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Neuroprotective role of Indirubin-3′-monoxime, a GSKβ inhibitor in high fat diet induced cognitive impairment in mice

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ABSTRACT

Recent studies have highlighted that diabetes mellitus (DM) is a strong risk factor for Alzheimer's disease (AD). Insulin resistance and/or hyperinsulinemia is one of the main characteristics of type 2 DM. Numerous epidemiological studies have demonstrated that insulin resistance contributes to AD pathogenesis. However the molecular mechanisms of association between these still remain elusive. Among the various possible mechanisms, the GSK-3β activity has been reported to be impaired in insulin-resistance, type 2 DM and AD. Thus, the present study was designed to explore the neuroprotective role of GSK3 β inhibitor, Indirubin-3′-monoxime (IMX) in insulin resistance induced cognitive impairment. Further, we have explored the possible molecular mechanism involved in cognitive impairment associated with insulin resistance. The mice subjected to high fat diet exhibited characteristic features of insulin resistance viz. increased serum glucose, triglycerides, cholesterol, insulin levels and impaired spatial learning and memory ability along with reduced brain insulin level, elevated oxidative stress and acetylcholinesterase (AChE) activity. The observed changes occurred concurrently with reduced brain derived neurotrophic factor. In contrast, the mice treated with IMX showed a significant reduction in plasma glucose, triglycerides, cholesterol, insulin levels and improvement in learning and memory performance, attenuated the oxidative stress and AChE activity. Moreover, IMX dose dependently augment the brain insulin and BDNF levels in HFD fed mice. Based upon these findings it could be suggested that GSK3 β inhibition could prove to be beneficial in insulin resistance induced cognitive deficit and this neuroprotection could be the result of enhanced BDNF based synaptic plasticity.

1. Introduction

The continued extension of human lifespan in modern society has led to a progressive increase in the incidence of age related neurodegenerative diseases such as Alzheimer's disease (AD). Recent studies have suggested that diabetes mellitus (DM) is a strong risk factor for AD and diabetic patients have a significantly higher risk of developing AD [1]. However, the precise molecular links between DM and AD still remain elusive. Many studies suggest that the risk of cognitive decline and neurodegeneration is increased not only in diabetic patients, but also in patients with prediabetic and metabolic syndrome [2]. Both diabetes and prediabetic conditions, are characterized by insulin resistance. Numerous studies have reported that insulin resistance contributes to AD pathogenesis [3]. Recently it has been demonstrated that peripheral insulin resistance influence central insulin resistance with reduced brain insulin level and impaired cognition [4]. This clinical study demonstrate that post mortem brain tissues from AD patients showed lesser activation of insulin signaling pathways when stimulated with insulin as compared to persons that don't have AD, suggesting impaired insulin signaling in AD [4]. In addition, intranasal insulin administration has been reported to improve learning and memory in AD patients [5], suggesting the role of insulin signaling in cognitive functions. Glycogen synthase kinase-3β (GSK3β) has been well known to play a leading role in the cascade of events initiated by insulin signaling [6]. Numerous studies have reported up-regulation in expression as well as increased activity of GSK3β in the frontal cortex and hippocampus of AD patients [7–8]. Furthermore, dysregulation of GSK 3β activity has been reported in insulin resistance, type 2 DM, neurodegeneration and it has been suggested that over-activity of GSK3β accounts for memory impairment, tau hyper-phosphorylation and increased amyloid-β (Aβ) production; all of which are hallmark characteristics of AD [6]. Conversely, treatment with GSK 3β inhibitors has been reported to prevent Aβ accumulation and tau hyperphosphorylation in transgenic mice over-expressing GSK3β [9]. Based on the aforementioned findings, it might be possible that

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GSK3β inhibition could result in cognitive improvement associated with insulin resistance. Therefore, the present study was designed to explore the role of GSK-3β inhibitor, IMX, in high fat diet (HFD) induced cognitive deficit in mice.

