International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2019.724.730

Research Article
Neuroprotective Role of Vitamin D3 Against Insulin Resistance and Diabetic Induced Memory Dysfunction in Rats

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Abstract

Background and Objective : Diabetes is a risk factor for cognitive dysfunction. Diabetes associated with impairment of insulin signaling and oxidative stress pathway in the brain. This study aimed to evaluate the neuroprotective role of vitamin D3 against diabetic induced memory dysfunction in rats of Alzheimer's disease. **Materials and Methods:** Type 3 diabetes were induced by a high-fat diet plus streptozotocin in Wistar rats. Diabetic rats were divided into six subgroups, positive control (non-treated), vitamin D3 groups (100,500 and 1000 IU/kg/day), vitamin D3 plus rivastigmine and rivastigmine monotherapy. Treatment started after the onset of hyperglycemia for 4 months. Novel object recognition test was used to assess cognitive after treatment followed by estimation of insulin, beta-amyloid-42, malondialdehyde, glutathione and superoxide dismutase level in the hippocampus by ELIZA kits. **Results:** Vitamin D3 significantly alleviated cognitive deficits ($p<0.001$) in novel object recognition test and further resulted in marked elevation ($p<0.001$) in hippocampal insulin level, which in turn reduced the accumulation of beta-amyloid-42 ($p<0.01$) as well as attenuated of oxidative stress via significantly elevated of glutathione level and marked reduction of malondialdehyde and superoxide dismutase level compared to positive control. **Conclusion:** These findings suggested that vitamin D3 treatment could alleviate cognitive deficit associated with type 3 diabetes due to its anti-oxidative and anti-diabetic potential.

Key words: Vitamin D3, cognitive dysfunction, anti-diabetic potential, rivastigmine monotherapy, neuroprotective, cognitive deficit

Received: January 13, 2019 Accepted: April 18, 2019 Published: August 01, 2019

Citation: Yahya A. Al-Zahrani, Mai A. Alim A. Sattar, Sameer E. Alharthi and Huda. M. Alkreathy, 2019. Neuroprotective role of vitamin D3 against insulin resistance and diabetic induced memory dysfunction in rats. Int. J. Pharmacol., 15: 724-730.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Alzheimer disease (AD) is a chronic neurodegenerative disease featured by progressive loss of memory capacity and cognitive function required daily for activities performance[1]. The neuropathological features of AD are deposition of β-amyloid as senile plaque and aggregation of tau protein to form neurofibrillary tangles[2]. Unfortunately, to date, there is no precise statistics on the prevalence of AD in Saudi Arabia. Saudi Alzheimer's Disease Association estimated that there are at least 50,000 patients in the Kingdom living with AD, most of them are women[3].

Many studies reported that the risk for AD developing is increased by 50-75% in diabetic patients compared to non-diabetic one[4-6]. Moreover, based on "ADPR" study by mayo clinic, 80% of AD patients suffer from T2DM or impaired fasting blood glucose[7]. Multiple studies suggested that AD is linked to impairment of insulin signaling pathway and glucose metabolism cascade in the brain and this theory caused some researchers to indicate to Alzheimer disease as type 3 diabetes or condition of brain resistance to insulin[8].

Insulin signaling is essential for regulations of numerous neuronal functions such as; synaptic activities, cognitive abilities, learning and memory. Moreover, insulin prevents intracellular Aβ deposition via elevates Aβ extracellular secretion and inhibits GSK-3β phosphorylation to block tau protein aggregation. Thus, recent studies concluded that brain insulin resistance (IR) lead to AD[9,10]. Also, diabetes and IR induce oxidative stress and decrease antioxidant defenses[11]. The impact of reactive oxygen species (ROS) on the development of AD confirmed by recent studies[12].

