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ORIGINAL ARTICLE

Urtica dioica modulates hippocampal insulin signaling and recognition memory deficit in streptozotocin induced diabetic mice

Sita Sharan Patel[1] · **Sahil Gupta**[1] · **Malairaman Udayabanu**[1]

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Abstract Diabetes mellitus has been associated with functional abnormalities in the hippocampus and performance of cognitive function. *Urtica dioica* (UD) has been used in the treatment of diabetes. In our previous report we observed that UD extract attenuate diabetes mediated associative and spatial memory dysfunction. The present study aimed to evaluate the effect of UD extract on mouse model of diabetes-induced recognition memory deficit and explore the possible mechanism behind it. Streptozotocin (STZ) (50 mg/kg, i.p. consecutively for 5 days) was used to induce diabetes followed by UD extract (50 mg/kg, oral) or rosiglitazone (ROSI) (5 mg/kg, oral) administration for 8 weeks. STZ induced diabetic mice showed significant decrease in hippocampal insulin signaling and translocation of glucose transporter type 4 (GLUT4) to neuronal membrane resulting in cognitive dysfunction and hypolocomotion. UD treatment effectively improved hippocampal insulin signaling, glucose tolerance and recognition memory performance in diabetic mice, which was comparable to ROSI. Further, diabetes mediated oxidative stress and inflammation was reversed by chronic UD or ROSI administration. UD leaves extract acts via insulin signaling pathway and might prove to be effective for the diabetes mediated central nervous system complications.

Keywords Cognition · Diabetes · GLUT4 · Insulin receptor · Novel object recognition · *Urtica dioica*

Introduction

Diabetes mellitus, a major epidemic of this century has increased in incidence by 50 % over the past 10 years (Danaei et al. 2011; Shaw et al. 2010). Diabetes mellitus, a most common metabolic disorder is associated with neuropathy, nephropathy, retinopathy, cardiomyopathy etc. Diabetic neuropathy is damage to nerves in the body that occurs due to high blood sugar level (Danaei et al. 2011). In the central nervous system, diabetes exacerbates depression, phobias, anorexia (Lustman et al. 1988; Nouwen et al. 2011) and reduces cognitive skills (Lupien et al. 2003). Clinically, patients with diabetes mellitus showed higher risk of cognitive impairment as compared to the general population (Lupien et al. 2003). Diabetes accelerates the progression from mild cognitive impairment to severe dementia (Yates et al. 2012) as well as induce recognition memory deficit (King et al. 2013). Diabetes induces oxidative stress and inflammation in the hippocampal neurons resulting in neurodegeneration (Alipour et al. 2012; Saravanan and Ponmurugan 2012). Insulin signaling in hippocampus is known to modulate cognitive performance. Experimental evidence suggests that, a decrease in the insulin receptor (IR) signaling impairs hippocampal synaptic neurotransmission and inflicts cognitive impairment (Grillo et al. 2009; Holscher 2011; Piroli et al. 2007). Further, dysregulation of glucose transporter type 4 (GLUT4) translocation in hippocampal neurons is associated with functional abnormalities in synaptic plasticity as observed in Alzheimer's disease and type 2 diabetes (Piroli et al. 2007).

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✉ Malairaman Udayabanu
m_udayabanu@rediffmail.com

[1] Department of Pharmacy, Jaypee University of Information Technology, Waknaghat, Himachal Pradesh 173234, India

Urtica dioica L (UD, Stinging nettle) is a perennial herb belonging to the family Urticaceae. UD leaves contain phytoconstituents such as scopoletin, gentisic acid, protocatechuic acid, quinic acid, caffeic acid, ferulic acid, quercetin, 5-O-caffeoylquinic acid, esculetin and rutin (Orcic et al. 2014). UD has been used as natural remedy for the treatment of diabetes. Studies on hydro-alcoholic leaves extract of UD have shown significant improvement in glycemic control during diabetes. In animal models, UD is reported to reduce blood glucose and glycated hemoglobin levels during streptozotocin (STZ)-induced diabetes (Qujeq et al. 2013). UD is known to exert glycemic control in type 2 diabetic patients by lowering the levels of fasting and postprandial blood glucose (Kianbakht et al. 2013). Previous studies reported that treatment of diabetic patients with UD significantly increased total antioxidant capacity with reduced inflammatory stress and glycated hemoglobin (Namazi et al. 2011, 2012). UD extract compensate granule cell density and astrocytes loss in the hippocampus of diabetic rat (Fazeli et al. 2008). Further, our earlier study demonstrated the beneficial effect of UD extract on diabetes mediated spatial and associative memory dysfunction (Patel and Udayabanu 2013, 2014). The present study was aimed to investigate the effect of UD extract on mouse model of diabetes-induced recognition memory dysfunction and attempts to explore the possible molecular mechanism.

Materials and methods

Plant material

Plant material was collected from the North Western Himalayan region and authenticated from Dr. Y.S. Parmar University of Horticulture & Forestry, India (specimen number 12,399). UD leaves were shade dried and powdered afterwards. The extraction of UD leaves (200 g) was performed at room temperature, with constant shaking during 48 h, using methanol and water (1:1) as solvent. Thereafter, the extract was filtered, centrifuged, evaporated under reduced pressure (Heidolph-Vap, Germany) and freez-dried (Allied Frost, Macflow Engineering).

Animals

Adult Swiss albino mice (aged 8–10 weeks, 25–30 g) of either sex were housed under a 12 h light/dark cycle at 26 ± 2 °C. The animals had access to food and water ad libitum. All experiments were carried out in accordance with CPCSEA guidelines and Institutional Animal Ethical Clearance. All efforts were made to reduce animals suffering and the number of animals used.

STZ induced diabetes and drug treatment

Animals were randomly divided into two groups, viz., G1 – normal control group (n = 6) and the rest of the animals (n = 18) were treated with STZ (50 mg/kg, i.p.) for 5 consecutive days (diabetic group). The animals which showed the blood glucose level ≥ 200 mg/dl were considered for further studies. The diabetic group animals were then randomly divided into three groups, viz., G2 – STZ (n = 6), G3 – STZ + hydro-alcoholic extract of UD (50 mg/kg) (n = 6) and G4 – STZ + rosiglitazone (ROSI) (5 mg/kg) (n = 6). The dose of UD used in the present experiment was selected from previous study (Patel and Udayabanu 2013). Hydro-alcoholic extract of UD or ROSI or vehicle (0.3 % CMC in water for injection) was administered once daily through oral gavage from the 6th day after STZ injection till 60th day (Fig. 1). On day 59–60, animals were subjected to behavioral studies to assess cognitive performance and locomotor activity. Immediately after behavioral studies, blood was collected via retro-orbital route in tubes containing 10 % sodium citrate and centrifuged at 1000 g for 20 min at 4 °C. Plasma aliquots were stored at −80 °C for biochemical estimations. Finally, hippocampus was dissected and used for further studies.

Oral glucose tolerance test (OGTT)

OGTT was performed on day 60 using 2 g glucose/kg body weight and blood was collected from the animals by tail snipping at 0, 0.5, 1.0, 1.5 and 2.0 h after glucose load. Blood glucose level was measured in blood samples using Accu-check (Roach Diagnostics GmbH, Germany) blood glucose monitoring system.