2. Materials and methods

2.1. Animals and study design

Male mice (5–6 weeks old) were randomly assigned into five groups (n = 10). Group 1: received normal pellet diet (NPD); Group 2: received a HFD; Group 3–5 received HFD for 8 weeks followed by IMX treatment (0.1, 0.2 and 0.4 mg/kg i.p, respectively) once daily for 1 week. IMX (Sigma Aldrich, USA) was dissolved in (2.5% v/v) DMSO in saline. The mice in NPD and HFD groups received an equivalent volume of vehicle (2.5% v/v DMSO in saline). The composition of HFD was similar as described by Srinivasan [10]. Doses of IMX were selected based on the reports available in literature [11]. Mice were kept under standard husbandry conditions (22 ± 1 °C and 60% humidity) and maintained on a 12/12-h light/dark schedule with free access to food and water for 8 weeks. Body weight was recorded weekly throughout the experimental period. All experimental procedures were approved by the Institutional Animal Ethics Committee of BITS, Pilani, India. The treatment schedule and intervals for various parameters are presented in Fig. 1A.

2.2. Serum metabolic parameters

Serum triglycerides (TGs) and total cholesterol (TC) levels were measured using UV-1700 Spectrophotometer, Shimadzu, Japan. The concentration of TGs and TC were measured using commercial kits (Coral clinical systems, India).

2.3. Serum glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and homeostatic model assessment of beta cell function (HOMA-β)

Fasting serum levels of glucose were measured by using commercial kit (Spinreact, Spain). Insulin was measured by enzyme linked immunosorbent assay kit from Crystal Chem Inc., USA. Insulin sensitivity was estimated using the HOMA index which is an arithmetic way of deriving indices of peripheral tissue insulin resistance and beta cell function (HOMA-β). HOMA-IR and HOMA-β was derived using online calculator at https://www.dtu.ox.ac.uk/homacalculator [12].

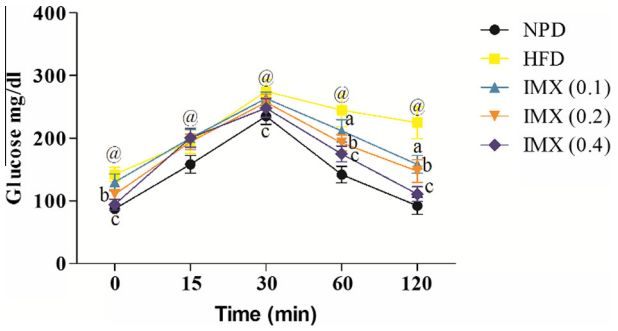

Fig. 1B. Effect of IMX on OGTT. ($^{@}P < 0.001$ vs NPD); {$^{a}P < 0.001$, IMX 0.1 mg/kg vs HFD}; {$^{b}P < 0.001$, IMX 0.2 mg/kg vs HFD}; {$^{c}P < 0.001$, IMX 0.4 mg/kg vs HFD}, (n = 6); (IMX-Indirubin-3′-monoxime).

2.4. Oral glucose tolerance test (OGTT)

In OGTT, the mice received (20% w/v) glucose solution (2 g/kg) through oral gavage after overnight fasting. Blood glucose level estimation was done from the tail using Accu check glucometer at 0, 15, 30, 60 and 120 min after glucose administration [12].

2.5. Behavioral assessment

2.5.1. Spontaneous locomotor activity (SLA)

Each animal was tested for SLA after the treatment phase. Animals were observed over a period of 10 min in a square closed arena (30 × 30 cm^2) equipped with infrared light sensitive photocells using a digital actophotometer (INCO, India) and the activity counts were recorded [13].

2.5.2. Passive avoidance task

Briefly, the apparatus consisted of two compartments. During the acquisition trial, each mice was placed in the illuminated compartment. After 60 s of habituation a guillotine door was opened and the initial latency to enter the dark chamber was recorded. As soon as the mice entered the dark compartment, the door was closed and an electric foot shock was delivered. The mice was then removed and placed back into its home cage. Retention latency was measured 24 h later in the same way as in the acquisition trial, but foot shock was not delivered [13]. The detailed procedure is supplied in SI Materials and Methods.