Vitamin D is a well-known steroid hormone which plays an important role in controlling bone levels of calcium, phosphorus and overall mineralization. Vitamin D deficiency defined by the American Association of clinical endocrinologists as 25 (hydroxy) vitamin D level less than 20 ng mL^{-1} and insufficiency of vitamin D as 25-(hydroxyl) vitamin D level between 2-29 ng mL^{-1}. Etgen *et al.*[13] concluded that patients whose have vitamin D deficiency have a twofold increase in risk for the impairment of cognitive function. Recently, one study conducted in an animal by Latimer *et al.*[14] resulted that the vitamin D for long term use may inhibit a cognitive decline in elder rats. There is several evidence supporting the antioxidant activity of vitamin D3 in the oxidative stress diabetes[15,16]. The results in some experimental studies implied that vitamin D3 administration in diabetic mice helps to diminish the ROS formation by the suppression of the gene expression of NADPH oxidase[17,18].

Although the impact of vitamin D3 on diabetes and cognitive decline have been independently reported, the possible beneficial effect of vitamin D3 on type 2 diabetic-associated cognitive decline in rats marked by brain insulin resistance has not yet been evaluated[19].

The current study aimed to evaluate the beneficial role of vitamin D3 against a model of type 3 diabetic induced memory dysfunction in rats. Furthermore, the underlying mechanism involving suppressing insulin resistance and oxidative stress pathway were also investigated.

MATERIALS AND METHODS

Experimental location and duration: This study was carried out in the labs of the King Fahd Center for Medical Research (KFCMR), King Abdul Aziz University (KAU), Jeddah, Saudi Arabia from January-May, 2018.

Ethical statement: All procedures applied in the current study were strictly conducted according to the ethical guidelines of medical ethics committee of the King Abdul-Aziz University (KAU). The animal protocol was approved by the research ethics committee at KAU (Approval number 488-17).

Chemicals and reagents: Streptozotocin and rivastigmine were obtained from Sigma Aldrich (CO., Saint Louis, MO, USA) as a white powder. Oral drops of cholecalciferol (Vitamin D3 4500 IU mL^{-1}, Novartis International AG, Basel, Switzerland) was used in this study. Rat ELISA kits for measurement of insulin, Beta-Amyloid peptide (Aβ), malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) were purchased from MyBiosource, Inc. (Southern California, San Diego, USA.

Drugs doses and preparations: Streptozotocin (STZ) was freshly prepared before use within 10 min by dissolving in 0.1 M sodium citrate buffer (pH to 4.5) and used intraperitoneally at a dose[20] of 40 mg kg^{-1} b.wt., Rivastigmine was prepared daily in sterile water and orally administered in a dose[21] of 1 mg/kg/day. Three graded doses of vitamin D3; 100,500 and 1000 IU/kg/day were selected and given orally[22]. High-fat diet (HFD) was prepared by using saturated animal fat (*beef tallow*) to form 40% kcal of total daily fatintake[20].

Animal and housing: Eighty four male albino rats, aged 6-9 weeks of average weight±SD (208 g±8.63) were

purchased from the experimental animal unit of King Fahd Center for Medical Research (KFCMR), KAU and housed in standard animal laboratory conditions; temperature ranged between 24-26°C, relative humidity was between 50-70% and a 12 h light/dark cycle. All animals were allowed to one week to acclimatize in animal housing conditions before being used for the experiment. All rats were fed with a regular diet and drinking water during the adapting period.

Experimental design: Rats were divided into regular diet group (negative control, n = 12) and high fat diet (HFD) group (n = 72). Type 3 diabetic was induced one month later of HFD feeding, all 72 rats in HFD group intraperitoneally injected after 12 h of fasting with previously prepared streptozotocin (40 mg kg^{-1}) followed by overnight administration of oral 5% glucose solution to prevent the hypoglycemic shock. Three days after streptozotocin administration, a rat with blood glucose levels >200 mg dL^{-1} measured by a glucometer (Accu-Chek, Roche, Basel, Switzerland) was considered diabetic rat and selected for this study[23]. All 84 rats grouped into 7 groups:

- **Group I:** Negative control rats: Injected with citrate buffer (pH 4.5) (1 mL kg^{-1}, i.p.)
- **Group II:** Non-treated T3D rats-positive control
- **Group III, Group IV and Group V:** These were T3D rats received oral Vitamin D3 100, 500 and 1000 IU kg^{-1}, respectively once daily
- **Group VI:** T3D rats received oral 500 IU kg^{-1} of Vitamin D3 once daily plus rivastigmine 1 mg/kg/day
- **Group VII:** T3D rats received oral rivastigmine 1 mg/kg/day. The treatment period lasted for sixteen

