ORIGINAL ARTICLE

Antidepressant and anxiolytic like effects of *Urtica dioica* leaves in streptozotocin induced diabetic mice

Sita Sharan Patel[1] · R. S. Ray[2] · Arun Sharma[1] · Vineet Mehta[1] · Anju Katyal[2] · Malairaman Udayabanu[1]

Abstract

The present study was aimed to investigate the effect of *Urtica dioica* Linn. (UD) extract against chronic diabetes mediated anxiogenic and depressive like behavior in mice. Streptozotocin (STZ) (50 mg/kg, i.p.) for 5 consecutive days was used to induce diabetes followed by treatment with UD leaves extract (50 mg/kg, p.o.) and rosiglitazone (ROSI) (5 mg/kg, p.o.) for 8 weeks. STZ induced chronic diabetes significantly induced anxiety and depressive like behavior in mice. Chronic diabetes significantly down-regulated BDNF ($p < 0.001$), TrKB ($p < 0.001$), Cyclin D1 ($p < 0.001$), Bcl2 ($p < 0.05$) and autophagy7 ($p < 0.001$), while upregulated iNOS ($p < 0.05$) mRNA expression in the hippocampus as compared to control mice. In addition, chronic diabetes significantly increased the expression of TNF-α in CA1 ($p < 0.001$), CA2 ($p < 0.01$), CA3 ($p < 0.001$) and DG ($p < 0.001$) regions of hippocampus as compared to control mice. Chronic diabetes mediated neuronal damage in the CA2, CA3 and DG regions of hippocampus. Chronic administration of UD leaves extract significantly reversed diabetes mediated anxiogenic and depressive like behavior in mice. Further, UD treatment significantly upregulated BDNF ($p < 0.01$), TrKB ($p < 0.001$), Cyclin D1 ($p < 0.001$), Bcl2 ($p < 0.01$), autophagy5 ($p < 0.01$) and autophagy7 ($p < 0.001$), while downregulated iNOS ($p < 0.05$) mRNA expression in the hippocampus of diabetic mice. Concomitantly, UD administration significantly decreased the expression of TNF-α in hippocampal CA1 ($p < 0.001$), CA2 ($p < 0.01$), CA3 ($p < 0.001$) and DG ($p < 0.001$) regions of diabetic mice. Diabetes mediated neuronal damage and DNA fragmentation in the hippocampus was substantially attenuated following UD treatment. UD leaves extract might prove to be effective for diabetes mediated anxiety and depressive like behavior.

Keywords Anxiety · Apoptosis · Depression · Diabetes · Inflammation · *Urtica dioica*

Introduction

Diabetes mellitus is a metabolic disorder characterised by neuropathy, retinopathy, cardiomyopathy, nephropathy, etc. The World Health Organization estimated that approximately 382 million of world population got afflicted with diabetes in 2013 (Danaei et al. 2011; Patel and Udayabanu 2017). The incidence of diabetes is increasing at an alarming rate and

estimates suggest that the diabetic cases will almost double by 2035 (Forbes and Cooper 2013). Neuropathy is an important sequela of diabetes which results in central and peripheral neuronal damage mainly due to persistent high blood glucose level (Danaei et al. 2011). In the central nervous system, diabetes is reported to induce depressive like behavior, anxiety, anorexia, phobias, hypolocomotion, cognitive dysfunction etc. (Lupien et al. 2003; Lustman et al. 1988; Nouwen et al. 2011; Patel et al. 2015). A cross-sectional study revealed that prevalence of depressive like behavior and anxiety symptoms is considerably higher in diabetic patients (Collins et al. 2009). Hyperglycemia is reported to induce inflammation and apoptosis in brain, resulting in neurological disorders such as depressive like behavior and anxiety (Aksu et al. 2012; Kuhad and Chopra 2007; Patel and Udayabanu 2014). Chronic diabetes downregulated brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) expression in brain which is known induce depression and anxiety like behavior (Gholamine et al. 2016; Wang et al. 2016). The

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downregulation of autophagy indices has been associated with increased apoptosis in hippocampus, dysfunctional synaptic plasticity and ensuing neurological disorders in diabetic mice (Li et al. 2016; Patel et al. 2016c).

Urtica dioica (UD) is an herbaceous plant and commonly known as stinging nettle belonging to the family Urticaceae. UD leaves are reported to contain active constituents like acetylcholine, esculetin, gentisic acid, rutin, scopoletin, 5-hydroxytryptamine and cholineacetyltransferase (Orčić et al. 2014; Patel et al. 2015; Patel and Udayabanu 2013). In clinical trial, UD is reported to exert glycemic control in type 2 diabetic patients by lowering the levels of fasting and postprandial blood glucose (Kianbakht et al. 2012). Earlier studies demonstrated that administration of UD extract to diabetic patients improves the antioxidant capacity and reduced inflammatory stress and glycated hemoglobin (Namazi et al. 2011; Namazi et al. 2012). Alcoholic and aqueous extract of UD leaves prevented the diabetes mediated pancreatic tissue injury in rats (Qujeq et al. 2013). In earlier studies from our lab, we documented that UD extract alleviated chronic diabetes related cognitive dysfunction via insulin and cholinergic signaling pathways (Patel et al. 2016a; Patel et al. 2015). UD treatment significantly reduced hyperglycemia, hypercorticosteronemia, oxidative stress and depressive like behavior in dexamethasone treated diabetic mice (Patel and Udayabanu 2014). The present study was designed to investigate the effect of UD extract on depressive and anxiogenic like behaviors in streptozotocin (STZ) induced male diabetic mice.

## Material and methods

Plant material

UD leaves were collected from the North Western Himalayan region and authenticated from Dr. Y.S. Parmar University of Horticulture & Forestry, India. Leaves of UD (specimen number-12399) were dried in shade, pulverized and passed through 40 mesh sieve. The extraction of UD leaves was performed at room temperature with constant shaking for 48 h using the solvent methanol and water (1:1). The extract thus obtained was filtered, centrifuged, evaporated and lyophilized. The yield of lyophilized UD extract was observed as 17.9% (*w*/w).