Novel object recognition task (NORT)

NORT was used to evaluate the recognition memory in rodents, is based on the tendency of animals to spend more time exploring a novel object than familiar object. Mice were placed into an open field apparatus with rectangular dimensions (50 cm wide X 50 cm long X 35 cm height). In the habituation phase, the animals were individually allowed to explore the empty open field for 5 min. Thereafter, mice were returned to its home cage. In the familiarization phase (twenty four hours after the habituation), the animals were presented with two identical objects and measured the time necessary to reach the 20-s criterion of total exploration, in a maximum period of 5 min. Investigative behaviors like head orientation, sniffing or any deliberate contact with each object from the distance of ≤2 cm or when touching with the nose were considered as object exploration. After two minutes of post-familiarization session, the test trial was performed in which one of the familiar objects was exchanged with another new

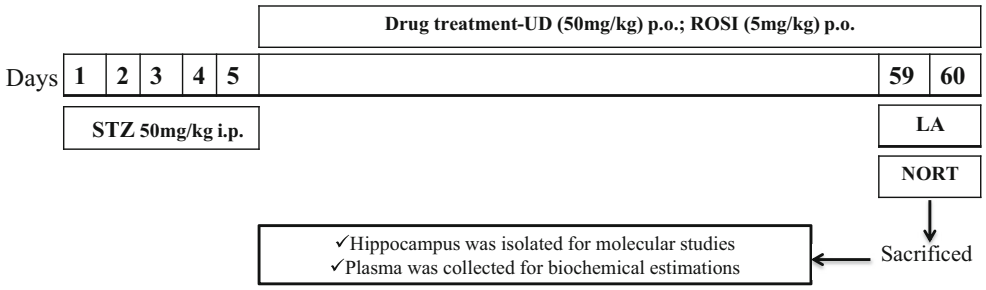

Fig. 1 Schematic representation of the experimental design: animals were administered with STZ (50 mg/kg, i.p.) for five consecutive days followed by drugs treatment. Thereafter, animals were subjected to different behavioral, molecular and biochemical studies. LA = locomotor activity, NORT = novel object recognition task

object and the mice were left in the open field for 5 min (test phase). The exploration time of familiar and novel object during test phase was recorded. The discrimination index of the novel object as the period of time spent exploring the novel object minus the period of time spent exploring the familiar one during test phase [discrimination index = (novel object exploration time/total exploration time) - (familiar object exploration time/total exploration time) × 100] was calculated. To exclude for odor cues, the open field apparatus and the objects were cleaned with 70 % ethanol.

Locomotor activity

Locomotor activity was examined in the open field task. The floor of the open field was subdivided into 9 vertical and horizontal (64 equal rectangles) black lines. Mice were individually placed at the centre of the open field arena of apparatus. After an initial familiarization period (5 min), the number of crossings across the lines (locomotion) was analyzed for ten minutes (Branco Cdos et al. 2013).

Real-time quantitative reverse transcription PCR (qPCR)

Animals were sacrificed and the total hippocampal RNA was isolated using TRIzol reagent (Invitrogen). The reliability of RNA was checked on 2 % agarose gel and quantified using nano-drop spectrophotometer (ND-2000C, Thermo Scientific). The reverse transcription of 3 μg of total RNA was then performed using cDNA synthesis kit (Fermentas life-sciences). qPCR amplification was performed in an CFX96™ Real-Time PCR Detection System (Bio-Rad) using the iQ™ SYBR green supermix dye (Bio-Rad). Reactions were carried out in total volumes of 12.5 μl containing 2.5 pM of each primer (PPARγ FP: 5′-AGG GCG ATC TTG ACA GGA AA-3′, RP: 5′-CGA AAC TGG CAC CCT TGA AA-3′; IR FP: 5′-TTT GTC ATG GAT GGA GGC TA-3′, RP: 5′-CCT CAT CTT GGG GTT GAA CT-3′; ILGF 1r FP: 5′-GTG GGG GCT GCT CGT GTT TCT C-3′, RP: 5′-GAT CAC CGT GCA GTT TTC CA-3′; GLP1 FP: 5′-TCA GAG ACG GTG CAG AAA TGG-3′, RP: 5′-ATC AAA GGT CCG GTT GCA GAA-3′; IRS1 FP: 5′-CGA TGG CTT CTC AGA CGT G-3′, RP: 5′-CAG CCC GCT TGT TGA TGT TG-3′; IRS2 FP: 5′-CTG CGT CCT CTC CCA AAG TG-3′, RP: 5′-GGG GTC ATG GGC ATG TAG C-3′; PI3K FP: 5′-CGA GAG TGT CGT CAC AGT GTC-3′, RP: 5′-TGT TCG CTT CCA CAA ACA CAG-3′; PKB FP: 5′-TGC CCA CAC GCT TAC TGA GA-3′, RP: 5′-CAA AGC AGA GGC GGT CGT-3′; GLUT4 FP: 5′-GAT GGG CTT TCT CCG TCC-3′, RP: 5′-GTG TGG CAA GAG TTC AGT GG-3′; INSG1 FP: 5′-CAC GAC CAC GTC TGG AAC TAT-3′, RP: 5′-TGA GAA GAG CAC TAG GCT CCG-3′; MAPK1 FP: 5′-CCC TTA GAC ACT GTG ACG GT-3′, RP: 5′-CAC AGT CCC AAA GCC ACA AA-3′; RAGE FP: 5′-GTG GAT CCT GCC TCT GAA CT-3′, RP: 5′-GGT GTC TCC TGG TCT CTT CC-3′ and GAPDH FP: 5′-TTC ACC ACC ATG GAG AAG GC-3′, RP: 5′-GGC ATG GAC TGT GGT CAT GA-3′) and 1 μl of cDNA template containing 100 ng cDNA. The thermal cycler conditions for cDNA amplification were as follows: Step 1, 95 °C for 3:00 min; Step 2, 95 °C for 10 s, 52–58 °C for 30 s and 72 °C for 2:20 s (35 cycles). Thermal cycler condition for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was as follows: Step 1, 95 °C for 3:00 min; Step 2, 95 °C for 10 s, 57.6 °C for 30 s and 72 °C for 2:20 s (35 cycles).

Immunoblot analysis

Hippocampal tissue was homogenized in 400 μl of ice-cold radioimmunoprecipitation assay buffer containing protease inhibitor cocktail (MP Biomedicals) and centrifuged at 4 °C 1, 000×g for 10 min. The supernatant was collected and further centrifuged at 16,000×g for 15 min to isolate cytosolic fraction. The pellet fraction was then resuspended in 100 μl of ice-cold cell lysis radioimmunoprecipitation assay buffer containing 1 % Triton X-100, centrifuged at 4 °C 16,000×g for 15 min and the supernatant was isolated as a crude membrane fraction (Potapenko et al. 2012). Protein concentration was estimated by the Bradford method at 595 nm. Thirty microgram of total protein from tissue homogenate was denatured

with Laemmli loading buffer (Himedia) by heating at 95 °C for 5 min. The samples were resolved by 10 % SDS-PAGE and electroblotted to nitrocellulose membrane using semi-dry transblot. Blots were then blocked with 3 % BSA in PBS overnight at 4 °C and then incubated with primary antibodies: GLUT4 (goat polyclonal IgG, 1:3500) and GAPDH (mouse monoclonal IgG, 1:3500) in PBS for 2.5 h at room temperature and then washed thrice with PBS. Thereafter, the blots were incubated with secondary antibodies: donkey anti-goat IgG-HRP (1:5000) and goat anti-mouse IgG-HRP (1:5000) with respect to primary antibodies in PBS at room temperature for 2 h. After washing, the blots were developed with 0.06 % 3, 3′-diaminobenzidine tetrahydrochloride, 0.025 % cobalt chloride in PBS and 0.01 % hydrogen peroxide. The blot images were captured and band density analysis was performed using densitometer (GS-800 Calibrated densitometer, BioRad). Values of the band density of GLUT4 were normalized with respect to the level of GAPDH intensity. We also calculated the ratio of GLUT4m/GLUTc (m/c) in hippocampus.

