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ORIGINAL PAPER

Urtica dioica extract attenuates depressive like behavior and associative memory dysfunction in dexamethasone induced diabetic mice

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Abstract Evidences suggest that glucocorticoids results in depression and is a risk factor for type 2 diabetes. Further diabetes induces oxidative stress and hippocampal dysfunction resulting in cognitive decline. Traditionally *Urtica dioica* has been used for diabetes mellitus and cognitive dysfunction. The present study investigated the effect of the hydroalcoholic extract of *Urtica dioica* leaves (50 and 100 mg/kg, p.o.) in dexamethasone (1 mg/kg, i.m.) induced diabetes and its associated complications such as depressive like behavior and cognitive dysfunction. We observed that mice administered with chronic dexamethasone resulted in hypercortisolemia, oxidative stress, depressive like behavior, cognitive impairment, hyperglycemia with reduced body weight, increased water intake and decreased hippocampal glucose transporter-4 (GLUT4) mRNA expression. *Urtica dioica* significantly reduced hyperglycemia, plasma corticosterone, oxidative stress and depressive like behavior as well as improved associative memory and hippocampal GLUT4 mRNA expression comparable to rosiglitazone (5 mg/kg, p.o.). Further, *Urtica dioica* insignificantly improved spatial memory and serum insulin. In conclusion, *Urtica dioica* reversed dexamethasone induced hyperglycemia and its associated complications such as depressive like behavior and cognitive dysfunction.

Keywords Cognition · Hyperglycemia · Rosiglitazone · Depression · Dexamethasone · *Urtica dioica*

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Introduction

Glucocorticoids (GC) are steroid hormones that potently suppresses a variety of inflammatory and autoimmune diseases make them among the most frequently prescribed classes of drugs. Moreover, chronic GC therapy is required in several cases such as pediatric nephrotic syndrome, graft-versus-host disease etc. (Park et al. 2013; Phan et al. 2013). However, chronic supraphysiological level of GC produces numerous side effects which include fluid and electrolyte abnormalities, hypertension, osteoporosis, myopathy, behavioral disturbances, cataracts, growth arrest etc. (Brunton et al. 2006). Prolonged therapy with GC inhibits insulin secretion from pancreatic β-cells, decrease glucose uptake and utilization, stimulate glucagon secretion, hepatic glucose production, decreased body weight and induce type 2 diabetes like state (Ghaisas et al. 2009; Jatwa et al. 2007). Evidences suggest that elevated cortisol level as observed in stress results in depression and is a risk factor for type 2 diabetes (Ghaisas et al. 2009; Haynes et al. 2001; Tongjaroenbuangam et al. 2011). Further, diabetes induces oxidative stress in hippocampus (Alipour et al. 2012), resulting in neurological disorders such as cognitive dysfunction, depression, anxiety etc. (Kuhad and Chopra 2007). Besides, GC treatment induces over production of reactive oxygen species and an elevation in cytosolic calcium that causes a subsequent increase in the calcium dependent death in neuronal cells (Suwanjang et al. 2013). Sustained stressors or pathophysiological conditions, such as affective disorders, have detrimental effects on cognition. The impairing effects have been attributed mainly to GC released from the adrenal gland (Tongjaroenbuangam et al. 2011). Moreover, stress levels of GC induce impairment in insulin-stimulated trafficking of glucose transporter-4 (GLUT4) in the hippocampus resulting in decreased metabolic activities and

plasticity of hippocampal neurons (Grillo et al. 2009; Piroli et al. 2007), which further leads to cognitive dysfunction (Huang et al. 2010). A similar alteration was also noted following the administration of dexamethasone, a synthetic GC-receptor agonist that induces neurological disorders (Brunton et al. 2006; Haynes et al. 2001).