Animals and drug treatment

Male Swiss albino mice (25-30 g) were housed under a 12 h light/dark cycle at 24 ± 2 °C. The mice had access to water and food ad libitum. All animal experiments were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional Animal Ethical Committee clearance. All efforts were made to reduce the number of animals used to minimize animal suffering. Animals ($n = 32$) were randomly divided into two groups, viz., G1 – normal control group ($n = 8$) and the rest of the animals ($n = 24$) were administered with STZ (50 mg/kg, i.p.) for 5 consecutive days. All groups were monitored for blood glucose levels. The STZ treated animals having consistently high blood glucose levels (≥ 200 mg/dl) were further randomly divided into three groups ($n = 8$), viz., G2 – STZ, G3 – STZ + UD (50 mg/kg) and G4 – STZ + rosiglitazone (ROSI) (5 mg/kg). The dose of UD extract and ROSI used in the present study was selected from previous report (Patel and Udayabanu 2013; Patel et al. 2015). Hydroalcoholic extract of UD or ROSI or vehicle (0.3% carboxymethyl cellulose in water for injection) was administered once daily through oral route from the 6th day after STZ injection till 60th day. Behavioural studies like forced swim test, tail suspension test and elevated plus maze task were performed after drug administration on day 60. On day 61, mice were sacrificed for qPCR and DNA fragmentation studies. Thereafter, animals were perfused and sacrificed for histology studies (Fig. 1).

Fig. 1 Schematic representation of the experimental design: animals were treated with STZ (50 mg/kg, i.p.) for five consecutive days followed by UD or ROSI treatment. Thereafter, animals were subjected to different behavioural, molecular and histology studies. FST = forced swim test, TST = tail suspension test, EPM = elevated plus maze task
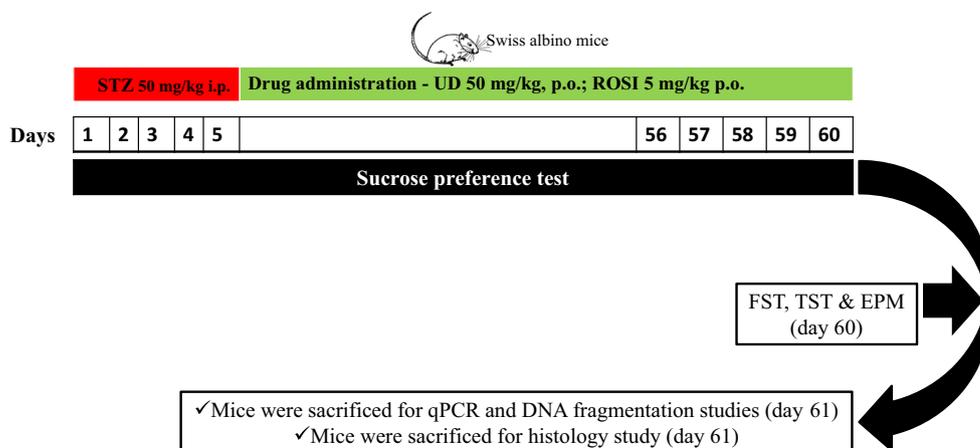
Behavioral studies

Forced swim test (FST)

Chronic diabetes is a risk factor for the progression of many psychopathological conditions including major depression. The FST is an animal behavioral test used for screening of antidepressant drugs, antidepressant efficacy of new drugs and experimental manipulations that are aimed to prevent or render depressive-like behavior. The animals were individually forced to swim in a cylinder having the radius of 24 cm and the height 25 cm filled with water (26 ± 2 °C) up to 18 cm of height. An animal was considered immobile whenever it remained floating passively in the water in upright position and its nose just above the surface of water. The total immobility time of each mouse during the 6 min test was recorded (Patel et al. 2016c).

Tail suspension test (TST)

The TST is a rodent behavioral test useful for the evaluation of potential antidepressant drugs and assessing other manipulations that are expected to affect depressive like state. In this test, mice were individually suspended on the edge of a shelf, 60 cm above a table top with the help of adhesive tape placed approximately 1 cm from the tip of the tail. Animals were considered immobile when they hang passively and motionless. The duration of immobility was recorded for the periods of 6 min (Patel et al. 2016c).

Sucrose preference test (SPT)

The SPT is a reward-based test used as an indicator of anhedonia in rodents. Anhedonia is the reduced ability to experience pleasure which represents the symptoms of depression. SPT was conducted as described previously (Banasr and Duman 2008). Briefly, mice were habituated for 48 h to 1% sucrose, and following a 4 h of deprivation, the preference for sucrose (1%) or water was determined for 1 h. Sucrose habituation test was performed during base line but not during diabetes. Sucrose preference was determined regularly and calculated using formula: Sucrose preference (%) = [Sucrose intake/(Sucrose intake + Water intake)]×100.

Elevated plus maze task (EPM)

The EPM is one of the most widely used tests for screening drugs against anxiety-like behaviour in rodents. Elevated plus maze apparatus consists of four arms including two open arms (50 cm × 5 cm) and two closed arms (50 cm × 5 cm) with 28-cm-high black walls, elevated 50 cm above the floor. The open arm and closed arm were connected via a central square (5 × 5 cm). The 10-min habituation test (day 59) was conducted before the main experiment. Thereafter, the animals were individually kept at the centre of EPM and the number of entries and the time spent in the open arms were recorded during the 5-min test (day 60) (Zhao et al. 2015).

Real-time quantitative reverse transcription PCR (qPCR)