Biochemical estimation

The level of thiobarbituric acid reactive substances (TBARS) in plasma was determined as per method described by Ohkawa et al. (1979). The mixture consists of 100 μl of plasma, 0.1 ml of 8 % sodium dodecyl sulfate, 1.0 ml of 20 % acetic acid and 1.0 ml of 0.67 % thiobarbituric acid. The mixture was boiled at 95 °C for 1 h and then cooled. One ml of double distilled water and five ml of n-butanol:pyridine (15:1, *v/v*) mixture was added and centrifuged at 5000 x rpm for 10 min. The absorbance of organic layer was measured at 540 nm. The level of plasma nitric oxide (NO) was determined as nitrite plus nitrate. The nitrate was reduced to nitrite in the presence of 2 % ammonium molybdate and 4 % ferrous ammonium sulphate and quantified by using Greiss reagent at 540 nm (Patel and Udayabanu 2014). Catalase level in plasma was determined by the method of Claiborne (1985). A total of 0.1 ml of plasma was added to the tube containing 1.9 ml of 50 mM phosphate buffer (pH 7). The reaction was initiated by addition of 1 ml freshly prepared 30 mM hydrogen peroxide. The rate of decomposition of hydrogen peroxide was measured at 240 nm. The level of total thiol in plasma was determined using 10 mM Ellman's reagent and measured at 412 nm as per More et al. (2012).

Statistical analysis

All the data were expressed as mean ± SEM. The statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test except for OGTT which was analyzed using two-way ANOVA followed by Bonferroni post test with a confidence level of $p < 0.05$.

Results

OGTT

In OGTT, glucose challenge dramatically raised the blood glucose level of STZ group compared with control group at 0–2.0 h intervals ($p < 0.001$) indicating impaired glucose tolerance. Chronic UD treatment showed significantly reduced glucose levels at 0 ($p < 0.001$), 0.5 ($p < 0.05$), 1.0 ($p < 0.001$), 1.5 ($p < 0.01$) and 2.0 h ($p < 0.001$) as compared to STZ group. Chronic ROSI administration significantly reversed STZ induced elevated levels of blood glucose ($p < 0.001$) at 0–2.0 h intervals (Fig. 2).

NORT

In NORT, diabetic mice showed no alteration in object exploration time between novel and familiar one during test phase. STZ induced diabetic mice showed significantly impaired novel object recognition performance in the task as compared to normal control mice ($p < 0.001$). Chronic UD or ROSI administration significantly increased ($p < 0.01$ and $p < 0.001$, respectively) the novel object recognition performance in diabetic mice (Fig. 3a). Further, the net preference between novel and familiar objects, the discrimination index in diabetic mice was reduced as compared to control mice ($p < 0.001$). Chronic UD or ROSI administration significantly increased ($p < 0.001$) the discrimination index in diabetic mice (Fig. 3b).

Locomotor activity

While assessing the locomotor performance in open field task, we observed that chronic diabetes significantly reduced the locomotor activity in mice ($p < 0.001$).

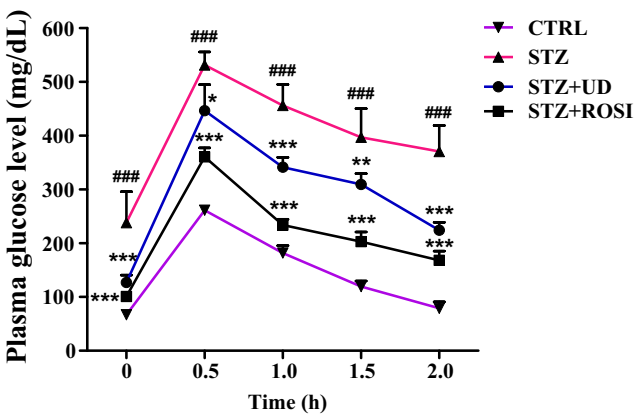

Fig. 2 Effect of UD extract on streptozotocin induced alteration in oral glucose tolerance test. Data were mean ± SEM values ($n = 6$). Significant differences: #CTRL vs. STZ, *STZ vs. STZ + UD and STZ + ROSI. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CTRL = control; STZ = streptozotocin; UD = *Urtica dioica*; ROSI = rosiglitazone

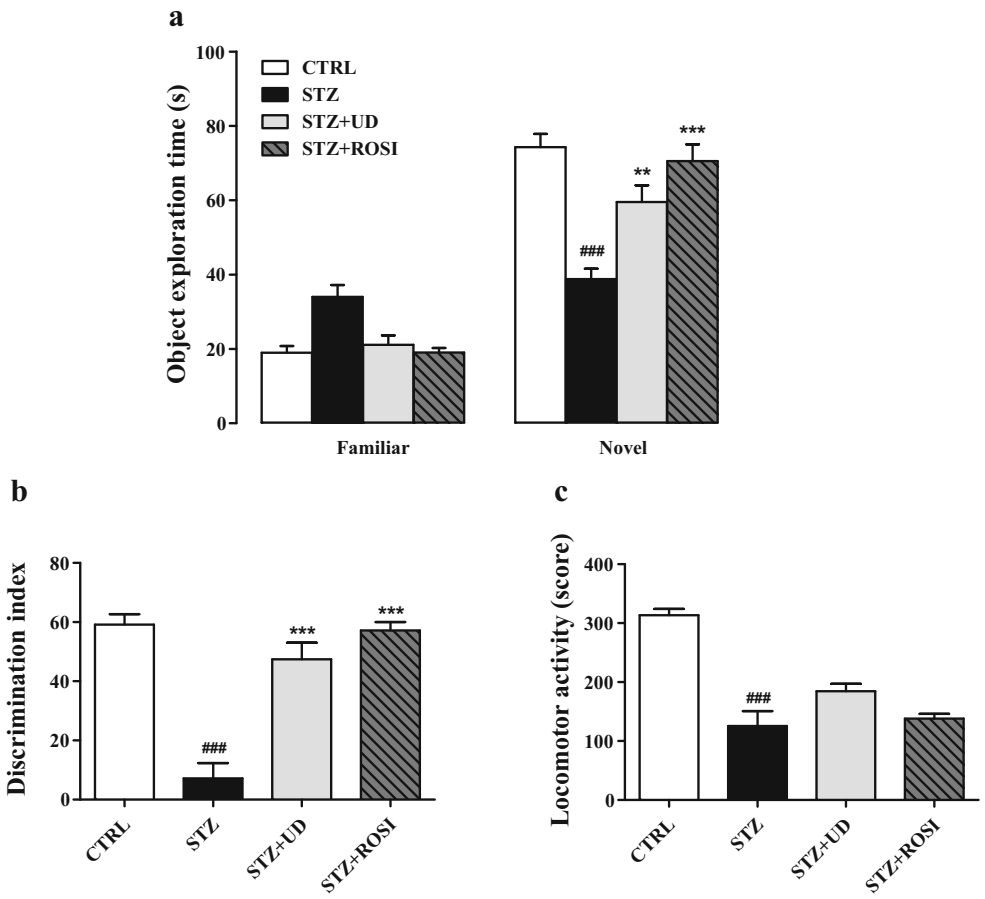

Fig. 3 Effect of UD extract on diabetes mediated alteration in object exploration time (**a**), discrimination index (**b**) and locomotor activity (**c**). Data were mean ± SEM values ($n = 6$). Significant differences: #CTRL vs. STZ, *STZ vs. STZ + UD and STZ + ROSI. **$p < 0.01$, ***$p < 0.001$. CTRL = control; STZ = streptozotocin; UD = *Urtica dioica*; ROSI = rosiglitazone