Urtica dioica (UD) belonging to the family Urticaceae have already been known and consumed for a long time as medicinal plant globally. Traditionally, UD has been used for a number of diseases including benign prostatic hyperplasia, urinary tract infections, neuralgia, allergic rhinitis, diuretic, cardiovascular diseases, anemia, hemorrhoids etc. Further, it shows protective effect against hepatic ischemic reperfusion (Kandis et al. 2010), hyperglycemia (Bnouham et al. 2003), hypercholesterolemia (Nassiri-Asl et al. 2009), diabetic neuropathy, etc. (Patel and Udayabanu 2013). A recent study demonstrates that, UD treatment induces GLUT4 translocation in muscle cells in vitro (Kadan et al. 2013). The major constituents of UD are known to be scopoletin, quercetin, carvacrol, 5-hydroxy tryptamine (5HT), acetylcholine, cholineacetyltransferase, etc. (Collier and Chesher 1956; Nahata and Dixit 2012; Otles and Yalcin 2012; Wessler et al. 2001). Carvacrol shows neuroprotective effect against focal cerebral ischemia/reperfusion injury (Yu et al. 2012), and modulates the levels of dopamine and serotonin in the prefrontal cortex and hippocampus (Zotti et al. 2013). Scopoletin increases the expression of peroxisome proliferator-activated receptor-γ and ameliorates insulin resistance in HepG2 cells (Zhang et al. 2010). Recent studies suggested that, peroxisome proliferator-activated receptor-γ agonist, reduce the level of systemic GC and improve learning & memory in both human and animals (Churi et al. 2008; Pipatpiboon et al. 2012; Torres et al. 2012). All these facts make UD an ideal plant for dexamethasone-induced diabetes and its associated complications such as depression and memory impairment, which has not been investigated yet. The present study was aimed to investigate the effect of UD on dexamethasone induced diabetes and its associated complications such as memory impairment and depressive like behavior.

Methods

Plant material

UD was collected from the North Western Himalayan region and authenticated from Department of Forest Product, Dr. Y.S. Parmar University of Horticulture & Forestry, INDIA. UD leaves were shade dried and subjected to hydro-alcoholic extraction with methanol and water (1:1). The extract was evaporated under reduced pressure and lyophilized.

Animals

Adult Swiss albino mice (24–30 g) of either sex were maintained in animal research facility with 12 h dark and light cycle at 25±2 °C; food and water ad libitum. All animal experiments were carried out in accordance with Institutional Animal Ethical Clearance. All efforts were made to minimize animals suffering and the number of animals used.

Experimental design

Animals were randomly divided into five groups (n=8–10) as follows: (1) Normal control (control), (2) Dexamethasone (D) (1 mg/kg/day, i.m.) for 12 weeks, (3) Dexamethasone for 12 weeks and UD extract (50 mg/kg/day, p.o.) from second week (D+UD50), (4) Dexamethasone for 12 weeks and UD extract (100 mg/kg/day, p.o.) from second week (D+UD100) and (5) Dexamethasone for 12 weeks and rosiglitazone (5 mg/kg/day, p.o.) from second week (D+rosi). The dose of UD was selected from a separate experiment in our laboratory, in which mice were orally administered with five different doses viz. 10, 15, 25, 50 and 100 mg/kg. After drug treatment acute stress was produced by immobilizing the animals for 150 min once only and the duration of immobility was recorded (Sheikh et al. 2007). We observed that 50 mg/kg of UD showed better efficacy and is comparable to 100 mg/kg (data not shown). 0.3 % carboxymethylcellulose in water for injection is used as a vehicle. The control animals also received the same throughout the treatment period.

On day 84 (60 min after drug administration), animals were subjected to different behavioral studies such as forced swim and tail suspension test. Morris water maze and passive avoidance step-through task were performed from day 80–83 and day 83–84, respectively. Apart from this body weight and water intake were constantly measured throughout the study. Immediately after behavioral studies the animals were euthanized, blood was collected via retro-orbital puncture in tubes containing 10 % sodium citrate and centrifuged at 1,000 g for 20 min at 4 °C. Plasma was separated and aliquots were stored at -80 °C for estimation of corticosterone, thiobarbituric acid reactive substances (TBARS), catalase and nitric oxide (NO) levels. Beside, serum was also separated for estimation of insulin level. Further, hippocampus was dissected for GLUT4 mRNA expression.

Forced swim test

The animals were individually forced to swim inside a cylinder with radius 24 cm and height 25 cm filled with water (25± 2 °C) up to a height of 18 cm. After the initial 2–3 min of vigorous swimming, the animals showed period of immobility by floating with minimum movements. An animal is considered immobile when it remains floating in the water without

struggle making very less movements of its limb necessary to keep its nose just above the water surface. The total immobility period of each mouse during the 6 min test was recorded (Kulkarni and Mehta 1985).

Tail suspension test

The mice were individually suspended on the edge of a shelf, 58 cm above a table top by adhesive tape, placed approximately 1 cm from the tip of the tail. The duration of immobility of each mouse was recorded for the periods of 6 min during the test. Animals were considered immobile when they hang passively and completely motionless (Steru et al. 1985).