Animals were sacrificed by cervical dislocation and the hippocampal RNA was isolated using TRIzol reagent (Invitrogen). The reliability of RNA was checked on 2.0% agarose gel electrophoresis and quantified using spectrophotometer (ND-2000C, Thermo Scientific). The reverse transcription of 3 μg of total RNA was performed using the First strand 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 (Bio-Rad). Reaction was carried out in total volume of 12.5 μl containing 2.5 pM of each primer (BDNF FP: 5′-ATG TCT ATG AGG GTT CGG CG-3′, RP: 5'-GCG AGT TCC AGT GCC TTT TG-3′; TrkB FP: 5'-ACT GTG AGA GGC AAC CCC AA-3′, RP: 5'-ATC ACC AGC AGG CAG AAT CC-3′; Cyclin D1 FP: 5'-GCG TAC CCT GAC ACC AAT CTC-3′, RP: 5'-ACT TGA AGT AAG ATA CGG AGG GC-3′; Bcl2 FP: 5'-GGC TGA GCA CTA CCT TCA GTA-3′, RP: 5'-TGG CGG TAT CTA TGG ATT CCA C-3′; AIP2 FP: 5'-AGC TTG GTG TCT GTT CTC TGT-3′, RP: 5'-TGG AGG GAA GAT AGG TCC CAC-3′; ATG5 FP: 5'-CTC GCT AGA TGG AAC CAC-3′, RP: 5'-AGT GGT CCT GTG TGT CTC-3′; ATG7 FP: 5'-TGG CTG CTA CTT CTG CAA TGA TGT-3′, RP: 5'-CAG GAC AGA GAC CAT CAG CTC CAC-3′; iNOS FP: 5'-GTT CTC AGC CCA ACA ATA CAA GA-3′, RP: 5'-GTG GAC GGG TCG ATG TCA C-3′; IL6 FP: 5′-GGT GCC CTG CCA GTA TTC TC-3′, RP: 5'-GGC TCC CAA CAC AGG ATG A-3′) and 1 μl of cDNA template containing 100 ng cDNA. The thermal cycler condition for cDNA amplification was kept at: 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 reference 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).
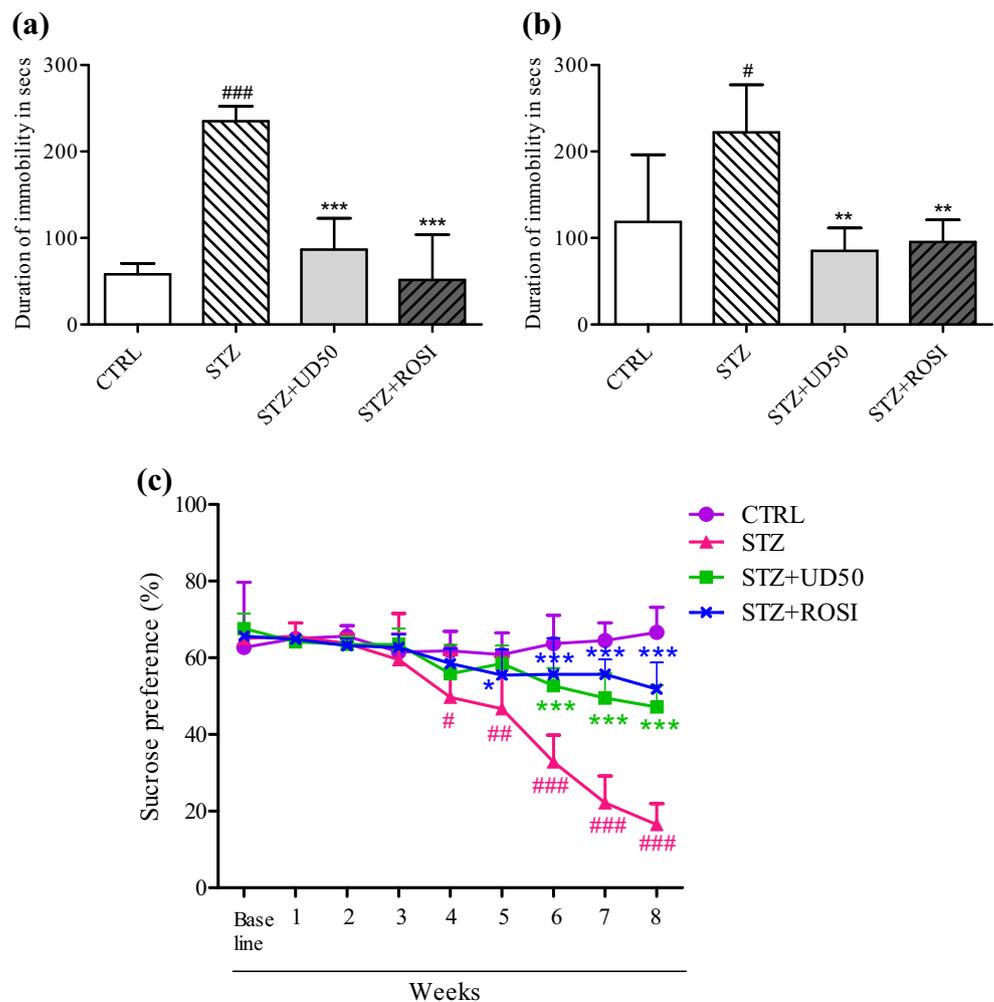
Immunofluorescence & Histopathology

The mice were anesthetized with pentobarbital sodium (50 mg/kg i.p.) and intracardially perfused with 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde contained in PBS. Thereafter, the whole brain was isolated and stored in 30% sucrose and 10% glycerol solution. Hippocampal sections of 4 μm were made using cryotome (CM 1850 Leica, Heidelberg, Germany). For immunofluorescence study, the hippocampal sections were permeabilized with 0.5% Triton X-100 in PBS for 10 min and then blocked with 3% BSA in PBS for 1 h at room temperature followed by overnight incubation with rabbit polyclonal IgG anti-mouse tumor necrosis factor-α

(TNF-α) (1 : 100) primary antibody. Thereafter, the sections were washed three times with PBS and incubated with fluorescein isothiocyanate (FITC) labeled respective secondary antibody for 2:00 h in a dark place and counterstained with DAPI. The sections were mounted using glycerol/PBS (9 : 1), observed at 40X objectives on Olympus microscope and images were captured. For histopathology study, the hippocampal sections were subjected to hematoxylin and eosin staining and images were captured at 40X using Olympus microscope.

DNA fragmentation

The hippocampal DNA was isolated using TRIzol reagent as per manufacturer instructions. Approximately 10 μg DNA was loaded in each lane of 0.8% agarose gel stained with ethidium bromide (0.5 mg/ml) and run at 70 V to separate the DNA. A 1 Kb DNA ladder was used as a standard (Dhote and Balaraman 2008). Because extensive DNA fragmentation is an important characteristic of programmed cell death, observation of DNA breaks could greatly facilitate the identification of apoptotic cells.

Statistical analysis

All the data were expressed as mean ± SD. The statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test using GraphPad Prism software. A confidence level of $p < 0.05$ was considered significant.

Results

Forced swim test

STZ induced diabetic mice showed significant increase in the duration of immobility in forced swim test ($p < 0.001$ vs. CTRL). Chronic UD administration significantly reduced the duration of immobility in diabetic mice ($p < 0.001$ vs. STZ) comparable to the chronic ROSI treatment ($p < 0.001$ vs. STZ). There is no statistically significant difference in ROSI and UD groups (Fig. 2a).

Fig. 2 Effect of UD extract on diabetes mediated depressive like behavior in FST (**a**), TST (**b**) and sucrose preference test (**c**). All data in the figures are represented as mean ± SD values ($n = 8$). 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

Tail suspension test

STZ treated diabetic mice showed significant increase in the duration of immobility in tail suspension test ($p < 0.05$ vs. CTRL). Chronic UD administration significantly reduced the duration of immobility in diabetic mice ($p < 0.01$ vs. STZ) comparable to chronic ROSI treatment ($p < 0.01$ vs. STZ). In addition, we did not observe any significant alteration in duration of immobility between UD and ROSI treated diabetic animals (Fig. 2b).