Assessments of cognitive function by novel object recognition (NOR) test: In NOR test, if memory is working normally, the rat will explore the novel object for a longer time compared to the familiar object. In contrast, if the exploration time for a familiar and novel object is similar, this indicates a memory deficit[24]. In the current study, each rat was exposed to a cage with two identical objects for 3 min then returned to its home cage (training phase). After 15 min inter-trial interval, the rat explored the cage in the presence of one familiar object and a novel object for 3 min (testing phase). The time spent for exploring each object in the testing phase was recorded. Exploration was defined as directly attending the object with the head while licking, sniffing or touching the object with its nose. The discrimination index (DI) data was analyzed by calculating a discrimination index (DI) for each rat, which was expressed as[24,25]:

$$DI = \frac{\text{Exploration of a novel object (EN)} - \text{An exploration of a familer object (EF)}}{EN+EF}$$

Hippocampus preparation and biochemical analysis: All rats were fasted overnight and euthanized by gentle decapitation under diethyl ether anesthesia. The rat hippocampus was immediately dissected, placed on ice, washed in cold 0.9% normal saline and weighed. Rat hippocampus subsequently homogenized in sodium phosphate buffer (pH 6.9) with a glass homogenizer on the ice and the homogenate centrifuged at 5,000 g. The clear supernatant obtained was used to estimate the hippocampal levels of insulin, Beta-Amyloid peptide (Aβ), malondialdehyde (MDA), glutathione (GSH) and Superoxide Dismutase (SOD) levels by quantification ELIZA kits., following the company's recommended protocol[26].

Statistical analysis: Statistical analysis was performed using SPSS (Statistical package of social sciences version 23). One-way analysis of variance (ANOVA) and two-way analysis of variance (ANOVA) followed by Tukey HSD *post hoc* test for multiple comparisons. The $p \leq 0.05$ was considered significant.

RESULTS

Effect on discrimination index using the novel object recognition test: The results from Fig. 1 revealed that oral administration of vit. D3 doses 100, 500 and 1000 IU/kg/day, combined vitamin D_3 and rivastigmine and rivastigmine alone have alleviated the acute decline in memory function that occurred in non-treated diabetic rats with a dose-dependent significant increase in discrimination index ($p<0.001$). Moreover, the discrimination index in all vitamin D3 treated groups was significantly increased ($p<0.05$) compared with rivastigmine group.

Effect on Aβ-42 level in hippocampal tissues: β-amyloid peptide (Aβ-42) exhibited twofold higher in non-treated diabetic rats compared with the negative control group (14.60 ± 0.58 vs. 6.20 ± 0.42 pg mL^{-1}, Fig. 2). This over expression was significantly decreased by chronic administration of vit. D3 doses (100, 500 and 1000 IU/kg/day) and vit. D_3 plus rivastigmine, respectively

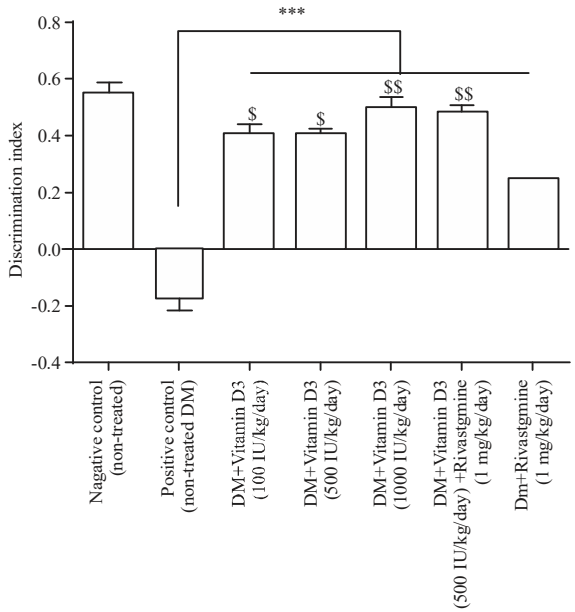

Fig. 1: Effect of vitamin D3 versus rivastigmine on discrimination index by novel object recognition test
***p<0.001 compared with positive control group values, $p<0.05 $$p<0.01 compared with rivastigmine group values, by one-way ANOVA and Tukey HSD *post hoc* test. Data expressed as the mean±SEM; n = 10 rats