Morris water maze task

Spatial memory was assessed using Morris water maze, which consisted of a white circular pool of 1 m diameter, filled with water at room temperature and has a submerged transparent escape platform kept 1 cm below the surface of water. The pool was made opaque with addition of nontoxic water-soluble white paint, which makes the submerged platform invisible to the mice. The pool was divided into four hypothetical quadrants. Initially, each mouse from all the groups were allowed to swim freely (habituation trial) in the maze for 5 min (without platform). During training trial, the platform was positioned in the center of quadrant and each mouse was released facing toward the wall of the pool in the randomly selected quadrant. Further, the position of the platform was changed in each trial. The mice were allowed to search the platform spontaneously within 60 s. Mice that failed to find the submerged platform within 60 s were placed onto the platform by the experimenter and allow to remain on the platform for 5 s (learning trial). Each mouse received three to four learning session per day with 10 min interval between each trial. In each trial, the time taken by the mouse to find the hidden platform was recorded as escape latency. Probe trial test was performed on day 84 to evaluate the index of memory in which the number of crossings across the platform area and the time spent in target quadrant were recorded (Udayabanu et al. 2012).

Passive avoidance step-through task

Passive avoidance step-through task was used to evaluate the associative memory in rodents, as described earlier (Patel and Udayabanu 2013). Briefly, mice were individually given one habituation trial to explore both light and dark chambers for 120 s. On day 1 of acquisition trial, the mice were individually placed in the light chamber for 5 s and the time taken by mouse to enter the dark chamber was recorded as step-through latency. Then an inescapable scrambled electric shock was delivered for 2 s on a grid floor in the dark chamber. On day 2 of acquisition, memory retention was tested for each animal in the same manner, but the shock was not delivered.

Reverse transcriptase PCR

Total RNA was isolated from hippocampus using TRI reagent (Invitrogen) according to the manufacturer's instructions. Total isolated RNA quantification and quality were assessed in spectrophotometer (Nanodrop-2000C, Thermo Scientific). The reverse transcription of 3 μg of total RNA was performed using First strand cDNA synthesis kit (Fermentas life sciences) according to the manufacturer's instructions. PCR amplification was carried out by employing a set of specific primers: GLUT4 FP-5′-GAT GGG CTT TCT CCG TCC-3′, RP-5′-GTG TGG CAA GAG TTC AGT GG-3′ and GAPDH FP-5′-TTC ACC ACC ATG GAG AAG GC-3′, RP-5′-GGC ATG GAC TGT GGT CAT GA-3′. For polymerase chain reaction 1 μl of cDNA, 1 μl of primers (each 5 Pico mole) and 10 μl of DreamTaq™ Green PCR Master Mix (2×) (Fermentas life sciences) was made up to 20 μl with DNase/RNase free water and amplified (Veriti® Thermal Cycler-Applied Biosystems). PCR products were resolved on 1.5 % agarose gel and subjected to densitometric analysis using AlphaImager® EP (Fisher Scientific), yielding an amplified product of GLUT4 (193 bp) and GAPDH (236 bp) respectively. Levels of PCR products were normalized relative to band intensity from corresponding GAPDH mRNA expression.

Estimation of corticosterone

An HPLC-UV system (Waters, USA) was used for quantification of plasma corticosterone according to Sheikh et al. (2007), using dexamethasone as an internal standard. Briefly, 500 μl of plasma containing known quantity of dexamethasone was extracted with 5 ml of dichloromethane (DCM). The DCM extract was evaporated to dryness and dissolved in 100 μl of mobile phase. Twenty microliter of extract was injected into HPLC system for quantification. Mobile phase consisted of methanol:water (70:30) at a flow rate of 1.2 ml/min and corticosterone was detected at 250 nm.

Estimation of blood glucose and serum insulin levels

Blood glucose levels were measured in blood samples collected from tail vein using Accu-check (Roach Diagnostics GmbH, Germany) blood glucose monitoring system. The levels of serum insulin were determined by chemiluminescent immunoassay using a commercially available kit (AccuLite CLIA Microwells, Monobind Inc., USA).