Sucrose preference test

Before STZ and drug treatment regimens, the basal level of sucrose preference was not significantly altered among the groups ($p > 0.05$). The sucrose preference parameter was not significantly altered up to 1–3 weeks in any of the groups. Chronic STZ treatment significantly reduced the sucrose preference on week 4 ($p < 0.05$ vs. CTRL), week 5 ($p < 0.01$ vs. CTRL), week 6 ($p < 0.001$ vs. CTRL), week 7 ($p < 0.001$ vs. CTRL) and week 8 ($p < 0.001$ vs. CTRL) as compared to control mice. The trend was not noticed following chronic UD or ROSI treated groups. Chronic UD administration significantly increased the sucrose preference on week 5 ($p < 0.05$ vs. STZ), week 6 ($p < 0.001$ vs. STZ), week 7 ($p < 0.001$ vs. STZ) and week 8 ($p < 0.001$ vs. STZ) in diabetic mice. Chronic ROSI administration significantly increased the sucrose preference on week 6 ($p < 0.001$ vs. STZ), week 7 ($p < 0.001$ vs. STZ) and week 8 ($p < 0.001$ vs. STZ) in diabetic mice (Fig. 2c).

Elevated plus maze task

STZ induced diabetic mice showed significantly decreased ($p < 0.001$ vs. CTRL) number of open arms entries in EPM as compared to control mice. Number of entries in open arms was significantly increased by chronic UD ($p < 0.05$ vs. STZ) or ROSI ($p < 0.01$ vs. STZ) administration in diabetic mice. In addition, the time spent in open arms was significantly reduced ($p < 0.01$ vs. CTRL) in diabetic mice as compared to control mice. Time spent in open arms was significantly increased by chronic UD ($p < 0.05$ vs. STZ) or ROSI ($p < 0.01$ vs. STZ) administration in diabetic mice. In addition, there is no statistically significant difference in number of open arms entries and time spent in open arms between ROSI and UD treated diabetic mice (Fig. 3a, b).

Molecular alterations in hippocampus

The relative expression of BDNF, TrkB and Cyclin D1 mRNA was significantly downregulated in the hippocampus of chronically diabetic mice ($p < 0.001$ vs. CTRL). Chronic UD administration significantly increased BDNF ($p < 0.01$ vs. STZ), TrkB ($p < 0.001$ vs. STZ) and Cyclin D1 ($p < 0.001$ vs. STZ) mRNA expression in the hippocampus of diabetic mice. Besides, chronic ROSI treatment also significantly increased ($p < 0.001$ vs. STZ) the mRNA expression of hippocampal BDNF, TrkB and Cyclin D1 in diabetic mice. There is no statistically significant difference in the level of BDNF expression in ROSI and UD groups. However, UD administration significantly increased TrkB ($p < 0.001$ vs. STZ + ROSI) and ROSI upregulated cyclin D1 expression ($p < 0.001$ vs. STZ + UD) in the hippocampus of diabetic mice (Fig. 4a-c).

Chronic diabetes significantly downregulated B-cell lymphoma 2 (Bcl2) and autophagy 7 (ATG7) ($p < 0.05$ vs. CTRL and $p < 0.001$ vs. CTRL, respectively), while did not modulate apoptosis inhibitory protein (AIP2) and autophagy 5 (ATG5) ($p > 0.05$ vs. CTRL) mRNA expressions in the hippocampus. Chronic UD administration significantly upregulated the
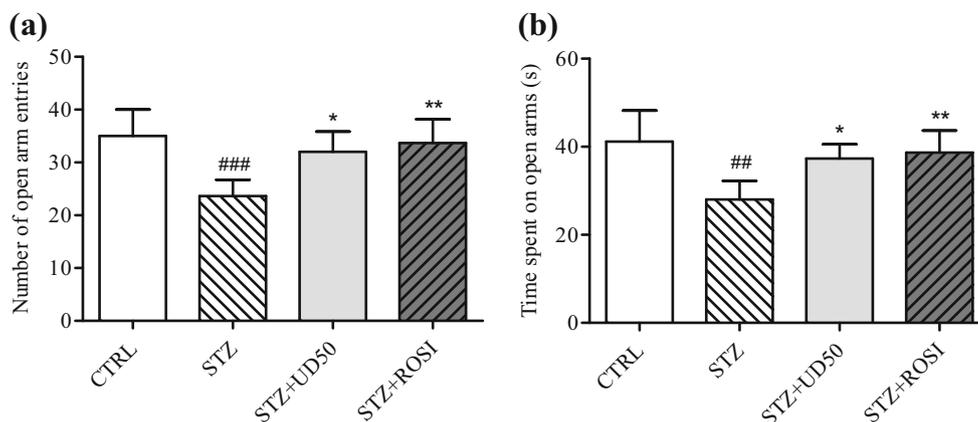

(a)

(b)

Fig. 3 Effect of UD extract on diabetes mediated anxiety like behavior in elevated plus maze task: number of entries in open arms (**a**) and time spent in open arms (**b**). All data in the figures are represented as mean ± SD values ($n = 8$). 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
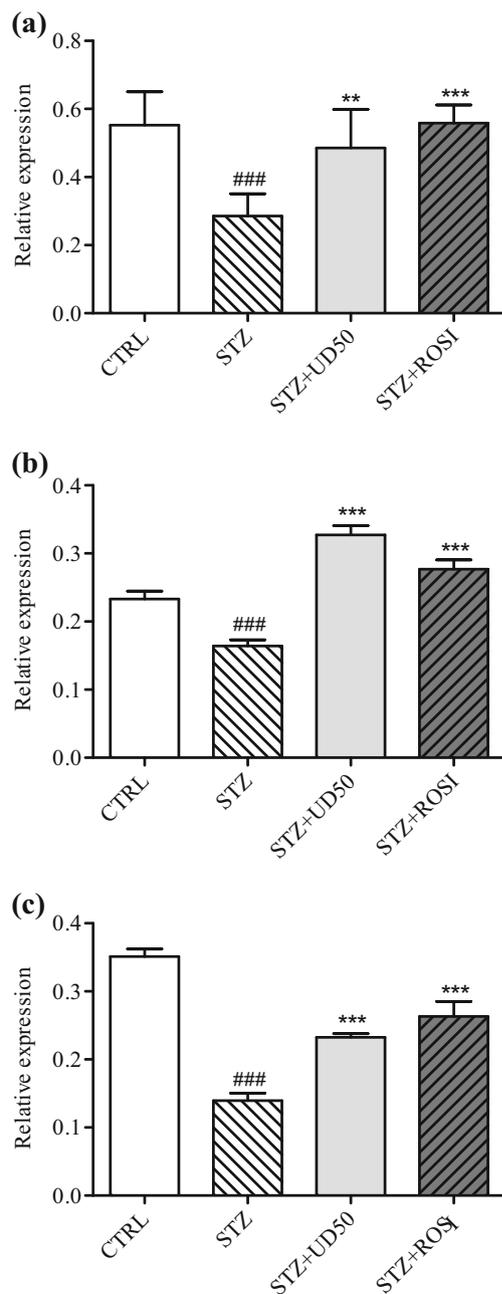
(a)