Chronic UD or ROSI administration did not reverse ($p > 0.05$) hypolocomotion induced by chronic diabetes (Fig. 3c).

mRNA expression in hippocampus

Peroxisome proliferator activated receptor gamma (PPARγ) mRNA expression was not altered in the STZ induced diabetic mice as compared to control mice ($p > 0.05$). Chronic ROSI or UD administration significantly upregulated ($p < 0.05$) the mRNA expression of hippocampal PPARγ in STZ induced diabetic mice (Fig. 4a). STZ induced diabetic mice showed significant decrease ($p < 0.05$) in the hippocampal IR mRNA expression as compared to control animals. Chronic UD or ROSI treatment significantly ($p < 0.01$) increased the mRNA expression of hippocampal IR in diabetic mice (Fig. 4b). Chronic diabetes did not modulate ($p > 0.05$) the expression level of hippocampal insulin-like growth factor 1 receptor (ILGF 1r) (Fig. 4c) and glucagon like peptide 1 (GLP1) (Fig. 4d) mRNA as compared to control. Further, chronic UD or ROSI administration did not alter the level of ILGF 1r and GLP1mRNA expression in diabetic mice ($p > 0.05$). STZ induced diabetic mice showed significant decrease ($p < 0.05$) in the hippocampal insulin receptor substrate 1 (IRS1) mRNA expression as compared to control animals. Chronic ROSI treatment significantly ($p < 0.05$) increased while chronic UD administration did not modulate ($p > 0.05$) the mRNA expression of hippocampal IRS1 in diabetic mice (Fig. 4e). Insulin receptor substrate 2 (IRS2) mRNA expression was significantly decreased ($p < 0.05$) in the hippocampus of diabetic mice as compared to control mice. Chronic UD or ROSI treatment significantly increased ($p < 0.05$) the mRNA expression of hippocampal IRS2 in diabetic mice (Fig. 4f). Phosphoinositide 3-kinase (PI3K) mRNA expression was significantly decreased ($p < 0.05$) in the hippocampus of diabetic mice as compared to control mice. Further, chronic UD or ROSI treatment significantly increased ($p < 0.05$) the mRNA expression of hippocampal PI3K in diabetic mice (Fig. 4g). Protein kinase B (PKB) mRNA expression was significantly decreased ($p < 0.05$) in STZ treated mice as compared to control mice. Chronic UD or ROSI administration significantly increased ($p < 0.05$) the mRNA expression of PKB in the hippocampus of diabetic mice (Fig. 4h). GLUT4 mRNA expression was significantly decreased ($p < 0.05$) in STZ treated animals as compared to control animals. Chronic UD or ROSI treatment significantly increased the mRNA expression of hippocampal GLUT4 ($p < 0.05$) in STZ treated animals (Fig. 4i). STZ induced diabetic mice showed significant decrease ($p < 0.001$) in

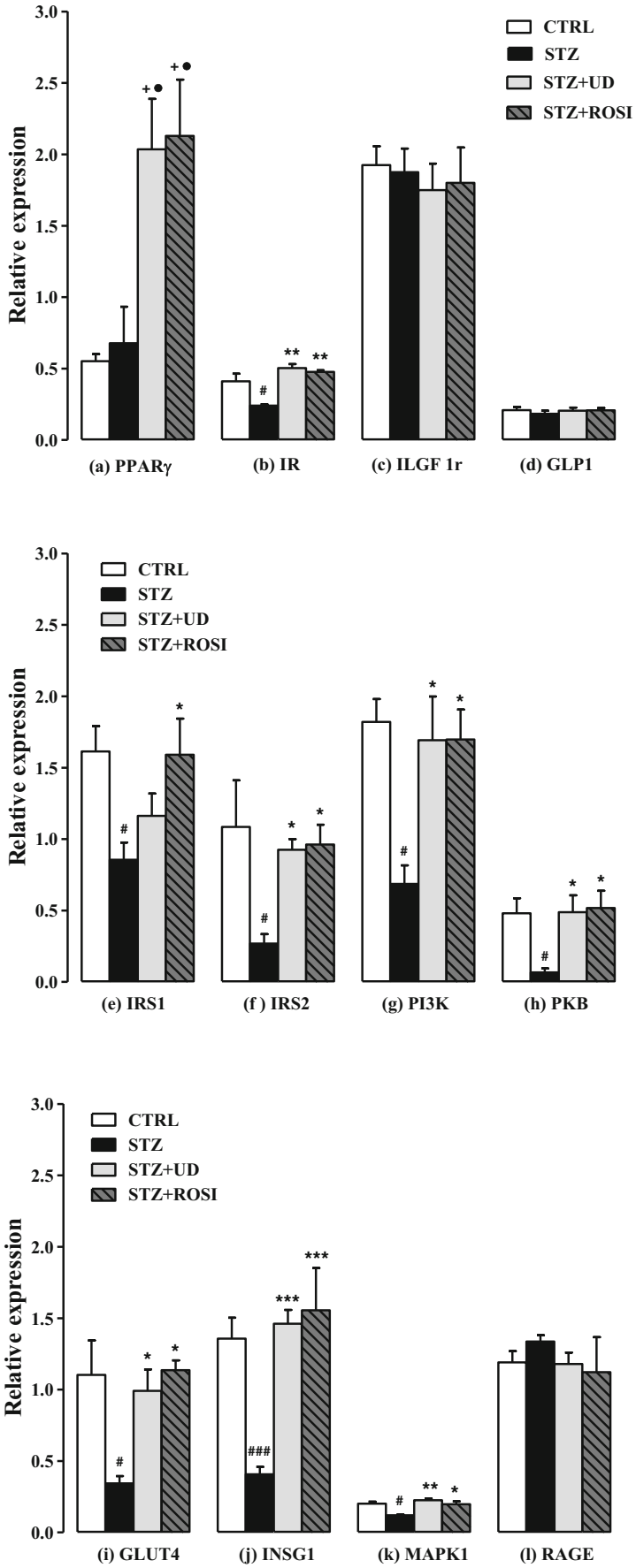

Fig. 4 Effect of UD extract on diabetes mediated alterations in the mRNA expression of hippocampal PPARγ (**a**), IR (**b**), ILGF 1r (**c**), GLP1 (**d**), IRS1 (**e**), IRS2 (**f**), PI3K (**g**), PKB (**h**), GLUT4 (**i**), INSG1 (**j**), MAPK1 (**k**) and RAGE (**l**). Data were mean ± SEM values ($n = 4$). Significant differences: $^{\#}$CTRL vs. STZ, *STZ vs. STZ + UD and STZ + ROSI, $^{\bullet}$CTRL vs. STZ + UD/STZ + ROSI, $^{+}$STZ vs. STZ + UD/STZ + ROSI. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. CTRL = control; STZ = streptozotocin; UD = *Urtica dioica*; ROSI = rosiglitazone

