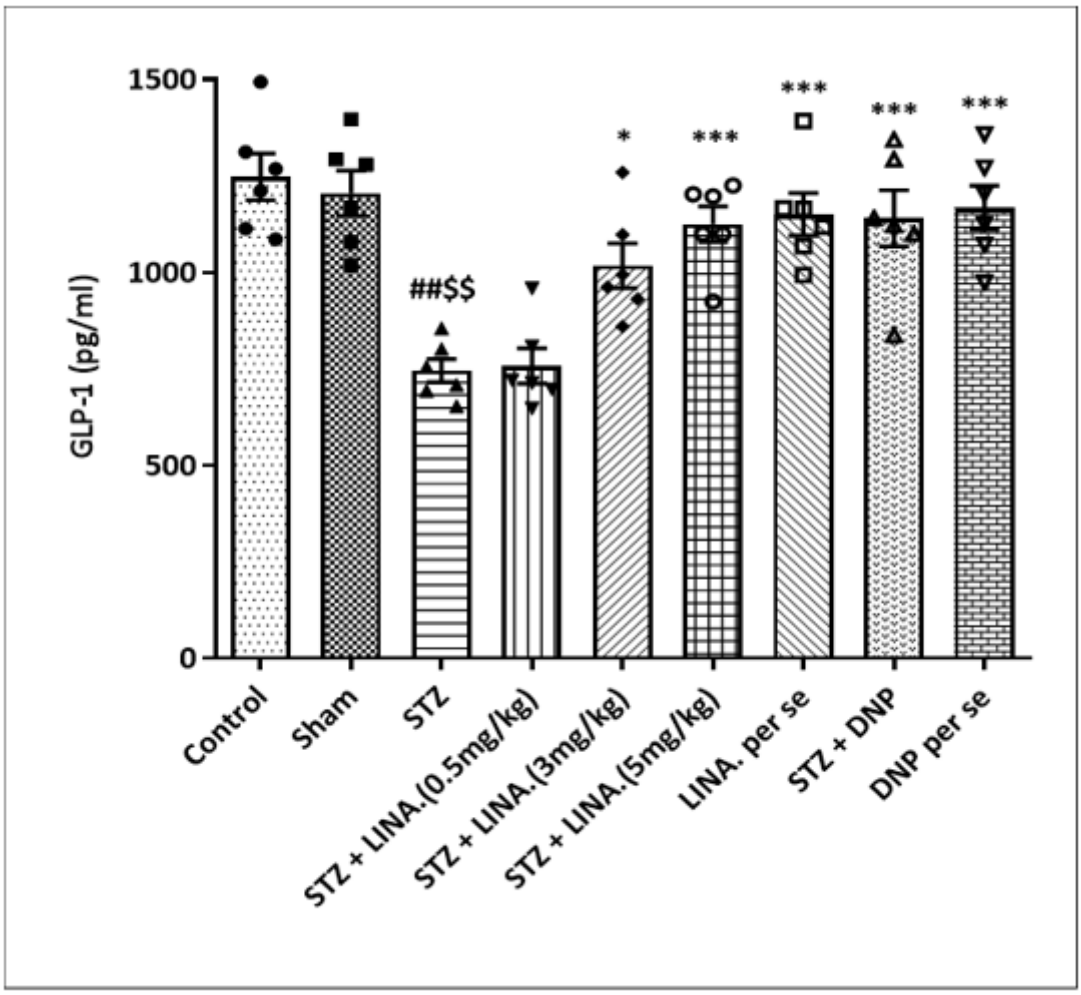

Figure 4

GLP-1 level in brain hippocampus after the oral administration of Linagliptin at 3 different doses. Each bar with vertical line represents the values as mean ± SEM. $^{\#\#\#\$\$\$}$ $p < 0.001$ vs. control and sham groups respectively. Linagliptin at dose 0.513mg/kg showed non-significant ($p > 0.05$) change. *$p < 0.05$; ***$p < 0.001$ vs. ICV-STZ infused toxic group. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