(b)

(c)

Fig. 4 Effect of UD extract on diabetes mediated alterations in the mRNA expression of hippocampal BDNF (**a**), TrKB (**b**) and cyclin D1 (**c**). All data in the figures are represented as mean ± SD values ($n = 4$). 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

mRNA expression of Bcl2 ($p < 0.01$ vs. STZ), ATG5 ($p < 0.01$ vs. STZ) and ATG7 ($p < 0.001$ vs. STZ) in the hippocampus of diabetic mice. Chronic ROSI administration also significantly upregulated the mRNA expression of Bcl2 ($p < 0.05$ vs. STZ), ATG5 ($p < 0.01$ vs. STZ) and ATG7 ($p < 0.001$ vs. STZ) in the hippocampus of diabetic mice. Neither UD nor ROSI administration modulate the mRNA expression of AIP2

($p > 0.05$ vs. STZ) in the hippocampus of diabetic mice. In addition, we did not observe any significant alteration in the mRNA expression of hippocampal Bcl2, AIP2, ATG5 and ATG 7 between UD and ROSI treated diabetic animals (Fig. 5a-d).

STZ induced diabetic mice showed significantly upregulated mRNA expression of inducible isoform of nitric oxide synthase (iNOS) ($p < 0.05$ vs. CTRL) in hippocampus. Chronic UD and ROSI administration significantly downregulated the mRNA expression of hippocampal iNOS in diabetic mice ($p < 0.05$ vs. STZ and $p < 0.01$ vs. STZ, respectively). Chronic diabetes did not alter the mRNA expression of interleukin 6 (IL6) in hippocampus ($p > 0.05$ vs. CTRL). In addition, chronic UD or ROSI treatment had no effect on the level of IL6 in hippocampus of diabetic mice. There is no statistically significant difference in the level of hippocampal iNOS and IL6 between ROSI and UD treated diabetic groups ($p > 0.05$ vs. STZ) (Fig. 5e-f).

STZ induced diabetes significantly upregulated expression level of TNF-α in CA1 ($p < 0.001$ vs. CTRL), CA2 ($p < 0.01$ vs. CTRL), CA3 ($p < 0.001$ vs. CTRL) and DG ($p < 0.001$ vs. CTRL) regions of hippocampus. Chronic UD administration significantly downregulated the expression level of TNF-α in CA1 ($p < 0.001$ vs. STZ), CA2 ($p < 0.01$ vs. STZ), CA3 ($p < 0.001$ vs. STZ) and DG ($p < 0.001$ vs. STZ) regions comparable to ROSI in the hippocampus of diabetic mice. In addition, we did not observe any significant alterations in the level of hippocampal TNF-α between UD and ROSI treated diabetic animals (Fig. 6a-d).

Neuronal damage

Examination of the hematoxylin & eosin stained brain sections showed that neither diabetes nor UD or ROSI administration altered the neuronal morphology in CA1 region of hippocampus. However, chronic diabetes induced neuronal damage in CA2, CA3 and dentate gyrus region of hippocampus. Besides, chronic UD or ROSI treated diabetic mice showed relatively restored neuronal damage in CA2, CA3 and dentate gyrus region of hippocampus (Fig. 7).

DNA fragmentation

On agarose gel electrophoresis, diabetic mice showed strong evidence of DNA fragmentation in the hippocampus as observed through DNA laddering pattern, which is a characteristic of oligoneucleosomal DNA fragmentation. Treatment with UD or ROSI resulted in relatively decreased DNA fragmentation in diabetic mice. Although, visualization of DNA fragmentation using ethidium bromide is not quantitative estimation, the observation of decreased DNA fragmentation in the UD or ROSI treated diabetic mice could be attributed to
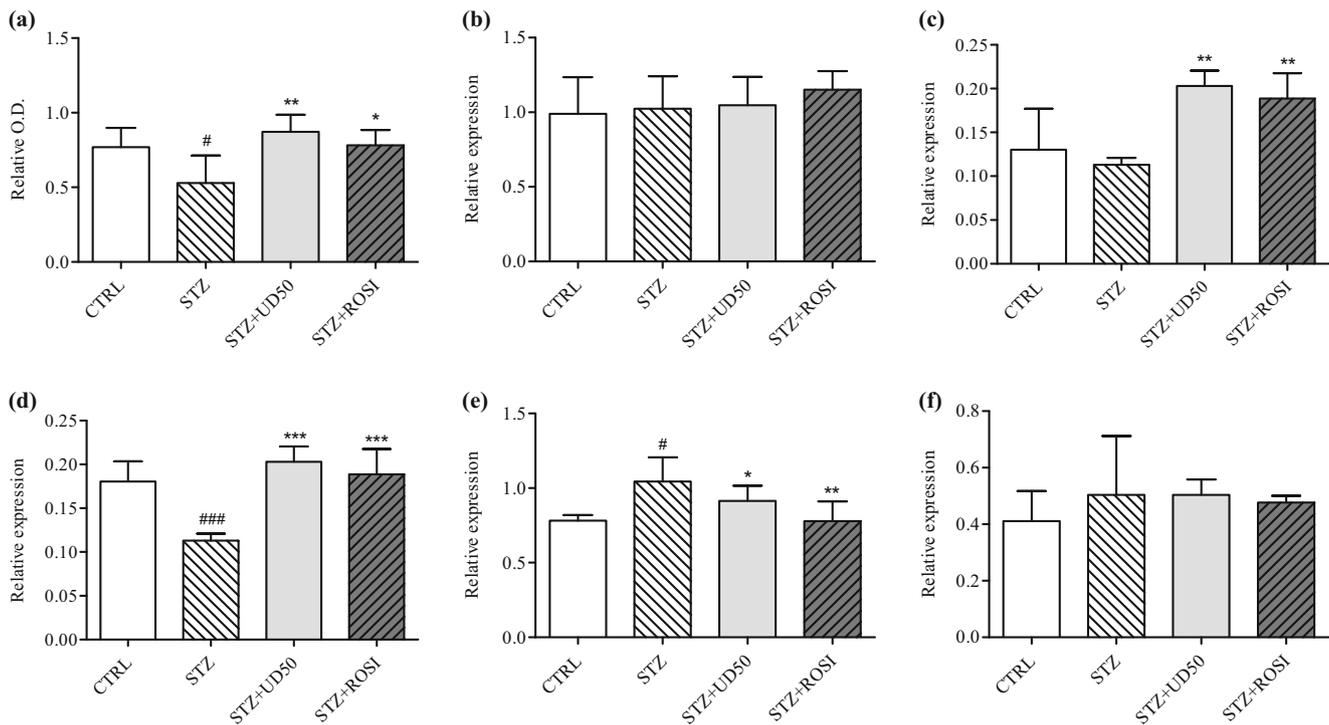
Fig. 5 Effect of UD extract on diabetes mediated alterations in the mRNA expression of hippocampal Bcl2 (**a**), AIP2 (**b**), ATG5 (**c**), ATG7 (**d**), iNOS (**e**) and IL6 (**f**). All data in the figures are represented as mean ± SD values ($n = 4$). Significant differences: [#]CTRL vs. STZ, [*]STZ vs. STZ + UD and STZ + ROSI. [*]$p < 0.05$, [**]$p < 0.01$, [***]$p < 0.001$. CTRL = control; STZ = streptozotocin; UD = *Urtica dioica*; ROSI = rosiglitazone