hippocampal insulin induced gene 1 (INSG1) mRNA expression as compared to control animals. Chronic UD or ROSI administration significantly increased ($p < 0.001$) the mRNA expression of hippocampal INSG1 in diabetic animals (Fig. 4j). STZ induced diabetic mice showed significant decrease ($p < 0.05$) in the hippocampal mitogen activated protein kinase 1 (MAPK1) mRNA expression as compared to control mice. Chronic UD or ROSI treatment significantly increased ($p < 0.01$ and $p < 0.05$, respectively) the mRNA expression of MAPK1 in diabetic mice (Fig. 4k). Chronic diabetes did not modulate ($p > 0.05$) the expression level of receptor for advanced glycation endproducts (RAGE) in the hippocampus as compared to control animals. Further, chronic UD or ROSI administration did not alter the expression of RAGE mRNA in diabetic mice ($p > 0.05$) (Fig. 4l).

GLUT4 translocation

STZ induced diabetic mice showed significant decrease ($p < 0.01$) in the level of cytosolic GLUT4 protein as compared to control animals. Chronic ROSI treatment significantly reversed ($p < 0.05$) while chronic UD treatment did not modulate the diabetes mediated downregulation of cytosolic GLUT4 protein in the hippocampus (Fig. 5a). The relative abundance of membrane GLUT4 protein was significantly reduced ($p < 0.05$) in STZ induced diabetic mice when compared to control mice. Chronic UD or ROSI treatment significantly increased ($p < 0.05$) the localization of membrane GLUT4 protein in the hippocampus of diabetic mice (Fig. 5b). Neither STZ nor drugs administration modulate the membrane to cytosol ratio (m/c) of GLUT4 protein in hippocampus (Fig. 5c).

Oxidative stress and inflammation

The level of plasma TBARS was significantly increased ($p < 0.001$) in diabetic animals as compared to control animals. Chronic treatment with UD extract and ROSI significantly decreased ($p < 0.001$ and $p < 0.05$, respectively) the elevated level of TBARS in diabetic animals (Fig. 6a). STZ induced long standing diabetes significantly elevated ($p < 0.001$) the level of plasma NO as compared to control animals. Chronic UD or ROSI administration significantly attenuated ($p < 0.05$ and $p < 0.01$, respectively) the elevated level of plasma NO in diabetic mice (Fig. 6b). STZ induced diabetes significantly decreased the level of plasma catalase ($p < 0.001$) as compared to control animals. Chronic UD or ROSI treatment significantly increased ($p < 0.01$) the level of catalase in diabetic mice (Fig. 6c). The level of plasma total thiol was significantly reduced in diabetic mice ($p < 0.01$) as

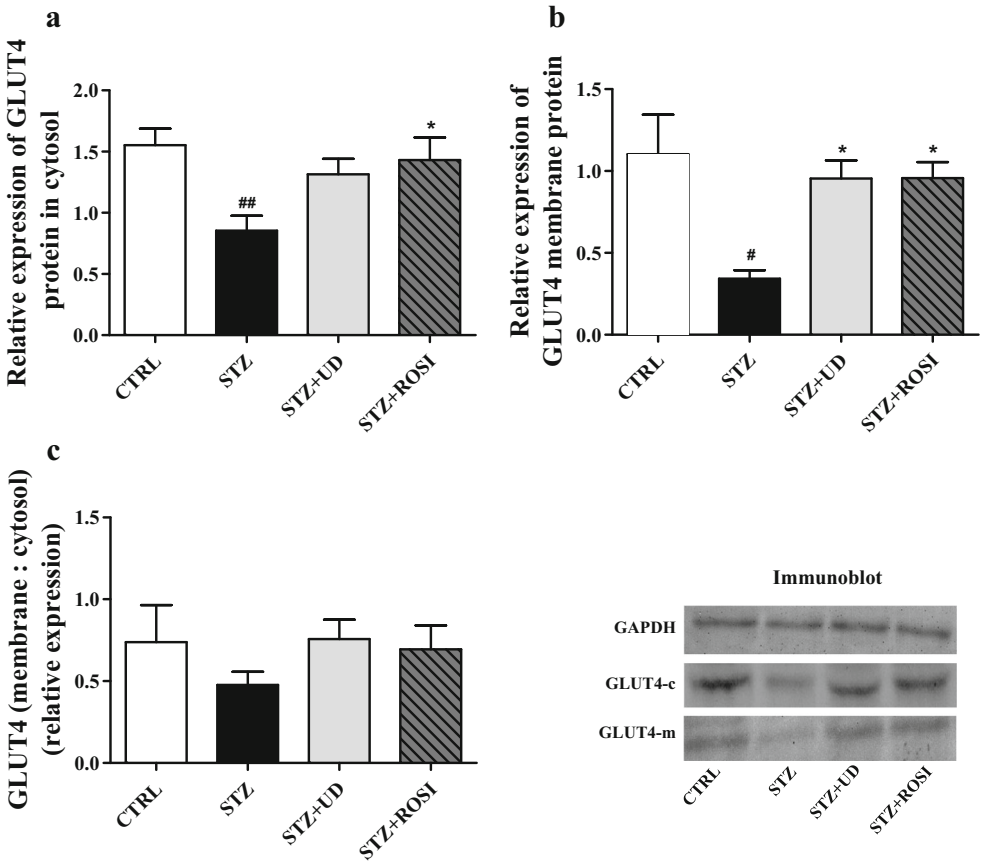

Fig. 5 Effect of UD extract on diabetes mediated alteration in the content of hippocampal GLUT4 protein in cytosol (**a**), plasma membrane (**b**) and membrane to cytosol ratio (m/c) (**c**). Data were mean ± SEM values ($n = 4$). Significant differences: #CTRL vs. STZ, *STZ vs. STZ + UD and STZ + ROSI. *$p < 0.05$, **$p < 0.01$. CTRL = control; STZ = streptozotocin; UD = *Urtica dioica*; ROSI = rosiglitazone; c = cytosol; m = membrane