2.5.3. Spatial navigation task

Spatial learning and memory was assessed in Morris water maze task (MWM) [14]. Mice received a training session consisting of 4 trials/session for 4 days. Each trial was having a ceiling time of 60 s. If the mice failed to locate the hidden platform within 60 s, it

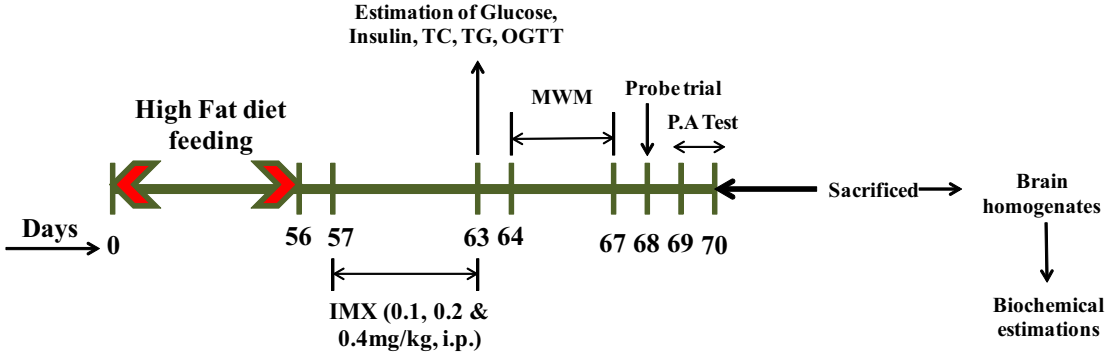

Fig. 1A. The design of treatment schedule and the intervals for estimation of various parameters. MWM = Morris water maze; P.A = Passive avoidance task; TC = total cholesterol; TG = triglyceride.

was gently placed on the platform and allowed to remain there for 30 s. The results were analyzed by using ANY-maze video tracking system (Stoelting, USA). For more details see SI Materials and methods.

2.5.4. Probe trial

Twenty-four hour after the last navigation trial, each mice was subjected to a probe trial. In probe trial, the hidden platform was removed and the time spent by each mice in the target quadrant, which previously contained the platform was recorded [13]. For details see SI Materials and methods.

2.6. Biochemical parameters

2.6.1. Brain homogenate preparation

Mice were sacrificed by decapitation at the end of behavioral study. The brains were removed and rinsed with ice-cold (0.9% w/v) isotonic saline. The brains were then homogenized with ice cold 0.1 M phosphate buffer (pH 7.4) in a volume 10 times (w/v). The homogenate was centrifuged at 10,000*g* for 15 min (4 °C) and aliquots of supernatant were separated and used for biochemical estimations.

2.6.2. Protein determination

Protein content in brain samples were measured by the method of Lowry [15] using bovine serum albumin (BSA) (1 mg/ml) as a standard.

2.6.3. Acetylcholinesterase (AChE) activity

The quantitative measurement of AChE activity was performed as described by Ellman [16]. The reaction mixture consists of supernatant, phosphate buffer (pH 8), acetylthiocholine iodide and DTNB. The change in absorbance was measured immediately at 412 nm spectrophotometrically. The detailed procedure is supplied in SI Materials and Methods.

2.6.4. Oxidative stress markers and antioxidant enzyme levels

The quantitative measurement of oxidative markers such as malondialdehyde (MDA) and nitrite was done using methods described by Wills [17] and Green [18], respectively. The level of endogenous antioxidant, reduced glutathione (GSH) was measured using the method described by Ellman [19]. The detailed procedures of these methods are supplied in SI Materials and Methods.

2.6.5. Brain Insulin levels

The amount of insulin was determined by ELISA Kit in the brain tissue homogenate as per manufacturer's instructions.

2.6.6. Brain Derived Neurotrophic Factor (BDNF)

BDNF level was determined by using a commercially available ELISA kit (Boster Biological Tech. Co., LTD., CA, USA) as per manufacturer's instructions.