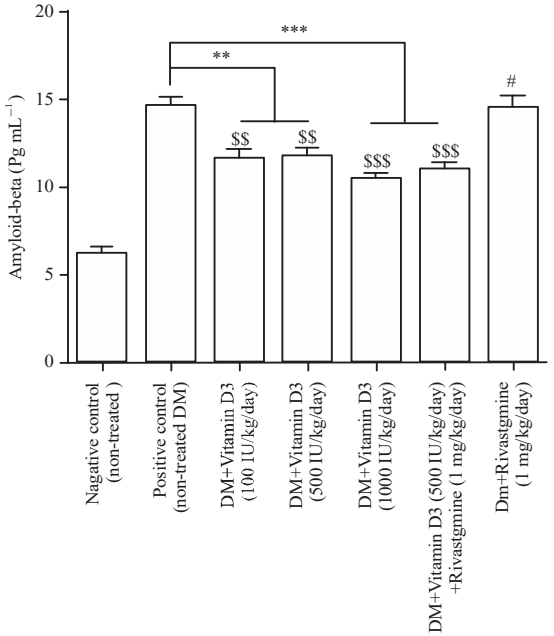

Fig. 2: Effect of vitamin D3 versus rivastigmine on amyloid-beta level in the hippocampal brain tissue by ELIZA
#p>0.05, **p<0.01, ***p<0.001 compared with positive control group values; $$p<0.01, $$$p<0.001 compared with rivastigmine group values; by one-way ANOVA and Tukey HSD *post hoc* test. Data expressed as the mean±SEM; n = 10 rats

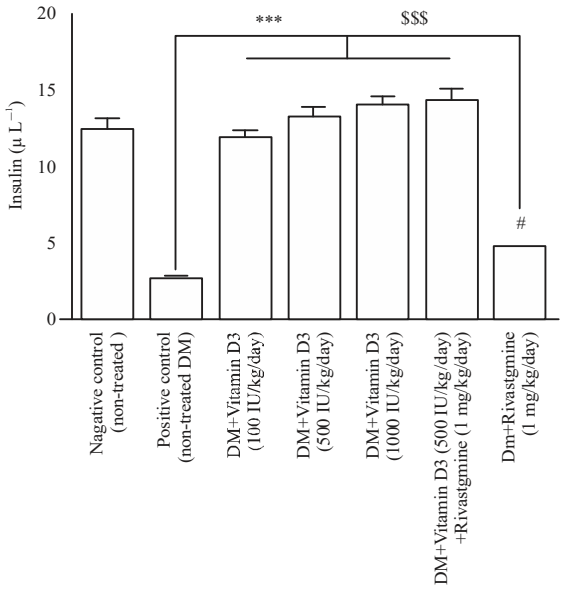

Fig. 3: Effect of vitaminD3 versus rivastigmine on insulin level in the hippocampal brain tissue by ELIZA
#p>0.05, ***p<0.001 compared with positive control group values; $$$p<0.001 compared with rivastigmine group values by one-way ANOVA and Tukey HSD post hoc test. Data expressed as the mean±SEM; n = 10 rats

versus positive control while a non-significant reduction of Aβ-42 level was obtained by administration of rivastigmine alone (p>0.05) as compare to positive control.

Effect on hippocampal insulin level: The results in Fig. 3 showed that hippocampal insulin level was significantly decreased in non-treated diabetic rats compared to negative control (2.52±0.23 vs. 12.26±0.88 μL^{-1}). Also, chronic administration of vitamin D3 doses (100, 500 and 1000 IU/kg/day) and vitamin D3 plus rivastigmine produced a significant dose-dependent elevation of insulin level compared to non-treated diabetic rats.