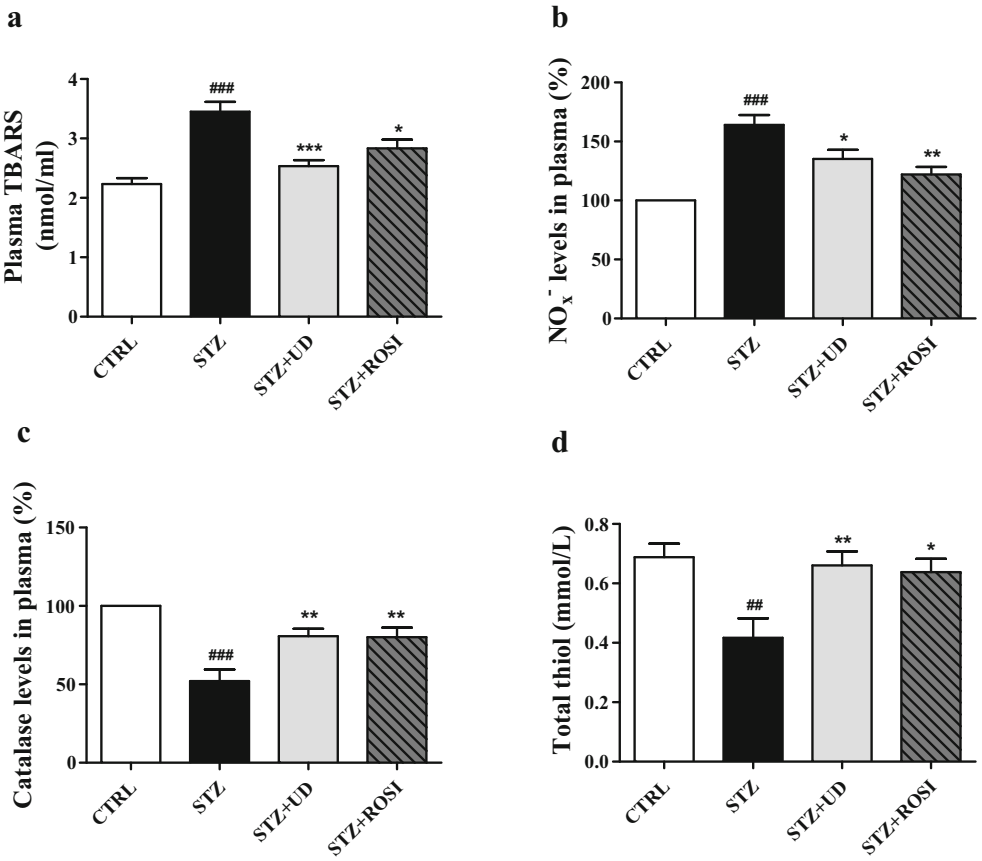

Fig. 6 Effect of UD on diabetes mediated alterations in TBARS level (**a**), nitric oxide level (**b**), catalase level (**c**) and total thiol level (**d**) in plasma. Data were mean ± SEM values ($n = 6$). Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD and STZ + ROSI. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. CTRL = control; STZ = streptozotocin; UD = *Urtica dioica*; ROSI = rosiglitazone

compared to control mice. Chronic UD or ROSI administration significantly increased the level of plasma total thiol in diabetic mice ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 6d).

Discussion

In the present study, STZ treatment significantly elevated the level of postprandial blood glucose depicting impaired glucose tolerance. Clinically, impaired glucose tolerance is associated with insulin resistance and diabetes mellitus (O'Rahilly et al. 1994). Treatment with UD or ROSI significantly reversed the STZ induced impaired glucose tolerance. In earlier study, we observed the presence of quercetin, scopoletin, esculetin, rutin and gentisic acid in UD leaves extract (Suppl. 1). UD leaves constituents like scopoletin, rutin, esculetin and quercetin are known to prevent hyperglycemia (Eid et al. 2015; Niture et al. 2014; Panda and Kar 2006; Prabakaran and Ashokkumar 2013). Current study confirms and extends earlier observations, in which UD extract has anti-diabetic property (Ahangarpour et al. 2012; Kianbakht et al. 2013; Patel and Udayabanu 2013; Qujeq et al. 2013).

STZ induced diabetic mice showed recognition memory deficit as evident from inability to recognize previously encountered object and novel object in NORT. Studies in rodents and primates revealed that visual object recognition memory process depends on the integrity and functions of hippocampus and cortex (Antunes and Biala 2012; Hammond et al. 2004). In the present study, chronic treatment with either UD or ROSI significantly reversed the recognition memory deficit associated with chronic diabetes. Quercetin attenuates colchicine induced cognitive impairment in rats (Kumar et al. 2008). Rutin is known to reverse trauma-induced memory deficit (Kumar et al. 2014). Scopoletin ameliorates long term potentiation in the hippocampus resulting in neurocognitive improvement (Hornick et al. 2011). Studies reported that PPARγ agonist ROSI improves cognitive performance in both human and animals (O'Reilly and Lynch 2012; Pipatpiboon et al. 2012).

Dysfunction in the insulin signaling alters hippocampal neurotransmitter release and cognition during diabetes (Holscher 2011). In earlier study, STZ induced diabetic mice have elevated level of fasting blood glucose which might be due to hypoinsulinemia (Patel and Udayabanu 2013) or insulin resistance. In both cases, the downstream insulin signaling impairs neuronal development and memory formation (Agrawal et al. 2011; Holscher 2011). We observed that, STZ induced diabetes significantly downregulated the mRNA expression of IR, IRS1/2, PI3K, PKB, GLUT4, INSG1 and MAPK1 in the hippocampus. We did not observe

any significant alteration in the mRNA expression of hippocampal PPARγ, ILGF 1r, GLP1 and RAGE in diabetic mice. Impairment of insulin signaling in the hippocampus is known to induce cognitive dysfunction (Agrawal et al. 2011; Holscher 2011). Chronic UD or ROSI administration upregulated PPARγ expression and restored diabetes induced alteration in mRNA expression of IR, IRS2, PI3K, PKB, GLUT4, INSG1 and MAPK1 in hippocampus. ROSI administration reversed the downregulation of IRS1 mRNA whereas UD extract administration did not modulate IRS1 mRNA expression in the hippocampus of diabetic mice. UD or ROSI administration has no effect on hippocampal ILGF 1r, GLP1 and RAGE mRNA expression. UD extract upregulated the expression level of GLUT4 mRNA in the hippocampus as well as reversed cognitive impairment in dexamethasone induced diabetic mice (Patel and Udayabanu 2014). Scopoletin, quercetin, rutin and esculetin are reported to induce the gene expression of PPARγ, a target receptor for ROSI (Cai et al. 2012; Lee et al. 2013; Surse et al. 2011; Zhang et al. 2010). ROSI mediates insulin sensitizing action in various cell types and attenuates memory deficits in STZ induced diabetic mice (Kavak et al. 2008; Liu et al. 2013).

Cognitive benefit is achievable with pharmacological interventions targeting glycemic control (Ryan et al. 2006). ROSI is known to induce IR expression via PPARγ gene transcription and ameliorate insulin sensitivity (Hernandez et al. 2003). Activation of MAPK via IR induces gene transcription, required for glucose homeostasis, cell growth, synapse growth, cell repair and maintenance (Holscher 2011). Further, MAPK pathway modulates induction of INSG1 resulting in cellular glucose homeostasis (Krapivner et al. 2007). Activation of IR attenuates apoptosis through stimulation of IRS1/2 → PI3K → Akt/PKB pathway (Holscher 2011; Schubert et al. 2003). In addition, IRS1 and 2 activations are associated with cellular glucose homeostasis and energy metabolism, achieved by activating Akt/PKB dependent transcription and translocation of GLUT4 (Gandhi et al. 2013; Holscher 2011). The level of cytosolic GLUT4 protein (Vannucci et al. 1998) and PI3K dependent GLUT4 membrane translocation in hippocampal neurons is reduced during diabetes (Kennedy et al. 1999; Piroli et al. 2007). In the present study, STZ induced diabetes showed reduced expression of cytosolic GLUT4 protein in the hippocampus of mice. Chronic UD administration did not modulate whereas ROSI treatment reversed diabetes mediated downregulation in the level of cytosolic GLUT4 protein. Moreover, chronic diabetes significantly reduced membrane GLUT4 translocation in hippocampal neurons, which was reversed by chronic UD or ROSI administration. Previous study demonstrated that, ROSI induces insulin-stimulated cellular glucose uptake via GLUT4 translocation to the plasma membrane (Hernandez et al. 2003). UD extract is known to ameliorate GLUT4 membrane translocation in L6-GLUT4myc muscle cells (Kadan et al. 2013).