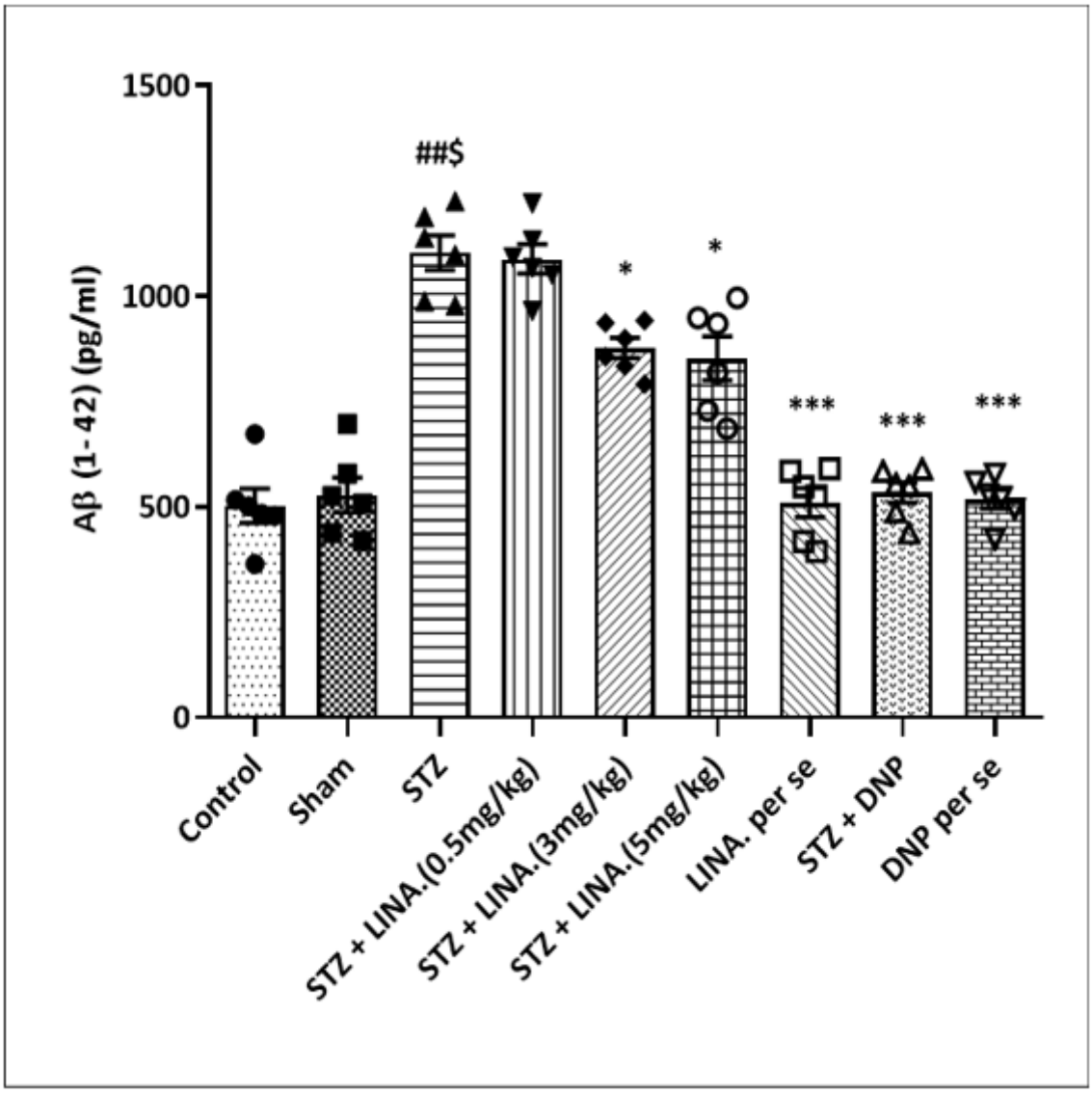

Figure 5

Level of Soluble Aβ (1–42) plaques in the hippocampus after the administration of Linagliptin at 3 different doses for the period of 8 weeks. Each bar with vertical line represents the values as mean ± SEM. $^{\#\#\#\$\$\$}$ $p < 0.001$ vs. control and sham groups respectively. 0.513 mg/kg dose of Linagliptin showed non-significant change ($p > 0.05$), whereas, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. ICV-STZ infused toxic group. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