Discussion

In our earlier study, we established that chronic low dose of STZ administration significantly induced type 2 diabetes in mice. In addition, chronic UD or ROSI administration significantly reduced hyperglycemia and postprandial blood glucose in diabetic mice (Suppl. 1). In the present study, we observed that chronic experimental diabetes resulted in depressive like behavior in mice as evidenced by increased immobility in FST and TST, which is in line with previous report (Tang et al. 2015). In addition, chronic diabetes significantly reduced the sucrose preference from week 4 onwards which also represents behavioral change like depression in mice (Wang et al. 2009). Chronic UD or ROSI treatment significantly reduced the duration of immobility associated with diabetes and reversed depressive behaviour from week 6 onwards in diabetic mice. Earlier report suggested that UD administration significantly reverse depressive like behavior against chronic dexamethasone induced diabetes. In our earlier study, we observed that hydroalcoholic extract of UD leaves contain scopoletin, quercetin, rutin, gentisic acid and esculetin (Suppl 2). UD constituents like scopoletin,

quercetin, rutin and esculetin are reported to alleviate depressive like behavior in rodents (Capra et al. 2010; Holzmann et al. 2015; Machado et al. 2008; Zhu et al. 2016). ROSI administration is also known to significantly alleviate depressive like behavior in diabetic mice (Patel and Udayabanu 2014). In EPM task, we observed that chronic diabetes mediate anxiety like behavior in mice evident from decreased number of entries and time spent in open arms which is according to the findings of Tang et al. (Tang et al. 2015). UD constituents like scopoletin, quercetin, rutin, gentisic acid and esculetin are reported to alleviate anxiety like behavior in rodents (Merzoug et al. 2014; Monsef-Esfahani et al. 2013; Su et al. 2014; Sulakhiya et al. 2016) although the mechanism of action is poorly understood in these studies. The standard drug ROSI also reduced the risk of anxiety associated with chronic diabetes (Montori et al. 2007). We speculate that constituents of UD leaf extract viz. scopoletin, quercetin, rutin, gentisic acid and esculetin can directly alleviate the anxiety behaviour of mice in the present experimental set up. In our earlier study, chronic UD or ROSI administration did not reverse hypolocomotion induced by chronic diabetes (Patel et al. 2016a), thus the antidepressant and anxiolytic like activity of UD leaves extract is unrelated to locomotor response.

It has been documented that depressive and anxiety like behavior depends on the integrity and function of hippocampal cells (Ge et al. 2016). We observed that, hippocampal

Fig. 6 Effect of UD extract on diabetes mediated alterations in the expression of TNF-α in CA1 (**a**), CA2 (**b**), CA3 (**c**) and DG (**d**) regions of hippocampus. All data in the figures are represented as mean ± SD values ($n = 4$). 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. Blue fluorescence in the figure indicates nucleus stained with DAPI whereas green fluorescence indicates TNF-α expression
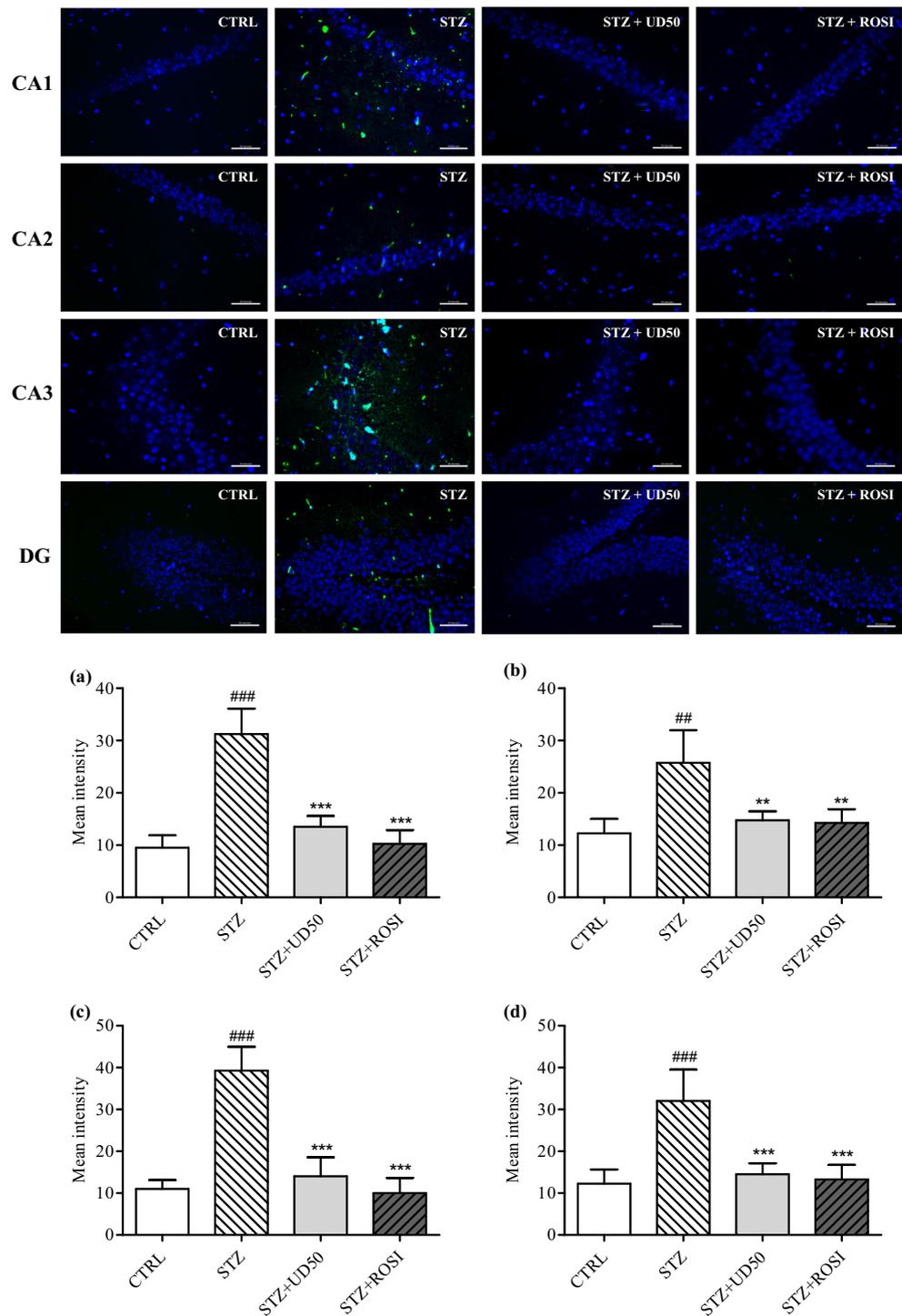
mRNA expression of BDNF, TrkB and cyclin D1 was downregulated in STZ induced diabetic mice. This is in consonance with a recent study documenting the downregulation of BDNF and tyrosine kinase B (TrkB) expression in the hippocampus of diabetic mice showing depressive like behavior (Wang et al. 2016). Chronic hypercorticosteronemia mediated diabetes (Patel and Udayabanu 2014) is also associated with decreased BDNF and its receptor TrkB expression in