Effect on oxidative stress markers in hippocampal tissues: The development of oxidative stress in diabetic rats was confirmed by increased of MDA level (0.96±0.02 nmol mL^{-1}), SOD level (104.40±2.66 ng mL^{-1}) and reduced of GSH level (4.34±0.46 µ mL^{-1}) compared to the negative control (MDA; 0.77±0.07 nmol mL^{-1}, SOD; 66.60±7.71 ng mL^{-1}, GSH; 10.42±0.49 µ mL^{-1}) as shown in Table 1.

DISCUSSION

The present work showed that administration of vitamin D3 produced a significant enhancement of cognitive

Table 1: Effect of Vitamin D3 on MDA, SOD and GSH levels in hippocampal tissue of diabetic-induced Alzheimer in rats

Groups	MDA (nmol mL^{-1})	SOD (ng mL^{-1})	GSH (μ mL^{-1})
Diabetic group			
Negative control (non-treated)	0.77± 0.07	66.60±7.71	10.42±0.49
Positive control (non-treated)	0.96± 0.02	104.40±2.66	4.34±0.46
Vit. D_3 (100 IU/kg/day)	0.47±0.07***+	69.60±5.82**+	7.08±0.36*$$$
Vit. D_3 (500 IU/kg/day)	0.55±0.03***+	72.60±6.31**+	7.86±0.39**$$
Vit. D_3 (1000 IU/kg/day)	0.33± 0.14***+	74.00±6.96**+	9.20±0.53***+
Vit. D_3 500 IU/kg/day (+Rivastigmine (1 mg/kg/day)	0.40± 0.06***+	72.40±3.16**+	11.34±0.76***+
Rivastigmine (1 mg/kg/day)	0.40± 0.04**	72.00±2.83**	10.82±0.60***

Values are expressed as the mean±SEM; n = 10 rats. Data were analyzed by one -way ANOVA followed by Tukey HSD *post hoc* test. +p>0.05, *p<0.05, **p<0.01, ***p<0.001 compared with positive control; +p>0.05, $$p<0.01, $$$p<0.001 compared with rivastigmine group, MDA: Malondialdehyde, SOD: Superoxide dismutase, GSH: Glutathione

function by the significant increase of DI in vitamin D3 doses (100, 500 and 1000 IU/kg/day) combined treatment of vitamin D3 and rivastigmine and rivastigmine monotherapy versus positive control. This finding illustrated the crucial role of vitamin D3 in the enhancement of hippocampus-dependent learning and memory. In accordance to the present results, Latimer *et al.*[14] reported the superiority of a high vitamin D diet over low vitamin D diet (1,000 and 100 IU kg^{-1}, respectively) in markedly enhancing of MWM escape performance.

Hippocampal insulin resistance (IR) is confirmed to be correlated with brain Aβ deposition; IR leads to increase of Aβ deposition intracellular and extracellular which to considered the hallmark of AD[27,28].

In the current study, hippocampal level of insulin level was significantly increased while the Aβ-42 level significantly decreased after chronic administration of vit. D3 compared with non-treated diabetic rats. In accordance with this work, in previous studied, it was found that the administration of intranasal insulin for healthy humans and AD patients elevated cognitive performance[29,30]. Also, it was observed that the vitamin D active form increase the efflux of Aβ out of the brain leading to enhancement of Aβ cerebral clearance[31]. Regarding to Aβ and insulin, present findings are reflecting the beneficial role of vitamin D3 in the improvement of insulin production and subsequently increased clearance of Aβ-42 and this may explained the neuroprotective role of vitamin D. Oxidative stress has a pivotal role in the pathogenesis of diabetes complications as excessive production of ROS during diabetic conditions contributed to the relatively reduced capability of the natural antioxidant systems leading to the neuronal apoptosis then AD[32]. Further confirmatory evidence was reported which supported the antioxidant activity of vitamin D3 in the oxidative stress diabetes[33].