Estimation of plasma TBARS, catalase and nitric oxide

The level of plasma TBARS was determined as per Ohkawa et al. (1979) with some modifications. The mixture consists of 100 μl of plasma, 0.1 ml of 8 % sodium dodecyl sulphate, 1.0 ml of 20 % acetic acid (pH 3.5) and 1.0 ml of 0.67 % thiobarbituric acid. The mixture was boiled at 95°C for 60 min and then cooled. 1.0 ml of double-distilled water and 5.0 ml of n-butanol:pyridine (15:1, *v/v*) mixture were added and centrifuged at 5,000 rpm for 10 min. The absorbance of organic layer was measured at 540 nm using spectrophotometer (Elico, SL 159 UV VIS spectrophotometer).

Catalase level was measured by the modified method of Claiborne (1985). A total of 0.1 ml of plasma was added to cuvette containing 1.9 ml of 50 mmol phosphate buffer (pH 7). The reaction was started by the addition of 1 ml freshly prepared 30 mmol H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm. Catalase level was expressed in percentage and the basal level of catalase was considered as 100 %.

The plasma NO was determined as nitrite plus nitrate. The NO is an unstable molecule, which spontaneously oxidizes to nitrite and nitrate (NO_x^-). The nitrates were reduced to nitrite by using 2 % ammonium molybdate and 4 % ferrous ammonium sulphate and quantified by using Greiss reagent (1 % sulphanilamide and 0.1 % naphthylethylenediamine dihydrochloride) at 540 nm spectrophotometrically. The nitrite concentration was calculated and expressed in percentage. The basal level of NO was considered as 100 % (Udayabanu et al. 2008).

Statistical analysis

All the data were expressed as mean ± SEM. The statistical significance was assessed by one-way analysis of variance followed by Tukey's post hoc test with a confidence level of $p<0.05$.

Results

Effect of UD extract on blood glucose, body weight and water intake

We observed that, chronic dexamethasone treatment significantly increased ($p<0.05$) the level of blood glucose (116.75 %) as compared to normal animals. Treatment with UD50 (*Urtica dioica*, 50 mg/kg), UD100 (*Urtica dioica*, 100 mg/kg) and rosiglitazone to dexamethasone treated mice upto 84th day significantly reduced ($p<0.05$) the blood glucose levels (53.09 %, 57.73 % and 55.41 %, respectively) (Fig. 1a).

Dexamethasone administration resulted in decreased ($p<0.05$) body weight (13.95 %) as compared with control mice. Chronic treatment with UD100 and rosiglitazone (5 mg/kg) significantly ($p<0.01$) ameliorated body weight loss (19.84 % and 18.16 %, respectively) as compared with dexamethasone treated mice. Further, chronic UD50 treatment significantly increased ($p<0.05$) the body weight (13.39 %) in dexamethasone treated mice but less than UD100 and rosiglitazone (Fig. 1b).

In our study, chronic dexamethasone administration significantly increased ($p<0.01$) the water intake (56.59 %) as compared to normal control mice, which is a classic sign of diabetes. Chronic administration of UD50, UD100 and rosiglitazone to dexamethasone treated mice significantly reduced ($p<0.05$) the water intake (24.98 %, 23.34 % and 23.30 %, respectively) when compared with dexamethasone treated mice (Fig. 1c).

Effect of UD extract on depressive like behavior

In this study, we used forced swimming and tail suspension test to assess the effect of UD against dexamethasone mediated depressive like behavior. In forced swim test, dexamethasone exhibited significant increase ($p<0.01$) in immobility periods as compared to control animals. Chronic UD treatment significantly improved ($p<0.05$) the mobility of dexamethasone treated mice. UD50 showed slight but insignificant increase in the mobility of dexamethasone administered mice as compared to UD100 treated animals. Rosiglitazone also reversed the immobility period in dexamethasone administered mice ($p<0.01$) (Fig. 2a).

In tail suspension test, dexamethasone exhibited significant increase in immobility period ($p<0.05$) as compared to control mice. Chronic treatment with UD50 and UD100 significantly increased ($p<0.01$ and $p<0.05$ respectively) the mobility in dexamethasone treated mice. Further, UD50 insignificantly reduced the immobility period in dexamethasone treated mice as compared to UD100. Rosiglitazone showed significant increase in the mobility period of dexamethasone treated mice ($p<0.01$) (Fig. 2b)

Effect of UD extract on spatial and associative memory

In Morris water maze task, dexamethasone treated mice showed significantly ($p<0.01$) increased escape latency on day 83 as compared to the control animals. The control animals showed improvement in learning between trials from day 80 to 83 as evident from decrease in the escape latency. But the chronically dexamethasone treated animals did not show any significant increase in learning between the trials (Fig. 2c). The time spent in target quadrant (Fig. 2d) and the number of crossings (Fig. 2e) across the platform area (probe trial) were also significantly decreased ($p<0.05$) in