hippocampus (Lee et al. 2015). Evidence suggests that, binding of BDNF to receptor TrkB activates MAPK/PI3K/ cyclic adenosine monophosphate response element binding protein pathway in hippocampus, resulting in cell growth, survival and synaptic plasticity. Activation of cyclic adenosine monophosphate -response element binding protein pathway mediates the expression of cyclin D1 (Patel et al. 2016b). Downregulation of cyclin D1 expression in hippocampus is
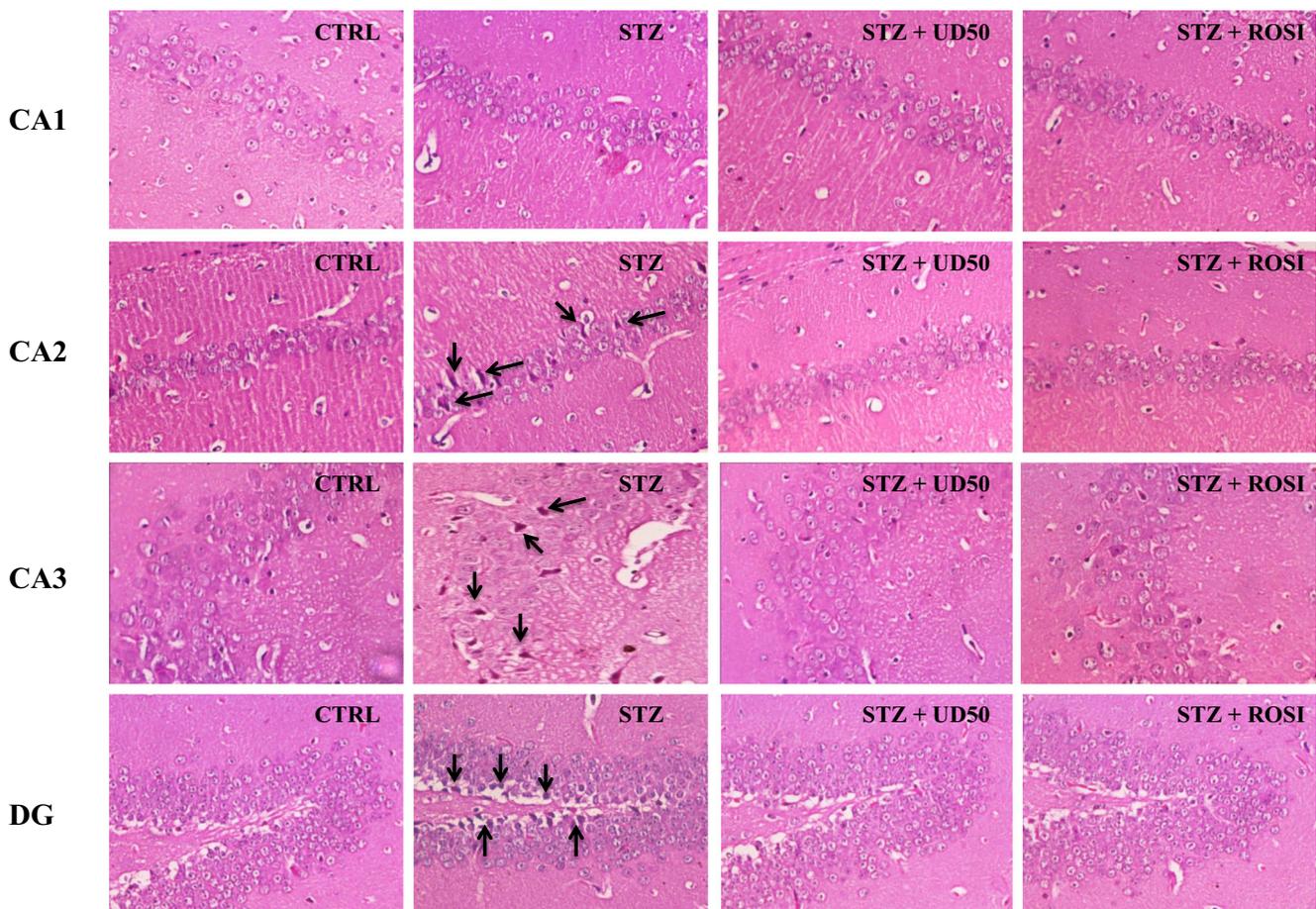
Fig. 7 Effect of UD extract on diabetes mediated neuronal damage in the CA1, CA2, CA3 and DG regions of hippocampus. Vehicle-treated STZ group shows neuronal loss, neuronal shrinkage and dark staining of neurons in CA2, CA3 and DG region. In contrast UD and ROSI treatment reduced this neuronal damage in diabetic mice (Magnification 400X). Arrow in the hippocampus of diabetic mice indicates neuronal damage

associated with anxiety and depressive like behavior in rodents. In contrast, upregulation of cyclin D1 expression in hippocampus exerted anxiolytic and antidepressant like effect (Ge et al. 2016). We observed that chronic UD or ROSI administration reverse the diabetes mediated downregulation of BDNF, TrkB and cyclin D1 expression in hippocampus. UD extract is reported to upregulate the mRNA expression of BDNF, TrkB and cyclin D1 expression in the hippocampus of chronically stressed mice in a separate study from our lab (Patel et al. 2016b). Quercetin is reported to increase the expression of BDNF and associated with the neuronal adaptation in hippocampus (Liu et al. 2015). Rutin activate BDNF gene expression in the hippocampus and attenuated neurotoxicity in rats (Moghbelinejad et al. 2014). Esculetin exhibited antidepressant like effects through the activation of BDNF/TrkB signaling in hippocampus (Zhu et al. 2016). Earlier study reported that ROSI administration ameliorates BDNF expression in hippocampus and reverse neuronal dysfunction in diabetic mice (Kariharan et al. 2015).