In the present study, chronic administration of vitamin D3 doses (100, 500 and 1000 IU/kg/day) and combined vit. D3 plus rivastigmine significantly decreased MDA level .These findings proved that vit. D3 has strong antioxidant properties in the brain tissues. These results are in agreement with those of Alatawi *et al.*[34], who reported that vitamin D and calcium had a significantly reduced plasma level of MDA in STZ-induced diabetic rats. Furthermore, the current study demonstrated a significant elevation of GSH levels in vitamin D3 treated diabetic rats. It appeared that the effect of vitamin D3 on GSH could be at two levels-either through increasing the biosynthesis of GSH or by inhibiting its utilization by reducing oxidative stress. Previous literature also showed a significant correlation between plasma GSH level in type 2 diabetic patients and plasma level of vitamin D[35]. It was also reported that vitamin D3 has an antioxidant effect as a result of an increase in hepatic GSH amounts in rats[36].

The activity of SOD is not fully understood in STZ-induced DM, variable responses to DM have been noted in brain tissues with observations of either unchanged, increased or decreased[37-39]. These results indicated that the antioxidant activity of SOD varies between tissue types and the severity of DM at various stages may be a major contributing factor. In the present study, diabetic rats treated with different vitamin D3 doses (100, 500 AND 1000 IU/kg/day), vitamin D3 combined with rivastigmine and rivastigmine monotherapy showed a significant decrease in the activity of SOD ($p<0.01$) compared with non-treated diabetic rats. In current work, vitamin D3 restored the SOD towards the control values. Inconsistent with current study Jang *et al.*[40] reported a significant increase in the activity of SOD (by+57 %) in non-treated diabetic rats compared with normal healthy rats. So it was suggested that vitamin D3 decrease in the need for activation of antioxidant defense of SOD due to its antioxidant activity.

CONCLUSION

It is concluded that Vitamin D3 considered a potential pharmacological agent for the management of cognitive dysfunction in T3DM and it was also attenuated the

oxidative stress by reducing the malondialdehyde and superoxide dismutase level and increasing the glutathione level compared to positive control.

SIGNIFICANCE STATEMENT

This study discovered the beneficial role of vitamin D3 for cognition deficits linked to diabetes via improving antioxidant, reducing insulin resistance as well as substantially abolishing Aβ accumulation. This study will help the researcher to uncover the critical areas of diabetes and cognitive deficits that many researchers were not able to explore.

ACKNOWLEDGMENTS

I wish to express my thanks and appreciation to King Abdulaziz city for Science and Technology for its financial support to the project of the research number (1-18-03-009-0054) and which enabled me in the provision of requirements and necessary chemical substances for the research.