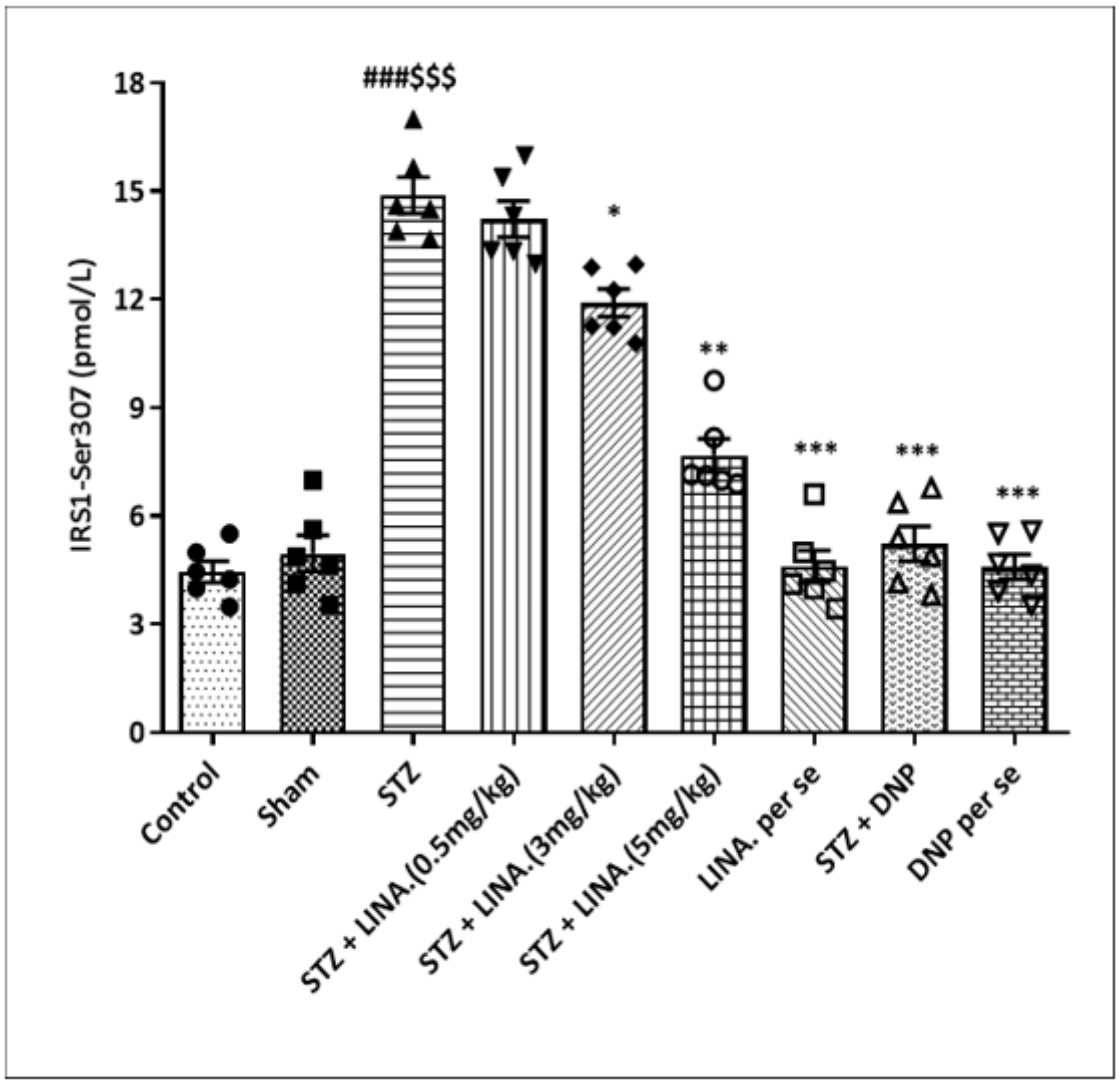

Figure 6

Level of Hippocampal IRS-1(s307) in STZ induced AD in rats. Each bar with vertical line represents the values as mean ± SEM. $^{\#\#\#\$\$\$}$ $p < 0.001$ vs. control and sham groups respectively. Non-significant ($p > 0.05$) difference was observed in treatment group with Linagliptin (0.513mg/kg) as compared to STZ group. ***$p < 0.001$ vs. ICV-STZ infused toxic group. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