2.6.7. Assessment of histological changes

The brains were rapidly removed and fixed by immersion in 10% formalin. Subsequently they were embedded in paraffin wax, cut into 5 μm thick sections and stained with haematoxylin eosin stain. Hippocampal CA1 and DG regions of brain were examined under bright field illumination using "Optika TCB5" microscope (Optika Research Microscope, Italy) at total of 40X and 100X.

3. Statistical analysis

The results are expressed as mean ± S.D. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using statistical GraphPad Prism software (version 5.0, La Jolla, CA, USA). The results of OGTT was analyzed using two way ANOVA followed by bonferroni's post hoc test. $P < 0.05$ was set to be statistically significant.

4. Results and discussion

The present study demonstrates the neuropharmacological effects of GSK3β inhibitor, IMX, in HFD induced cognitive deficit in mice. The results obtained in the present study suggests that treatment with IMX dose dependently reversed the cognitive impairment and combat the elevated oxidative stress markers in HFD fed mice.

The HFD induced rodent model is widely used to study the pathophysiology of obesity; insulin resistance and its complications [20–22]. In the present study, HFD fed mice gained significantly more weight than the NPD fed mice after 8 weeks of diet feeding (1). In addition, there was a significant increase in serum parameters, characteristic of insulin resistance viz. glucose ($F_{(4,29)} = 28.72$; $P < 0.001$), TGs ($F_{(4,29)} = 64.46$; $P < 0.001$), TC ($F_{(4,29)} = 39.47$; $P < 0.001$) and insulin levels ($F_{(4,29)} = 38.85$; $P < 0.001$) in HFD fed mice (Table 1). Treatment with IMX, reduced body weight although not statistically significant at lower doses, but at 0.4 mg/kg, the reduction was statistically significant ($P < 0.05$) as compared to HFD. Moreover, IMX dose dependently lowered the serum glucose, TGs, TC and insulin levels in HFD fed mice. These results indicate the beneficial effects of IMX treatment on insulin resistance state.

The HFD fed mice showed marked glucose intolerance as assessed by OGTT. Blood glucose levels were found to be significantly higher in HFD fed mice as compared with NPD mice at 0, 15, 30, 60 and 120 min after oral glucose administration. Treatment with IMX results in dose dependent improvement in glucose tolerance as significant reduction in blood glucose was observed at 0, 30, 60 and 120 min after glucose administration ($P < 0.001$; Fig. 1B).

The results of HOMA-IR of HFD fed mice were significantly higher than that of NPD fed mice ($P < 0.001$). IMX treatment significantly decreased HOMA-IR levels as compared to HFD group ($F_{(4,29)} = 41.76$; $P < 0.001$; 1). This data suggests that insulin

Table 1
Effect of IMX on body weight, glucose, triglyceride, cholesterol, insulin, HOMA-IR and HOMA-β.

Parameter	Normal diet	High fat diet	HFD + IMX (0.1)	HFD + IMX (0.2)	HFD + IMX (0.4)
Body weight (g)	29.90 ± 4.38	37.6 ± 4.88$^{@}$	34.8 ± 3.80	34.1 ± 4.67	31.7 ± 4.42*
Glucose (mg/dl)	81.17 ± 9.88	138.0 ± 14.35$^{@}$	129.7 ± 13.44	110.2 ± 7.985$^{*,\#}$	90.83 ± 8.40$^{*\$}$
Triglycerides	53.67 ± 13.13	201.7 ± 22.23$^{@}$	165.8 ± 19.11*	143.7 ± 11.59*	113.8 ± 17.01$^{*\$}$
Total cholesterol	114.3 ± 20.56	275.2 ± 29.41$^{@}$	222.3 ± 26.28*	174.2 ± 23.96$^{*\#}$	126.7 ± 29.48$^{*\$}$
Insulin (pmol/l)	74.25 ± 5.88	180.8 ± 23.16$^{@}$	150.7 ± 18.79*	135.2 ± 17.28*	108.0 ± 7.58$^{*\$}$
HOMA-IR	1.20 ± 0.17	3.45 ± 0.54$^{@}$	2.88 ± 0.27	2.26 ± 0.27$^{*\#}$	1.65 ± 0.32$^{*\$}$
HOMA-β	130.0 ± 5.17	66.67 ± 12.32$^{@}$	78.67 ± 5.50	103.8 ± 5.74$^{*\#}$	122.2 ± 15.72$^{*\$}$