In the present study, the levels of plasma TBARS and NO were increased whereas the level of catalase and total thiol were decreased in STZ induced diabetic mice. Studies revealed that elevated levels of oxidative and inflammatory markers in plasma are involved in pathophysiology of neurological disorders (Buhmann et al. 2004; Marioni et al. 2010). Our data is consistent with earlier findings that, diabetes induces oxidative and nitrative stress in hippocampus which aggravates cognitive decline in rodents (Saravanan and Ponmurugan 2012; Wang and Jia 2014). Chronic treatments with UD or ROSI reversed STZ-induced alteration in the plasma level of antioxidants catalase and total thiol as well as attenuate oxidative stress and inflammation. In earlier study, we observed that UD treatment reverse STZ induced elevated levels of oxidative and inflammatory markers in hippocampus (Patel et al. 2015). Our observations are in line with earlier studies, which showed antioxidant and anti-inflammatory effects of UD extract (Patel et al. 2015; Toldy et al. 2005).

In conclusion, UD extract modulated glucose homeostasis in hippocampus as well as showed anti-inflammatory and antioxidant effects resulting in neurocognitive improvement. Phytochemical analysis revealed the presence of flavonoids and phenolics in UD extract which is known to improve cognitive performance. UD extract might prove to be effective for diabetes and its associated central nervous system complications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

References

Agrawal R, Tyagi E, Shukla R, Nath C (2011) Insulin receptor signaling in rat hippocampus: a study in STZ (ICV) induced memory deficit model. Eur Neuropsychopharmacol 21:261–273

Ahangarpour A, Mohammadian M, Dianat M (2012) Antidiabetic effect of hydroalcholic *Urtica dioica* leaf extract in male rats with fructose-induced insulin resistance. Iran J Med Sci 37:181–186

Alipour M, Salehi I, Ghadiri Soufi F (2012) Effect of exercise on diabetes-induced oxidative stress in the rat hippocampus. Iran Red Crescent Med J 14:222–228

Antunes M, Biala G (2012) The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process 13: 93–110

Branco Cdos S, Scola G, Rodrigues AD, Cesio V, Laprovitera M, Heinzen H, Dos Santos MT, Fank B, de Freitas SC, Coitinho AS, Salvador M (2013) Anticonvulsant, neuroprotective and behavioral effects of organic and conventional yerba mate (*Ilex paraguariensis*

St. Hil.) on pentylenetetrazol-induced seizures in Wistar rats. Brain Res Bull 92:60–68

Buhmann C, Arlt S, Kontush A, Möller-Bertram T, Sperber S, Oechsner M, Stuerenburg HJ, Beisiegel U (2004) Plasma and CSF markers of oxidative stress are increased in Parkinson's disease and influenced by antiparkinsonian medication. Neurobiol Dis 15:160–170

Cai Y, Fan C, Yan J, Tian N, Ma X (2012) Effects of rutin on the expression of PPARγ in skeletal muscles of db/db mice. Planta Med 78: 861–865

Claiborne A (1985) Handbook of methods for oxygen radical research. CRC Press, Florida

Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M (2011) National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 378:31–40

Eid HM, Nachar A, Thong F, Sweeney G, Haddad PS (2015) The molecular basis of the antidiabetic action of quercetin in cultured skeletal muscle cells and hepatocytes. Pharmacogn Mag 11:74–81

Fazeli SA, Gharravi AM, Ghafari S, Jahanshahi M, Golalipour MJ (2008) The granule cell density of the dentate gyrus following administration of *Urtica dioica* extract to young diabetic rats. Folia Morphol (Warsz) 67:196–204

Gandhi GR, Stalin A, Balakrishna K, Ignacimuthu S, Paulraj MG, Vishal R (2013) Insulin sensitization via partial agonism of PPARγ and glucose uptake through translocation and activation of GLUT4 in PI3K/p-Akt signaling pathway by embelin in type 2 diabetic rats. Biochim Biophys Acta 1830:2243–2255

Grillo CA, Piroli GG, Hendry RM, Reagan LP (2009) Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent. Brain Res 1296:35–45

Hammond RS, Tull LE, Stackman RW (2004) On the delay-dependent involvement of the hippocampus in object recognition memory. Neurobiol Learn Mem 82:26–34

Hernandez R, Teruel T, Lorenzo M (2003) Rosiglitazone produces insulin sensitisation by increasing expression of the insulin receptor and its tyrosine kinase activity in brown adipocytes. Diabetologia 46: 1618–1628

Holscher C (2011) Diabetes as a risk factor for Alzheimer's disease: insulin signaling impairment in the brain as an alternative model of Alzheimer's disease. Biochem Soc Trans 39:891–897

Hornick A, Lieb A, Vo NP, Rollinger JM, Stuppner H, Prast H (2011) The coumarin scopoletin potentiates acetylcholine release from synaptosomes, amplifies hippocampal long-term potentiation and ameliorates anticholinergic- and age-impaired memory. Neuroscience 197: 280–292

Kadan S, Saad B, Sasson Y, Zaid H (2013) *In vitro* evaluations of cytotoxicity of eight antidiabetic medicinal plants and their effect on GLUT4 translocation. Evid Based Complement Alternat Med 2013:549345

Kavak S, Ayaz L, Emre M, Inal T, Tamer L, Gunay I (2008) The effects of rosiglitazone on oxidative stress and lipid profile in left ventricular muscles of diabetic rats. Cell Biochem Funct 26:478–485

Kennedy JW, Hirshman MF, Gervino EV, Ocel JV, Forse RA, Hoenig SJ, Aronson D, Goodyear LJ, Horton ES (1999) Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. Diabetes 48:1192–1197

Kianbakht S, Khalighi-Sigaroodi F, Dabaghian FH (2013) Improved glycemic control in patients with advanced type 2 diabetes mellitus taking *Urtica dioica* leaf extract: a randomized double-blind placebo-controlled clinical trial. Clin Lab 59:1071–1076

King MR, Anderson NJ, Guernsey LS, Jolivalt CG (2013) Glycogen synthase kinase-3 inhibition prevents learning deficits in diabetic mice. J Neurosci Res 91:506–514

Krapivner S, Chernogubova E, Ericsson M, Ahlbeck-Glader C, Hamsten A, van't Hooft FM (2007) Human evidence for the involvement of insulin-induced gene 1 in the regulation of plasma glucose concentration. Diabetologia 50:94–102

Kumar A, Sehgal N, Kumar P, Padi SS, Naidu PS (2008) Protective effect of quercetin against ICV colchicine-induced cognitive dysfunctions and oxidative damage in rats. Phytother Res 22:1563–1569

Kumar A, Rinwa P, Dhar H (2014) Possible nitric oxide modulation in the protective effects of rutin against experimental head trauma-induced cognitive deficits: behavioral, biochemical, and molecular correlates. J Surg Res 188:268–279

Lee SM, Moon J, Cho Y, Chung JH, Shin MJ (2013) Quercetin up-regulates expressions of peroxisome proliferator-activated receptor γ, liver X receptor α, and ATP binding cassette transporter A1 genes and increases cholesterol efflux in human macrophage cell line. Nutr Res 33:136–143