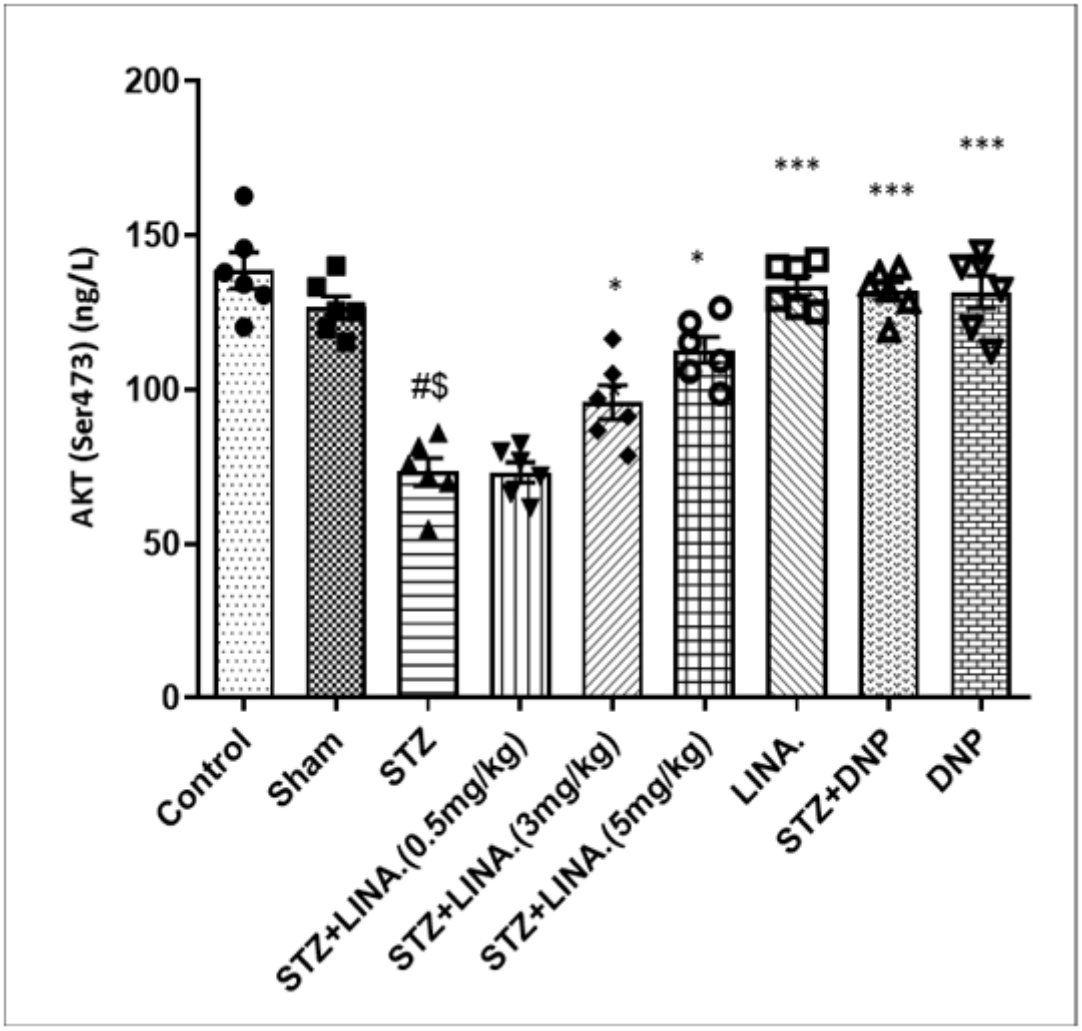

Figure 7

Effect of Linagliptin on AKT (Ser473) level after the administration of Linagliptin for 8 weeks. Each bar with vertical line represents the values as mean ± SEM. $^{\#\#\#\$\$\$}$ $p < 0.001$ vs. control and sham group respectively. Dose 0.513mg/kg of Linagliptin exhibits non-significant ($p > 0.05$) change when compared with toxic rats. *$p < 0.05$ and ***$p < 0.001$ vs. STZ infused toxic group. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