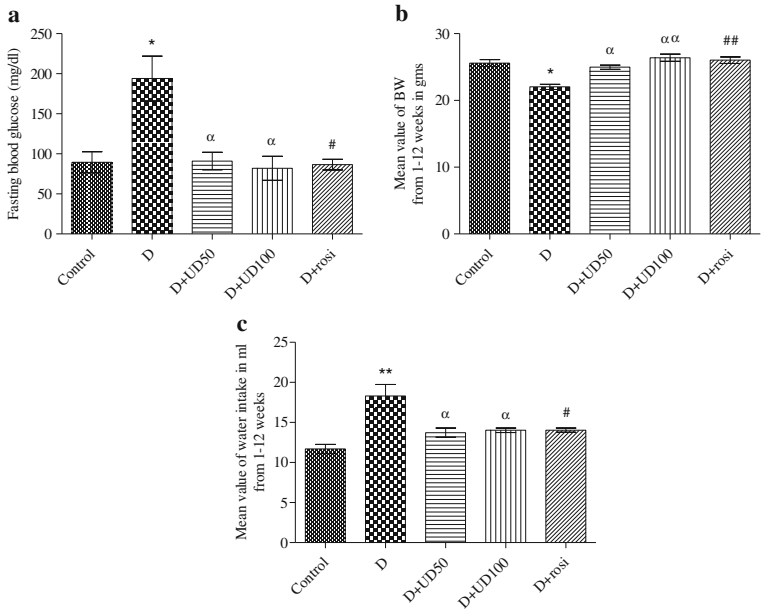

Fig. 1 Effect of *Urtica dioica* on dexamethasone-induced alteration in blood glucose level (**a**), body weight (**b**) and water intake (**c**). *control vs D, $^{\alpha}$D vs D+UD50/D+UD100, $^{\#}$D vs D + rosi. *$p<0.05$, **$p<0.01$. *D* dexamethasone; *UD50, UD100 Urtica dioica* 50 mg/kg, 100 mg/kg respectively; *rosi* rosiglitazone

dexamethasone treated animals as compared to normal animals. UD50 and UD100 treatment insignificantly decreased the escape latency, improved the time spent in target quadrant and the number of crossings across the platform area (probe trial) as compared to dexamethasone treated mice ($p>0.05$). Rosiglitazone treated animals showed significant increase in learning between the trials on day 83 as evident from the decrease in escape latency ($p<0.01$), and improved the time spent in target quadrant and the number of crossings across the platform area ($p<0.05$) during the probe trial as compared to dexamethasone treated animals.

In passive avoidance step-through task, the dexamethasone treated mice showed no significant alteration in step-through latency on day 84 (memory retention trial) when compared with their respective day 83 (acquisition trial) step-through latency. During memory retention trial, the dexamethasone treated animals showed significantly decreased step-through latency as compared to normal animals ($p<0.05$). Further we observed that treatment with UD50, UD100 and rosiglitazone significantly ($p<0.05$, $p<0.001$ and $p<0.01$, respectively) increased the step-through latency during memory retention trial as compared with dexamethasone treated mice (Fig. 2f).

Effect of UD extract on corticosterone and insulin levels

We observed that, chronic dexamethasone treatment significantly elevated ($p<0.05$) the level of plasma corticosterone (206.87 %) but insignificantly reduced the level of circulating