Liu LP, Yan TH, Jiang LY, Hu W, Hu M, Wang C, Zhang Q, Long Y, Wang JQ, Li YQ, Hu M, Hong H (2013) Pioglitazone ameliorates memory deficits in streptozotocin-induced diabetic mice by reducing brain β-amyloid through PPARγ activation. Acta Pharmacol Sin 34:455–463

Lupien SB, Bluhm EJ, Ishii DN (2003) Systemic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. J Neurosci Res 74:512–523

Lustman PJ, Griffith LS, Clouse RE (1988) Depression in adults with diabetes: results of 5-yr follow-up study. Diabetes Care 11:605–612

Marioni RE, Strachan MW, Reynolds RM, Lowe GD, Mitchell RJ, Fowkes FG, Frier BM, Lee AJ, Butcher I, Rumley A, Murray GD, Deary IJ, Price JF (2010) Association between raised inflammatory markers and cognitive decline in elderly people with type 2 diabetes: the Edinburgh type 2 diabetes study. Diabetes 59:710–713

More AS, Kumari RR, Gupta G, Kathirvel K, Lonare MK, Dhayagude RS, Kumar D, Kumar D, Sharma AK, Tandan SK (2012) Effect of S-methylisothiourea in acetaminophen-induced hepatotoxicity in rat. Naunyn Schmiedeberg's Arch Pharmacol 385:1127–1139

Namazi N, Esfanjani AT, Heshmati J, Bahrami A (2011) The effect of hydro alcoholic Nettle (*Urtica dioica*) extracts on insulin sensitivity and some inflammatory indicators in patients with type 2 diabetes: a randomized double-blind control trial. Pak J Biol Sci 14:775–779

Namazi N, Tarighat A, Bahrami A (2012) The effect of hydro alcoholic nettle (*Urtica dioica*) extract on oxidative stress in patients with type 2 diabetes: a randomized double-blind clinical trial. Pak J Biol Sci 15:98–102

Niture NT, Ansari AA, Naik SR (2014) Anti-hyperglycemic activity of rutin in streptozotocin-induced diabetic rats: an effect mediated through cytokines, antioxidants and lipid biomarkers. Indian J Exp Biol 52:720–727

Nouwen A, Nefs G, Caramlau I, Connock M, Winkley K, Lloyd CE, Peyrot M, Pouwer F (2011) Prevalence of depression in individuals with impaired glucose metabolism or undiagnosed diabetes: a systematic review and meta-analysis of the European Depression in Diabetes (EDID) research consortium. Diabetes Care 34:752–762

O'Rahilly S, Hattersley A, Vaag A, Gray H (1994) Insulin resistance as the major cause of impaired glucose tolerance: a self-fulfilling prophesy? Lancet 344:585–589

O'Reilly JA, Lynch M (2012) Rosiglitazone improves spatial memory and decreases insoluble Aβ(1–42) in APP/PS1 mice. J NeuroImmune Pharmacol 7:140–144

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Orcic D, Franciskovic M, Bekvalac K, Svircev E, Beara I, Lesjak M, Mimica-Dukic N (2014) Quantitative determination of plant phenolics in *Urtica dioica* extracts by high-performance liquid chromatography coupled with tandem mass spectrometric detection. Food Chem 143:48–53

Panda S, Kar A (2006) Evaluation of the antithyroid, antioxidative and antihyperglycemic activity of scopoletin from *Aegle marmelos* leaves in hyperthyroid rats. Phytother Res 20:1103–1105

Patel SS, Udayabanu M (2013) Effect of *Urtica dioica* on memory dysfunction and hypoalgesia in an experimental model of diabetic neuropathy. Neurosci Lett 552:114–119

Patel SS, Udayabanu M (2014) *Urtica dioica* extract attenuates depressive like behavior and associative memory dysfunction in dexamethasone induced diabetic mice. Metab Brain Dis 29:121–130

Patel SS, Parashar A, Udayabanu M (2015) *Urtica dioica* leaves modulates muscarinic cholinergic system in the hippocampus of streptozotocin-induced diabetic mice. Metab Brain Dis 30: 803–811

Pipatpiboon N, Pratchayasakul W, Chattipakorn N, Chattipakorn SC (2012) PPARγ agonist improves neuronal insulin receptor function in hippocampus and brain mitochondria function in rats with insulin resistance induced by long term high-fat diets. Endocrinology 153: 329–338

Piroli GG, Grillo CA, Reznikov LR, Adams S, McEwen BS, Charron MJ, Reagan LP (2007) Corticosterone impairs insulin-stimulated translocation of GLUT4 in the rat hippocampus. Neuroendocrinology 85: 71–80

Potapenko ES, Biancardi VC, Zhou Y, Stern JE (2012) Altered astrocyte glutamate transporter regulation of hypothalamic neurosecretory neurons in heart failure rats. Am J Physiol Regul Integr Comp Physiol 303:R291–R300

Prabakaran D, Ashokkumar N (2013) Protective effect of esculetin on hyperglycemia-mediated oxidative damage in the hepatic and renal tissues of experimental diabetic rats. Biochimie 95:366–373

Qujeq D, Tatar M, Feizi F, Parsian H, Sohan Faraji A, Halalkhor S (2013) Effect of *Urtica dioica* leaf alcoholic and aqueous extracts on the number and the diameter of the islets in diabetic rats. Int J Mol Cell Med 2:21–26

Ryan CM, Freed MI, Rood JA, Cobitz AR, Waterhouse BR, Strachan MW (2006) Improving metabolic control leads to better working memory in adults with type 2 diabetes. Diabetes Care 29:345–351

Saravanan G, Ponmurugan P (2012) Antidiabetic effect of S-allylcysteine: effect on thyroid hormone and circulatory antioxidant system in experimental diabetic rats. J Diabetes Complicat 26:280–285

Schubert M, Brazil DP, Burks DJ, Kushner JA, Ye J, Flint CL, Farhang FJ, Dikkes P, Warot XM, Rio C (2003) Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. J Neurosci 23:7084–7092

Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87:4–14

Surse VM, Gupta J, Tikoo K (2011) Esculetin induced changes in Mmp13 and Bmp6 gene expression and histone H3 modifications attenuate development of glomerulosclerosis in diabetic rats. J Mol Endocrinol 46:245–254

Toldy A, Stadler K, Sasvari M, Jakus J, Jung KJ, Chung HY, Berkes I, Nyakas C, Radak Z (2005) The effect of exercise and nettle supplementation on oxidative stress markers in the rat brain. Brain Res Bull 65:487–493

Vannucci SJ, Koehler-Stec EM, Li K, Reynolds TH, Clark R, Simpson IA (1998) GLUT4 glucose transporter expression in rodent brain: effect of diabetes. Brain Res 797:1–11

Wang SB, Jia JP (2014) Oxymatrine attenuates diabetes-associated cognitive deficits in rats. Acta Pharmacol Sin 35:331–338

Yates KF, Sweat V, Yau PL, Turchiano MM, Convit A (2012) Impact of metabolic syndrome on cognition and brain: a selected review of the literature. Arterioscler Thromb Vasc Biol 32:2060–2067

Zhang WY, Lee JJ, Kim Y, Kim IS, Park JS, Myung CS (2010) Amelioration of insulin resistance by scopoletin in high-glucose-induced, insulin-resistant HepG2 cells. Horm Metab Res 42:930–935