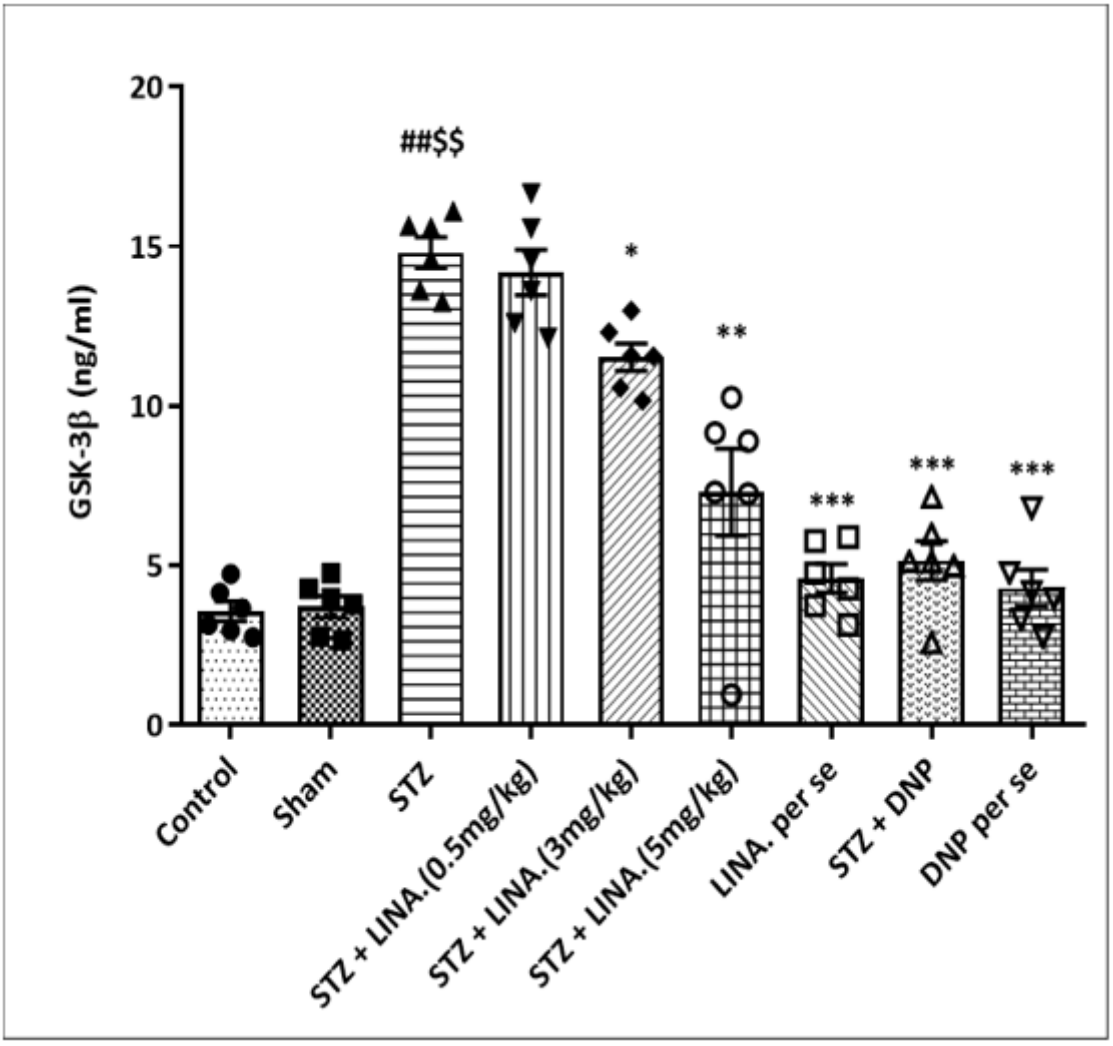

Figure 8

Linagliptin significantly attenuates the level of hippocampal GSK-3β in ICV-STZ induced AD in rats. Each bar with vertical line represents the values as mean ± SEM. $^{###\$\$\$}$ $p < 0.001$ vs. control and sham group respectively. STZ + Lina 0.513mg/kg group does not exhibit any significant ($p > 0.05$) change as compared to STZ group. *$p < 0.05$ and ***$p < 0.001$ vs. STZ infused toxic group. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