REFERENCES

1. Rizzi, L., I. Rosset and M. Roriz-Cruz, 2014. Global epidemiology of dementia: Alzheimer's and vascular types. BioMed Res. Int., Vol. 2014. 10.1155/2014/908915.
2. Kumar, A., A. Singh and Ekavali, 2015. A review on Alzheimer's disease pathophysiology and its management: An update. Pharmacol. Rep., 67: 195-203.
3. Albugami, M., N. Qadi, F. Almugbel, A. Mohammed and A. Alttas *et al*., 2018. The demographic characteristics and the risk factors of dementia in SAUDI elderly. Am. J. Psychiatry Neurosci., 6: 1-8.
4. Ott, A., R.P. Stolk, F. van Harskamp, H.A.P. Pols, A. Hofman and M.M.B. Breteler, 1999. Diabetes mellitus and the risk of dementia: The Rotterdam study. Neurology, 53: 1937-1937.
5. Biessels, G.J. and L.J. Kappelle, 2005. Increased risk of Alzheimer's disease in type II diabetes: Insulin resistance of the brain or insulin-induced amyloid pathology? Biochem. Soc. Trans., 33: 1041-1044
6. .Brands, A.M.A., G.J. Biessels, E.H.F. de Haan, L.J. Kappelle and R.P.C. Kessels, 2005. The effects of type 1 diabetes on cognitive performance: A meta-analysis. Diabetes Care, 28: 726-735.
7. Irie, F., A.L. Fitzpatrick, O.L. Lopez, L.H. Kuller, R. Peila, A.B. Newman and L.J. Launer, 2008. Enhanced risk for Alzheimer disease in persons with type 2 diabetes and APOE ε4: The cardiovascular health study cognition study. Arch. Neurol., 65: 89-93.
8. De la Monte, S.M. and J.R. Wands, 2008. Alzheimer's disease is type 3 diabetes-evidence reviewed. J. Diabetes Sci. Technol., 2: 1101-1113.
9. Kim, B. and E.L. Feldman, 2012. Insulin resistance in the nervous system. Trends Endocrinol. Metab., 23: 133-141.
10. Kim, B. and E.L. Feldman, 2015. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Exp. Mol. Med., Vol. 47, No. 3. 10.1038/emm.2015.3.
11. Chikezie, P.C., O.A. Ojiako and A.C. Ogbuji, 2015. Oxidative stress in diabetes mellitus. Int. J. Biol. Chem., 9: 92-109.
12. Wojtunik-Kulesza, K.A., A. Oniszczuk, T . Oniszczuk and M. Waksmundzka-Hajnos, 2016. The influence of common free radicals and antioxidants on development of Alzheimer's disease. Biomed. Pharmacother., 78: 39-49.
13. Etgen, T., D. Sander, H. Bickel, K. Sander and H. Forstl, 2012. Vitamin D deficiency, cognitive impairment and dementia: A systematic review and meta-analysis. Dementia Geriatr. Cognit. Disord., 33: 297-305.
14. Latimer, C.S., L.D. Brewer, J.L. Searcy, K.C. Chen and J. Popovic *et al*., 2014. Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. Proc. Natl. Acad. Sci. USA., 111: E4359-E4366.
15. Droge, W., 2002. Free radicals in the physiological control of cell function. Phsyol. Rev., 82: 47-95.
16. Asemi, Z., T . Hashemi, M. Karamali, M. Samimi and A. Esmaillzadeh, 2013. Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation and oxidative stress in gestational diabetes: A double-blind randomized controlled clinical trial. Am. J. Clin. Nutr., 98: 1425-1432.
17. Labudzynskyi, D.O., O.V. Zaitseva, N.V. Latyshko, O.O. Gudkova and M.M. Veliky, 2015. Vitamin D_3 contribution to the regulation of oxidative metabolism in the liver of diabetic mice. Ukr. Biochem. J., 87: 75-90.
18. Kono, K., H. Fujii, K. Nakai, S. Goto and R. Kitazawa *et al*., 2013. Anti-oxidative effect of vitamin D analog on incipient vascular lesion in non-obese type 2 diabetic rats. Am. J. Nephrol., 37: 167-174.
19. Banerjee, A., V.K. Khemka, A. Ganguly, D. Roy, U. Ganguly and S. Chakrabarti, 2015. Vitamin D and Alzheimer's disease: Neurocognition to therapeutics. Int. J. Alzheimer's Dis., Vol. 2015. 10.1155/2015/192747.
20. Gheibi, S., K. Kashfi and A. Ghasemi, 2017. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed. Pharmacother., 95: 605-613.
21. Bihaqi, S.W., M. Sharma, A.P. Singh and M. Tiwari, 2009. Neuroprotective role of *Convolvulus pluricaulis* on aluminium induced neurotoxicity in rat brain. J. Ethnopharmacol., 124: 409-415.