($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD), ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 6$); (IMX-Indirubin-3′-monoxime).

resistance was prevented in the IMX treated groups. Moreover, the pancreatic β-cell function (HOMA-β) in HFD fed mice was found to be significantly lower than that of NPD fed mice. Treatment with IMX dose dependently improved the β-cell functioning in HFD mice ($F_{(4,29)} = 45.59$; $P < 0.001$; Table 1).

The behavioral data obtained in the present study indicated cognitive impairment in HFD fed mice, which is in agreement with previous studies [21–22]. The changes in locomotor activity have been suggested to modulate performance in learning and memory tasks [13]. However, in present study, no significant difference was observed between any of the groups suggesting no effect whatsoever of HFD or IMX on the locomotion activity of animals ($P > 0.05$) [$F_{(4,49)} = 0.70$] (Fig. 2A).

MWM has been widely used to evaluate spatial learning and memory in rodents since its first introduction in 1984 [11]. The mean escape latency (EL) to reach the target platform reduced gradually in all the groups during 4 days (day 64–67) of training. The mean EL was significantly ($P < 0.001$) higher in the HFD fed mice as compared with the NPD fed mice on days 66 and 67, showing poorer learning performance. The increased EL in the HFD group was significantly attenuated by IMX treatment at dose (0.4 mg/kg) on day 66 ($P < 0.01$) and at (0.2 and 0.4 mg/kg) on day 67 ($P < 0.001$) (Fig. 2B).

During the probe trial, HFD fed mice failed to remember the precise location of the platform, spending significantly lesser time in the target quadrant ($P < 0.001$, Fig. 2C). Whereas, HFD fed mice treated with IMX spent significantly more time in the target quadrant indicating improved consolidation of memory [$F_{(4,49)} = 17.01$; $P < 0.001$]. Although, this improvement was not significant at lowest dose of IMX (0.1 mg/kg), but at higher doses the effect was statistically significant.

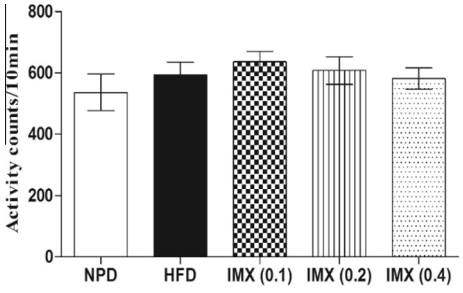

Fig. 2A. Effect of IMX on SLA. Values are expressed as mean ± S.D. ($n = 10$); (IMX-Indirubin-3′-monoxime).

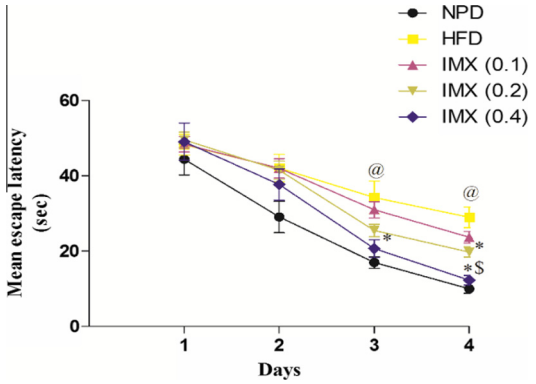

Fig. 2B. Effect of IMX on MWM task. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 10$); (IMX-Indirubin-3′-monoxime).

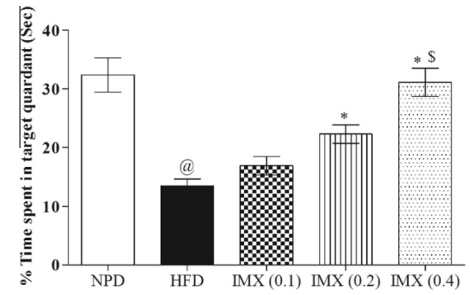

Fig. 2C. Effect of IMX on probe trial. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 10$); (IMX-Indirubin-3′-monoxime).