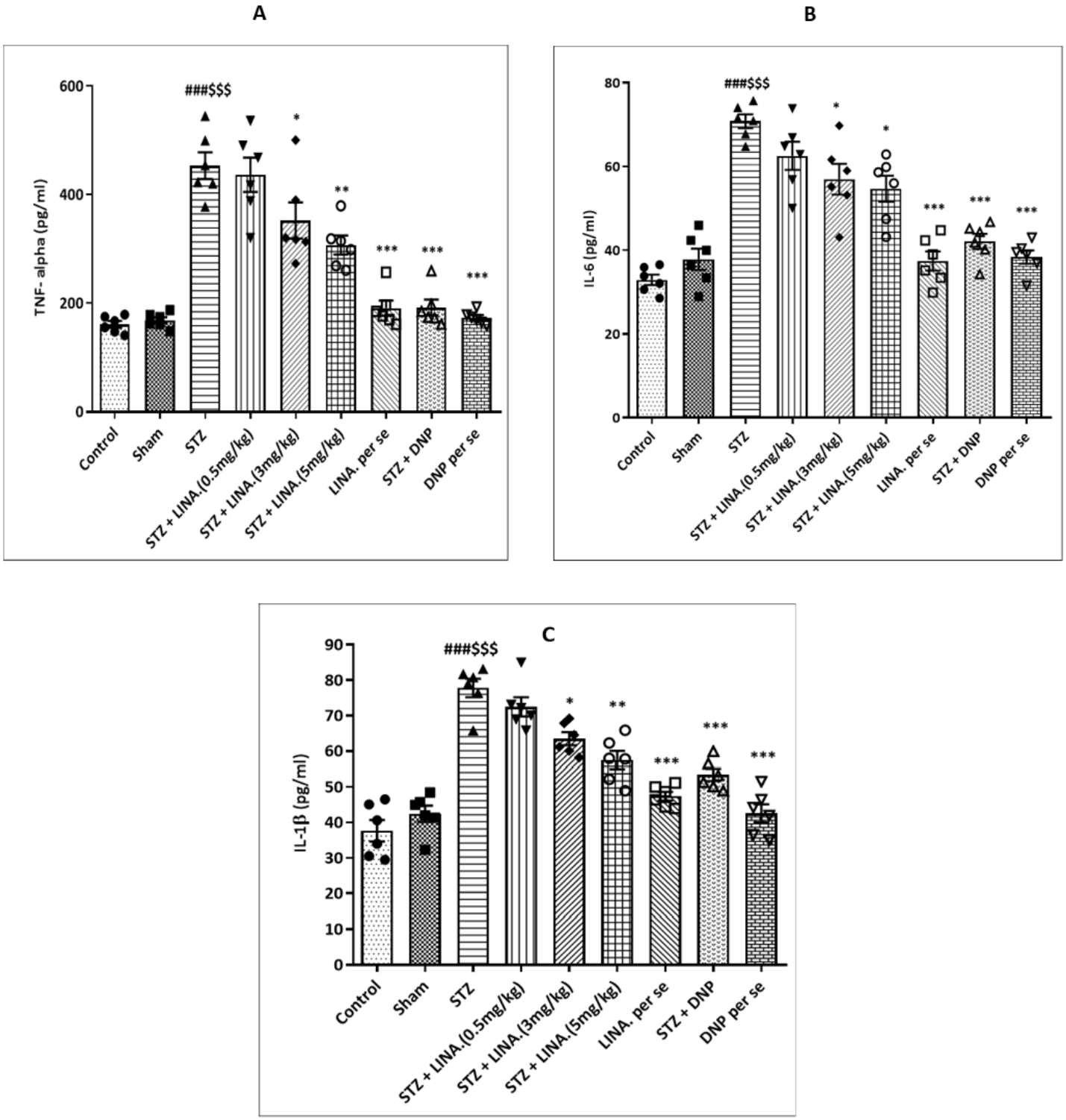

Figure 9

Linagliptin effect on proinflammatory cytokines TNF-α, IL-6 and IL-1β in ICV-STZ induced AD in rats. Each bar with vertical line represents the values as mean ± SEM in a, b and c. $^{###\$\$\$}$ $p < 0.001$ vs. control and sham group respectively. Dose 0.513mg/kg of Linagliptin exhibits non-significant ($p > 0.05$) change in all the three parameters when compared with toxic rats.*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. STZ infused toxic group. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