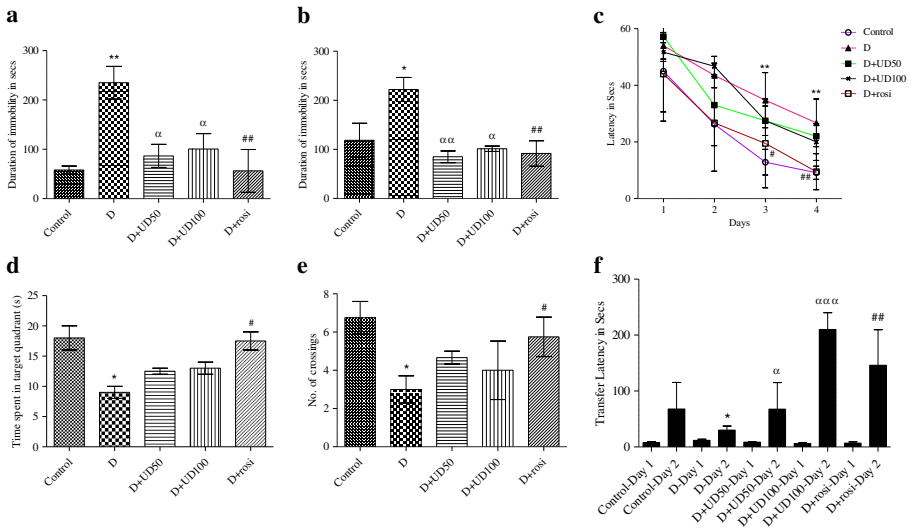

Fig. 2 Effect of *Urtica dioica* on dexamethasone-induced behavioral alterations in forced swim test (**a**), tail suspension test (**b**), Morris water maze task (**c**), probe trial (time spent in target quadrant) (**d**), probe trial (number of crossing across platform) (**e**) and passive avoidance step through task (**f**). *control vs D, $^{\alpha}$D vs D+UD50/D+UD100, $^{\#}$D vs D + rosi. *$p<0.05$, **$p<0.01$, ***$p<0.001$. *D* dexamethasone; *UD50, UD100 Urtica dioica* 50 mg/kg, 100 mg/kg respectively, *rosi* rosiglitazone

insulin (41.05 %) as compared to normal animals. Treatment with UD50, UD100 and rosiglitazone to dexamethasone treated mice up to 84th day significantly reduced ($p<0.05$) the plasma corticosterone level (41.37 %, 54.02 % and 48.27 %, respectively) (Fig. 3a). Further, there was an insignificant increase in circulating insulin level in UD50 & UD100 treated animals (76.78 % & 90.17 %, respectively) as compared to dexamethasone treated animals (Fig. 3b).

Effect of UD extract on Hippocampal GLUT4 mRNA expression

GLUT4 mRNA expression was significantly decreased ($p<0.01$) in chronic dexamethasone treated animals (66.93 %) as compared to control animals in hippocampus region of brain. Chronic UD50 and UD100 treatment significantly increased ($p<0.05$ and $p<0.01$, respectively) the mRNA expression of GLUT4 (93.57 % and 226.10 %, respectively) in hippocampus as compared to dexamethasone treated mice. Further, rosiglitazone treatment showed significant increase in mRNA expression of GLUT4 as compared to dexamethasone ($p<0.01$) (191.56 %) and UD50 ($p<0.05$) (50.62 %) treated animals. UD100 also showed significant increase ($p<0.05$) in mRNA expression of GLUT4 (68.46 %) as compared to UD50 treated animals (Fig. 4).

Effect of UD extract on oxidative/nitrative stress

Chronic dexamethasone treatment significantly increased ($p<0.05$) TBARS (142.85 %) and decreased ($p<0.05$) catalase (53.67 %) levels in plasma as compared with normal animals. Chronic UD50, UD100 and rosiglitazone treatment significantly decreased ($p<0.05$) TBARS (52.94 %, 41.17 % and 41.17 %, respectively) (Fig. 5a) and increased ($p<0.05$) catalase (122.31 %, 132.37 % and 113.68 %, respectively) (Fig. 5b) levels in plasma as compared with dexamethasone treated animals. Further, we observed that chronic dexamethasone treatment insignificantly increased (93.15 %) the level of NO_x^- as compared to control animals. However, UD50, UD100 and rosiglitazone treatment insignificantly decreased (22.61 %, 28.79 % and 12.53 %, respectively) the level of NO_x^- as compared to dexamethasone treated mice (Fig. 5c).

Discussion

The current study demonstrates that chronic dexamethasone treatment causes depressive like behavior, as observed by performance in forced swim and tail suspension test, which is in line with previous report (Haynes et al. 2001). Chronic treatment with either UD or rosiglitazone significantly reversed the duration of immobility associated with dexamethasone. Moreover, UD50 showed better efficacy than UD100 against dexamethasone induced depressive like behavior. In line with our current results it was reported that rosiglitazone significantly reverse depressive like behavior (Sharma et al. 2012). Besides, chronic dexamethasone administration impaired the spatial memory, as observed by performance in Morris water maze task. It was also observed that dexamethasone leads to associative memory dysfunction in mice evident from decreased step-through latency in passive avoidance step-through task. Associative and spatial memory performance depends on the integrity and functions of certain brain regions such as hippocampus, striatum etc. (Ambrogi Lorenzini et al. 1999). Evidences suggest that GC deleteriously affects survival and function of hippocampal neurons, and associated with diminished memory and mood (Haynes et al. 2001). In the present study, chronic treatment with rosiglitazone significantly improved dexamethasone mediated spatial and associative memory impairment, which is in line with previous reports (Pipatpiboon et al. 2012). However, chronic UD treatment significantly improved associative memory but only a slight improvement was observed in the case of spatial memory. Further we observed that UD100 showed better efficacy than UD50 to improve associative memory performance. Earlier studies suggests that scopoletin, a chemical constituent of UD modulates acetylcholine release, hippocampal long term potentiation and memory functions (Hornick et al. 2011). It also possesses a specific antidepressant-like activity as observed in the tail suspension test (Capra et al. 2010).