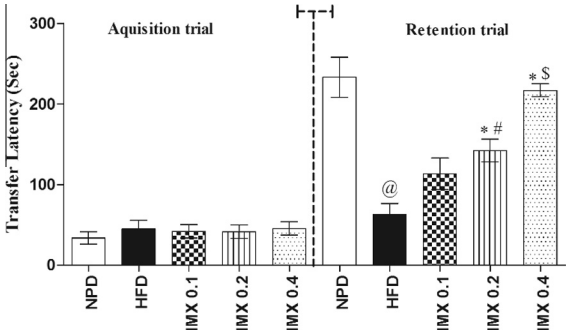

Fig. 2D. Effect of IMX on passive avoidance task. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 10$); (IMX-Indirubin-3′-monoxime).

Passive avoidance learning refers to the learned inhibition of behavior in order to avoid punishment [13]. The initial latency in the acquisition trial did not differ significantly ($P > 0.05$) between all the groups. Twenty-four hours later, the mice were tested again and it was found that the retention latency was significantly decreased in the HFD fed group as compared with NPD fed mice ($P < 0.001$) (Fig. 2D). Decreased retention latency in the HFD fed mice was significantly attenuated by IMX treatment in a dose dependent manner ($F_{(4,49)} = 29.19$; $P < 0.05$).

Along with the cognitive impairments observed in these behavioral tasks, the HFD feeding also results in significant morphological changes in DG and CA1 region of hippocampal neurons. The H&E stained sections showed healthy neurons in DG and CA1 region in the NPD fed mice (Fig. 2E, Panel A and F). Healthy neurons were robust in shape, had a spherical or slightly oval nucleus and a single large nucleolus with clear visible cytoplasm as indicated by yellow arrows. The HFD results in significant neuronal degeneration in CA1 and DG regions of hippocampus. Decreased neuronal density and increased pyknotic neurons were observed in DG and CA1 regions of HFD mice. The pyknotic neurons were darkly stained with no nucleus or visible nucleolus and few of the cells were shrunken and sickle shaped in brain sections of HFD fed mice as indicated by red arrows (Panel B and G). Treatment with IMX (0.1 and 0.2 mg/kg) (Panel C-D and H-I) attenuated HFD induced cell loss and pyknotic cells but some degenerating cells with morphological changes were still observed at these lower doses. However, there was marked improvement in neuronal density and reduction in pyknotic neurons following IMX (0.4 mg/kg), indicating the dose dependent neuroprotective actions of IMX (Panel E and J).

These behavioral and morphological changes could be well co-related with the elevated AChE activity observed in brain

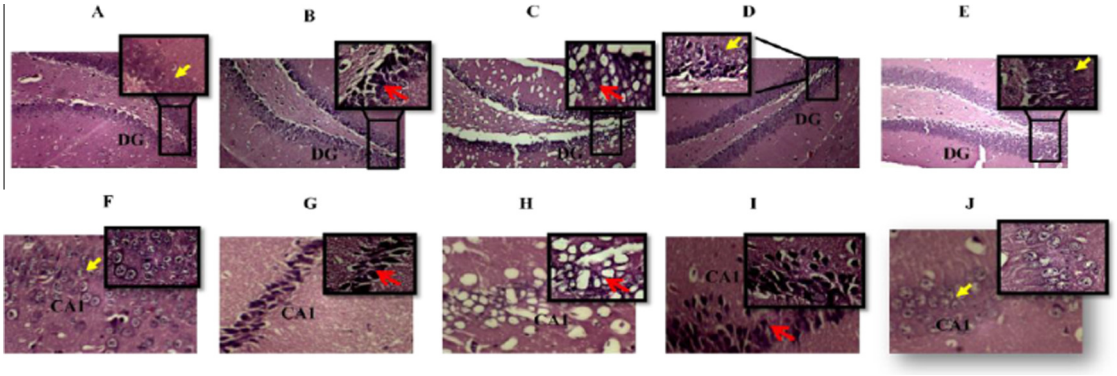

Fig. 2E. Effect of IMX on dentate gyrus and CA1 region. Panel A–E shows photomicrographs of Dentate gyrus (DG); Panel F–J shows photomicrographs of CA1 regions of hippocampus.