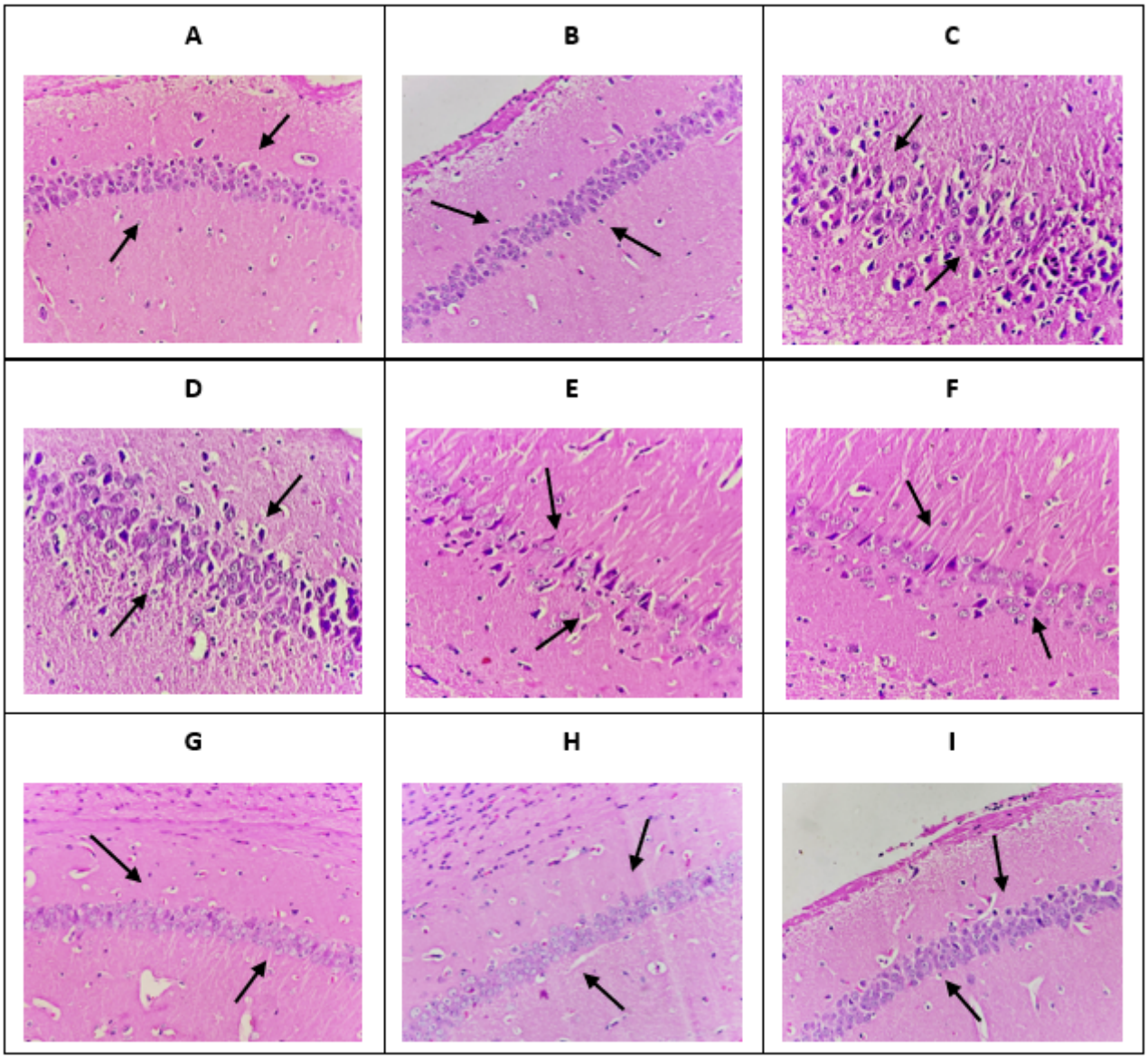

Figure 10

Histopathology of CA1 region of hippocampus with H&E staining after 8 weeks of experimental protocol at 40X magnification (100 micrometre). **(A)** Control rat's exhibits healthy neurons with prominent nuclei. **(B)** Sham rats also showed healthy neurons with prominent nuclei. **(C)** STZ infused toxic group rats represents neuronal damage, vacuolization, neuronal shrinkage and eosinophilic stained cytoplasm. **(D)** STZ + Lina. 0.513mg/kg treatment rats showed non-significant ($p > 0.05$) difference when compared to toxic group. **(E)** Treatment group with Lina. 3mg/kg represented moderate neuronal injury with decreased number of eosinophilic stained neurons as compared to STZ group and **(F)** Lina. 5mg/kg treatment group showed mild neuronal injury with much more less number of eosinophilic stained neurons as compared to toxic rats. **(G)** Lina. Per se, **(H)** STZ + DNP group and **(I)** DNP per se showed intact neurons with prominent nuclei. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