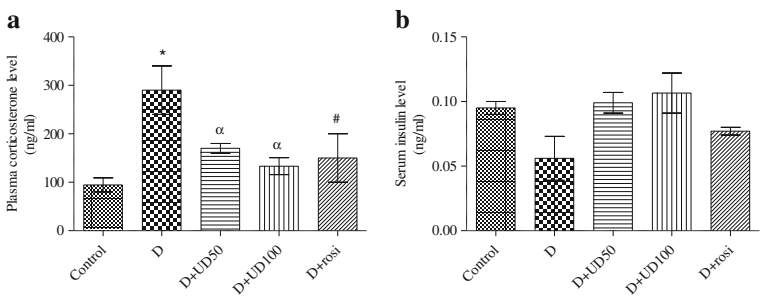

Fig. 3 Effect of *Urtica dioica* on dexamethasone-induced alterations in plasma corticosterone (**a**) and serum insulin levels (**b**). *control vs D, $^{\alpha}$D vs D+UD50/D+UD100, $^{\#}$D vs D + rosi. *$p<0.05$. *D* dexamethasone, *UD50, UD100 Urtica dioica* 50 mg/kg, 100 mg/kg respectively, *rosi* rosiglitazone

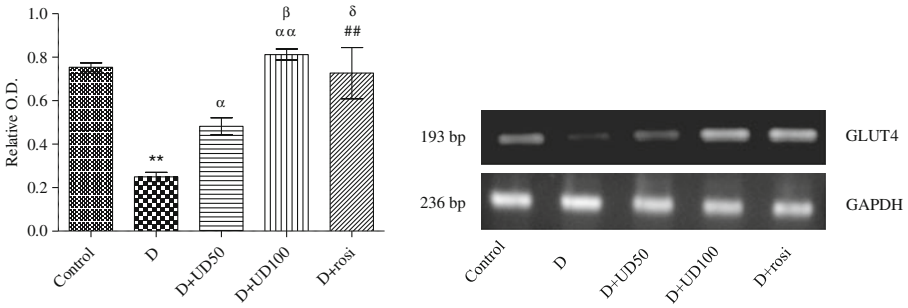

Fig. 4 Effect of *Urtica dioica* on dexamethasone-induced alteration in GLUT4 mRNA expression in hippocampus. *control vs D, $^{\alpha}$D vs D+UD50/D+UD100, $^{\#}$D vs D + rosi, $^{\beta}$D+UD50 vs D+UD100, $^{\delta}$D+UD50 vs D + rosi. *p<0.05, **p<0.01. *D* dexamethasone, *UD50, UD100 Urtica dioica* 50 mg/kg, 100 mg/kg respectively, *rosi* rosiglitazone

Chronic dexamethasone administration significantly induces diabetes mellitus in both animals and humans. Diabetes is clinically characterized by growth retardation, polydypsia, hyperglycemia and associated with a number of complications such as diabetic neuropathy, depression, cognitive dysfunctions, etc. (Brunton et al. 2006; Sullivan et al. 2013). Herein, we observed that, chronic dexamethasone administration up to 12 weeks significantly increased blood glucose, polydypsia and body weight loss similar to diabetic state. However, the level of circulating insulin was not significantly altered. Reports suggest that, GC showed variable effect on insulin secretion in rodents with respect to the dose of GC, the length of treatment and the susceptibility of the animal strain to develop diabetes (Lambillotte et al. 1997; Rafacho et al. 2010). In addition, GC induces insulin resistance and accelerates the progression from prediabetes to diabetes (Colvin et al. 2013). In the present study, both UD and rosiglitazone reversed the dexamethasone mediated diabetic like state by reducing the level of blood glucose, restoring body weight loss and polydypsia. UD100 showed better efficacy than UD50 against dexamethasone mediated body weight loss and hyperglycemia. However, both doses of UD showed good efficacy against polydypsia. It has been reported that UD extract reduces the intestinal absorption of glucose and showed antihyperglycemic effect in rodents (Bnouham et al. 2003). Reports also suggest that, UD is a rich source of 5HT (Collier and Chesher 1956), which is known for its association with insulin secretion (Adeghate et al. 1999; Patel and Udayabanu 2013), memory and depression (Cowen and Sherwood 2013). Further, UD treatment increased the level of circulating insulin upto the basal level when compared with control but insignificant when compared to dexamethasone treated animals. Moreover, UD100 showed good efficacy than UD50 against dexamethasone induced hypoinsulinemia. We also observed that, rosiglitazone did not alter the level of circulating insulin as compared with dexamethasone treated animals, which is understandable that, rosiglitazone cannot induce insulin secretion under glucotoxicity condition (Marshall et al. 2007).