Chronic diabetes mediates apoptosis, autophagic inhibition and inflammation of hippocampal neurons resulting in behavioral dysfunctions (Li et al. 2016; Patel et al. 2015). Herein, chronic diabetes significantly decreased the mRNA expression of antiapoptotic Bcl2 gene in hippocampus. Report suggests that, lowered level of Bcl2 gene expression in hippocampus is associated with depressive and anxiogenic like behavior (Liu et al. 2016). Chronic diabetes did not modulate the level of AIP2 in the hippocampus. Chronic diabetes significantly downregulated the levels of ATG7, while did not modulate ATG5 levels in the hippocampus. On agarose gel electrophoresis, diabetic mice showed strong evidence of hippocampal DNA fragmentation as observed through DNA laddering pattern, which has been established as characteristic feature of oligonucleosomal DNA fragmentation and apoptosis by several scientists (Dhote and Balaraman 2008). Chronic diabetes also leads to significant increase in the level of inflammatory marker iNOS in hippocampus. Gene expression results indicate that, chronic UD or ROSI administration significantly increased Bcl2, ATG5 and ATG7, and decreased the expression of iNOS in hippocampus. We suggest the antiapoptotic, autophagic stimulation and anti-inflammatory action of UD and ROSI in the diabetic mice. Chronic UD or ROSI administration
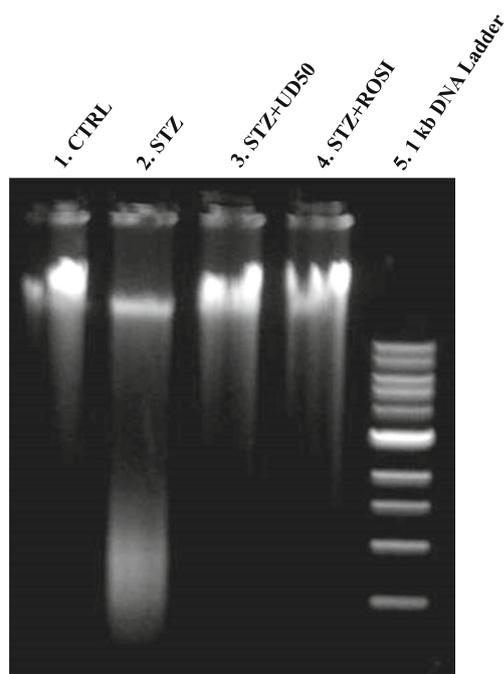
Fig. 8 Effect of UD extract on diabetes mediated fragmentation of DNA in hippocampus: Lane-1, control mice without any prominent DNA fragmentation; Lane-2, STZ-treated diabetic mice displaying a laddering pattern; Lane-3, UD (50 mg/kg) treated diabetic mice with reduced DNA laddering pattern; Lane-4, ROSI (5 mg/kg) treated diabetic mice showing reduced DNA fragmentation; Lane-5, 1 kb DNA standard

did not modulate the level of hippocampal AIP2 and IL6 in diabetic mice. In the present study, chronic diabetes significantly increased the expression of TNF-α in hippocampal regions including CA1, CA2, CA3 and dentate gyrus. Earlier study suggests that, diabetes leads to substantial increase of TNF-α levels in hippocampus (Jawale et al. 2016) which is known to mediate anxiety and depressive-like behavior (Jangra et al. 2016). Chronic UD or ROSI administration significantly reduced the expression of TNF-α in CA1, CA2, CA3 and dentate gyrus regions of hippocampus. UD extract was found to be an effective anti-inflammatory and antiapoptotic supplement promoting neuronal survival (Patel and Udayabanu 2014; Toldy et al. 2005). UD constituents like scopoletin, quercetin, rutin, gentisic acid and esculetin are known to exert anti-inflammatory (Anand et al. 2013; Ding et al. 2008; Fernandez et al. 1998; Guardia et al. 2001) and antiapoptotic effects (Ishikawa and Kitamura 2000; Jeong et al. 2009; Karnewar et al. 2016). Besides, ROSI is reported to attenuate inflammation and apoptosis in hippocampus and alleviated depressive like behaviour in mice (Patel et al. 2016c).

Histological analysis revealed that chronic diabetes mediates progressive alterations (disorganization in the pyramidal cellular arrangement, dense cytosolic staining and disruption of nucleus) in the CA2, CA3 and DG regions of the hippocampus. Study suggests that STZ induced diabetes is known to disorganize neuronal morphology in the hippocampus resulting in

neuronal damage (El-Akabawy and El-Kholy 2014). Interestingly, UD or ROSI administration noticeably attenuated those alterations and improved the neuronal architecture. These results demonstrated that UD reduce brain damage and ameliorate functional outcome during chronic diabetes. The beneficial effect of UD administration on N-methyl-D-aspartate induced brain lesion in rat model has been reported (Toldy et al. 2009). Earlier studies reported that, UD leaves constituents reduce diabetes as well as neurological deficits in different experimental models (Capra et al. 2010; Holzmann et al. 2015; Machado et al. 2008; Patel et al. 2016a; Zhu et al. 2016), which might indicate UD extract constituents cross the blood brain barrier. Although, we did not performed the estimation of UD constituent in brain thus the anti-depressant and anti-anxiety effects might be related to its anti-hyperglycemic effect.

In conclusion, UD extract attenuated anxiogenic and depressive like behavior alongside autophagic stimulation, antiapoptotic and anti-inflammatory effects. Phytochemical analysis revealed the presence of gentisic acid, quercetin, scopoletin, esculetin and rutin in UD leaves extract, which might be involved in the reversal of diabetes mediated anxiogenic and depressive like behaviour and needs further investigation. UD extract might prove to be effective for diabetes mediated anxiety and depressive like behavior.

Compliance with ethical standards

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

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