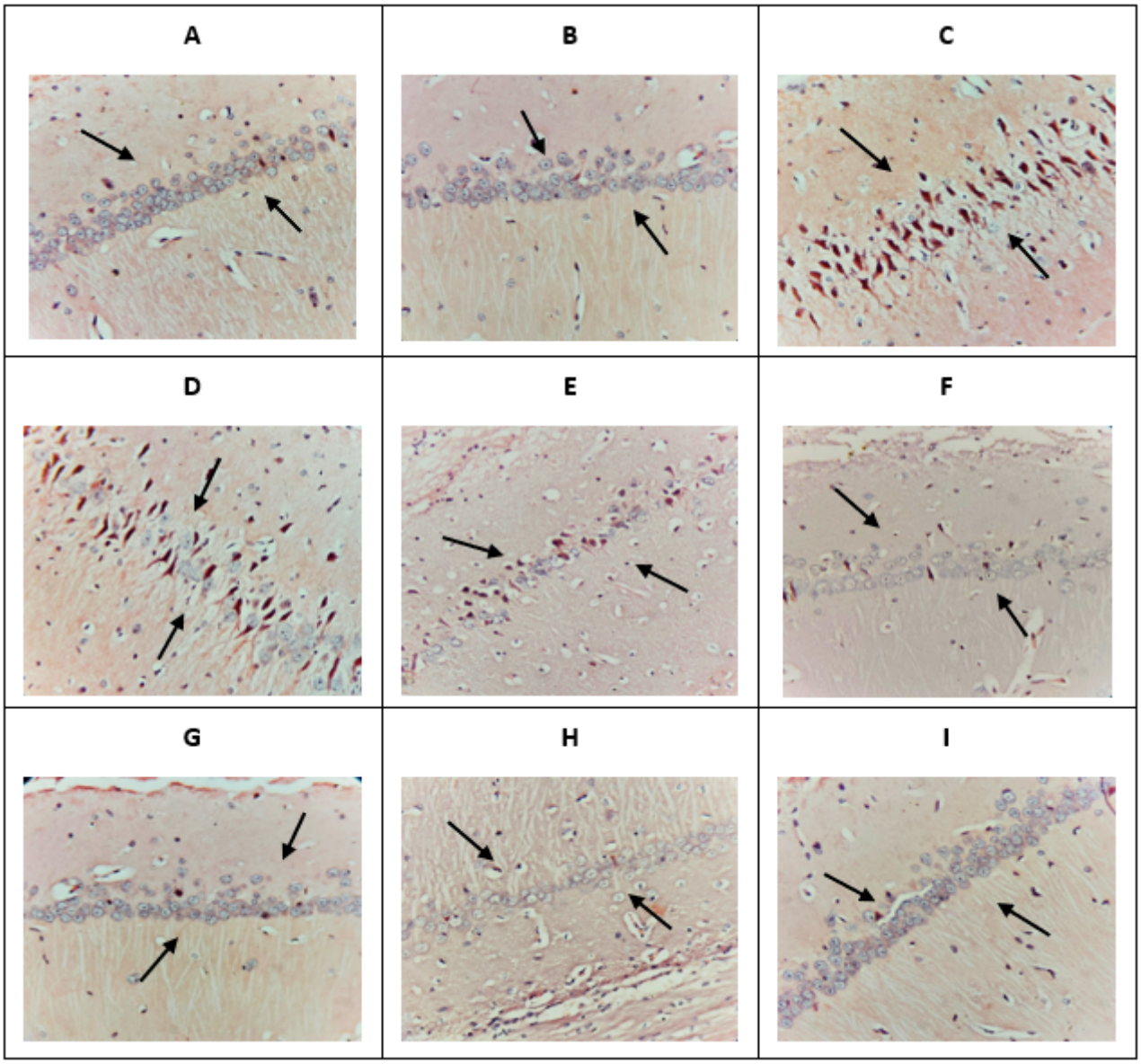

Figure 11

Histological analysis of CA1 region of hippocampus with Congo red staining after 8 weeks of experimental protocol at 40X magnification (100 micrometre). **(A)** Control animals showed healthy neurons with no deposition of Aβ. **(B)** Sham group animals also represented intact neurons with no Aβ accumulation. **(C)** STZ infused toxic group animals represented neuronal damage with extensive deposition of Aβ when compared to control and sham group **(D)**STZ + Lina. 0.513mg/kg treatment group does not show any significant change when compared to STZ infused toxic rats ($p > 0.05$). **(E)** Treatment group with Lina. 3mg/kg represented moderate accumulation of Aβ with neurodegeneration as compared to toxic rats and **(F)** Lina. 5mg/kg treatment group showed significant decrease in the Aβ deposition when compared to STZ group. **(G)**Lina. Per se, **(H)** STZ + DNP group and **(I)** DNP per se showed intact neurons with no accumulation of Aβ. STZ: Streptozotocin; Lina: Linagliptin; DNP: Donepezil.

Supplementary Files

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- Highlights.docx