homogenates of HFD fed mice (Fig. 3A). Acetylcholine (Ach) as a neurotransmitter has been well documented for its role in learning and memory [23]. AChE is the enzyme responsible for the breakdown of Ach into choline and acetate. Increased AChE activity has been observed around Aβ-plaques in AD patients [24]. In the present study, the AChE activity was found to be elevated in HFD fed mice as compared NPD fed mice [$F_{(4,29)} = 49.68$; $P < 0.001$] (Fig. 3A). IMX treatment dose dependently attenuate HFD induced elevation in AChE activity ($P < 0.001$). These results suggest that the pharmacological inhibition of GSK3β could improve the memory performance in HFD fed mice by enhancing cholinergic functions.

Oxidative stress appears to be a deleterious factor leading to insulin resistance, β-cell dysfunction and eventually, type 2 DM [25]. Furthermore, increased oxidative stress markers and reduced endogenous antioxidant enzyme level, such as GSH has been reported in post mortem AD brains [26–27]. In the present study, increased level of oxidative stress markers such as MDA, nitrite and reduction in GSH level was reported in brain homogenates of HFD fed mice ($P < 0.001$). Treatment with IMX dose dependently attenuated MDA ($F_{(4,29)} = 62.35$; $P < 0.001$; Fig. 3B) and nitrite levels ($F_{(4,29)} = 36.71$; $P < 0.001$; Fig. 3C) and restored GSH level ($F_{(4,29)} = 24.79$; $P < 0.001$; Fig. 3D) in HFD fed mice. These results are in line with previous studies indicating reduced oxidative stress markers as a result of GSK3 inhibition [28].

To reveal the effects of peripheral insulin resistance on central insulin signaling we measured the level of brain insulin and found that insulin resistance (with high levels of insulin in the body) paradoxically leads to lower-than-normal levels of insulin in the brains of HFD fed mice ($P < 0.001$), which could possibly be the reason for observed cognitive deficit in these mice. This data is consistent with results obtained from a recent clinical study indicating insulin resistance along with reduced brain insulin levels and insulin signaling in AD patients [29]. The results obtained in this study can further be warranted by the observation that intranasal administration of insulin results in learning and memory in AD [29]. Thus, restoration of brain insulin level could be an alternative therapeutic strategy for patients with AD, especially in those with DM. In the present study, treatment with IMX augmented the brain insulin levels in HFD fed mice at (0.2 and 0.4 mg/kg) but not at lowest dose (0.1 mg/kg) [$F_{(4,9)} = 38.60$] (Fig. 3E).

The association between GSK3β and BDNF has been well reported in AD such as, reduction in BDNF level has been reported in hippocampal and cortical tissues of AD patients and is associated with increased GSK3β activity [30–31]. Further, it has been proposed that BDNF activation of TrkB receptors is responsible for activation of the PI3K/Akt pathway followed by GSK3β inhibition

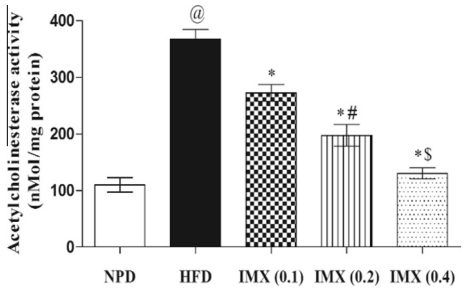

Fig. 3A. Effect of IMX on AChE activity. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 6$); (IMX-Indirubin-3′-monoxime).