22. Salum, E., P. Kampus, M. Zilmer, J. Eha and M. Butlin *et al*., 2012. Effect of vitamin D on aortic remodeling in streptozotocin-induced diabetes. Cardiovasc. Diabetol., Vol. 11. 10.1186/1475-2840-11-58.
23. Mahajan, S., P. Chauhan, M. Mishra, D. Yadav, M. Debnath and G. Prasad, 2018. Antidiabetic potential of eugenia jambolana ethanolic seed extract: effect on antihyperlipidemic and antioxidant in experimental streptozotocin-induced diabetic rats. Adv. Complement. Altern. Med., Vol. 2, No. 3. 10.31031/ACAM.2018.02.000540.
24. Antunes, M. and G. Biala, 2012. The novel object recognition memory: Neurobiology, test procedure and its modifications. Cognit. Process., 13: 93-110.
25. Ennaceur, A., 2010. One-trial object recognition in rats and mice: Methodological and theoretical issues. Behav. Brain Res., 215: 244-254.
26. Engvall, E. and P. Perlmann, 1971. Enzyme-Linked Immunosorbent Assay (ELISA) quantitative assay of immunoglobulin G. Immunochemistry, 8: 871-874.
27. Matioli, M.N.P.S. and R. Nitrini, 2015. Mechanisms linking brain insulin resistance to Alzheimer's disease. Dementia Neuropsychol., 9: 96-102.
28. Zhao, L., B. Teter, T. Morihara, G.P. Lim and S.S. Ambegaokar *et al*., 2004. Insulin-degrading enzyme as a downstream target of insulin receptor signaling cascade: Implications for Alzheimer's disease intervention. J. Neurosci., 24: 11120-11126.
29. Benedict, C., W.H. Frey II, H.B. Schioth, B. Schultes, J. Born and M. Hallschmid, 2011. Intranasal insulin as a therapeutic option in the treatment of cognitive impairments. Exp. Gerontol., 46: 112-115.
30. Reger, M.A., G.S. Watson, P.S. Green, C.W. Wilkinson and L.D. Baker *et al*., 2008. Intranasal insulin improves cognition and modulates beta-amyloid in early AD. Neurology, 70: 440-448.
31. Ito, S., S. Ohtsuki, Y. Nezu, Y. Koitabashi, S. Murata and T. Terasaki, 2011. 1α,25-Dihydroxyvitamin D_3 enhances cerebral clearance of human amyloid-β peptide(1-40) from mouse brain across the blood-brain barrier. Fluids Barriers CNS, Vol. 8. 10.1186/2045-8118-8-20.
32. Giacco, F. and M. Brownlee, 2010. Oxidative stress and diabetic complications. Circ. Res., 107: 1058-1070.
33. Mokhtari, Z., Z. Hekmatdoost and M. Nourian, 2017. Antioxidant efficacy of vitamin D. J. Parathyroid Dis., 5: 11-16.
34. Alatawi, F.S., U.A. Faridi and M.S. Alatawi, 2018. Effect of treatment with vitamin D plus calcium on oxidative stress in streptozotocin-induced diabetic rats. Saudi Pharmaceut. J., 26: 1208-1213.
35. Jain, S.K., D. Micinski, L. Huning, G. Kahlon, P.F. Bass and S.N. Levine, 2014. Vitamin D and L-cysteine levels correlate positively with GSH and negatively with insulin resistance levels in the blood of type 2 diabetic patients. Eur. J. Clin. Nutr., 68: 1148-1153.
36. Sardar, S., A. Chakraborty and M. Chatterjee, 1996. Comparative effectiveness of vitamin D_3 and dietary vitamin E on peroxidation of lipids and enzymes of the hepatic antioxidant system in Sprague-Dawley rats. Int. J. Vitamin Nutr. Res., 66: 39-45.
37. Shanmugam, K.R., K. Mallikarjuna, K. Nishanth, C.H. Kuo and K.S. Reddy, 2011. Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues. Food Chem., 124: 1436-1442.
38. Erejuwa, O.O., S.A. Sulaiman, M.S.A. Wahab, K.N.S. Sirajudeen, M.S.M. Salleh and S. Gurtu, 2010. Antioxidant protective effect of glibenclamide and metformin in combination with honey in pancreas of streptozotocin-induced diabetic rats. Int. J. Mol. Sci., 11: 2056-2066.
39. Ulusu, N.N., M. Sahilli, A. Avci, O. Canbolat and G. Ozansoy *et al*., 2003. Pentose phosphate pathway, glutathione-dependent enzymes and antioxidant defense during oxidative stress in diabetic rodent brain and peripheral organs: effects of stobadine and vitamin E. Neurochem. Res., 28: 815-823.
40. Jang, Y.Y., J.H. Song, Y.K. Shin, E.S. Han and C.S. Lee, 2000. Protective effect of boldine on oxidative mitochondrial damage in streptozotocin-induced diabetic rats. Pharmacol. Res., 42: 361-371.