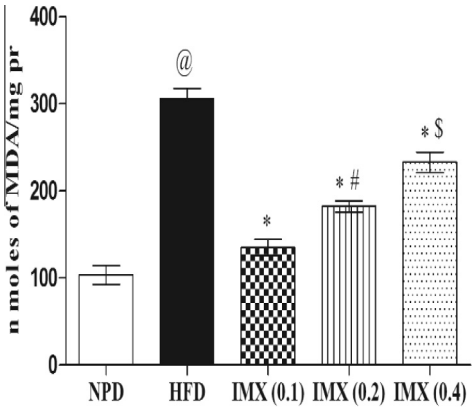

Fig. 3B. Effect of IMX on Malondialdehyde. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 6$); (IMX-Indirubin-3′-monoxime).

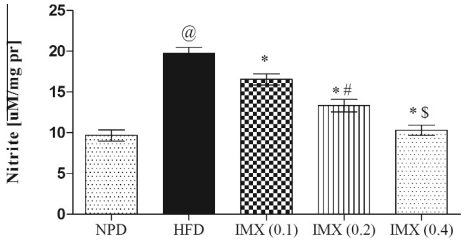

Fig. 3C. Effect of IMX on nitrite. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 6$); (IMX-Indirubin-3′-monoxime).

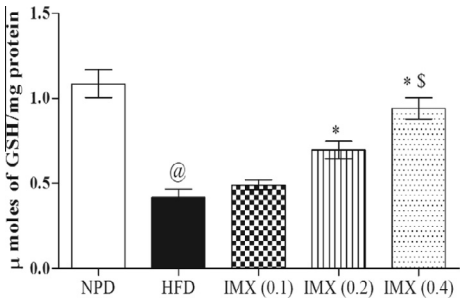

Fig. 3D. Effect of IMX on reduced glutathione. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 6$); (IMX-Indirubin-3′-monoxime).

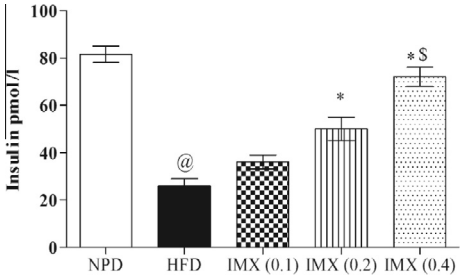

Fig. 3E. Effect of IMX on brain insulin. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 2$); (IMX-Indirubin-3′-monoxime).

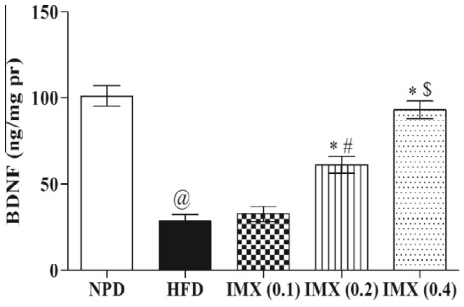

Fig. 3F. Effect of IMX on BDNF. ($^{@}P < 0.001$ vs NPD); ($^{*}P < 0.001$ vs HFD); ($^{\#}P < 0.001$ vs IMX (0.1 mg/kg); ($^{\$}P < 0.001$ vs IMX 0.2 mg/kg), ($n = 2$); (IMX-Indirubin-3′-monoxime).

[30]. In the present study, we found reduced BDNF level in HFD fed mice as compared with NPD fed mice ($P < 0.001$). Treatment with GSK3β inhibitor, IMX, significantly and dose dependently ameliorate the HFD induced decrease in BDNF level ($F_{(4,9)} = 47.13$; $P < 0.001$; Fig. 3F), which could possibly improve neuronal plasticity and results in neuroprotection.

Based on the aforementioned findings, it can be concluded that IMX treatment significantly and dose dependently improved the HFD induced cognitive deficit. The observed beneficial effects of IMX may be related to its actions on improved glucose tolerance, combating insulin resistance or oxidative stress. Moreover, these neuroprotective and cognitive enhancing actions of IMX could also be attributed to improved brain insulin and BDNF levels. Thus, it would be safe to conclude that the GSK3 β inhibition would be therapeutic in cognitive impairment associated with insulin resistance and type 2 DM.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.09.034.

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