Studies from human type 2 diabetes or animal models (*ob/ob* and *db/db* mice) suggest that GLUT4 gene expression is controlled at the transcriptional level and tissue specific knock

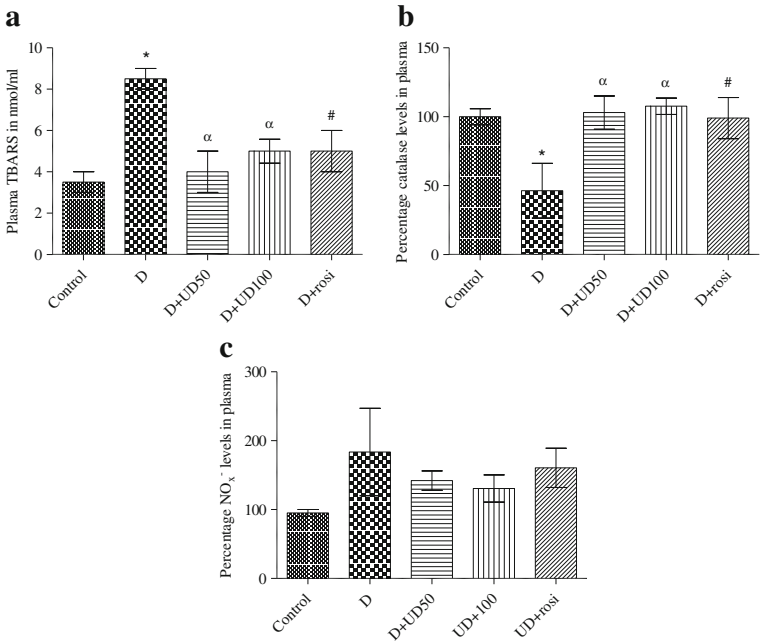

Fig. 5 Effect of *Urtica dioica* on dexamethasone-induced alterations in plasma TBARS (**a**), catalase (**b**) and nitric oxide (**c**) levels. *control vs D, $^{\alpha}$D vs D+UD50/D+UD100, $^{\#}$D vs D + rosi. *p<0.05. *D* dexamethasone, *UD50, UD100 Urtica dioica* 50 mg/kg, 100 mg/kg respectively, *rosi* rosiglitazone

out of GLUT 4 gene resulted in insulin resistance whereas overexpression resulted in restoration of whole body glucose disposal and insulin sensitivity (Zisman et al. 2000; Carvalho et al. 2005; Im et al. 2007). Further, down regulation in GLUT4 mRNA have been observed in type 2 diabetic patients, which is reversed by peroxisome proliferator-activated receptor-γ agonists (Karnieli and Armoni 2008; Kolak et al. 2007). In the present study we observed that, chronic dexamethasone treatment significantly increased the level of plasma corticosterone and reduced the expression of GLUT4 mRNA in hippocampus. Previous studies demonstrated that dexamethasone treatment affects hippocampal insulin signaling pathway and contribute to the functional deficits observed in hypercortisolemic states, such as diabetes (Piroli et al. 2007). These functional activities of insulin signaling in the hippocampus contribute to the neurological complications of diabetes in brain, including cognitive deficits and increased risk of co-morbid neurological disorders such as depressive like behavior (Grillo et al. 2009; Iizuka et al. 1996; Piroli et al. 2007). We observed that chronic treatment with either UD or rosiglitazone significantly reversed the dexamethasone induced alteration in plasma corticosterone and GLUT4 expression in hippocampus. Moreover, UD100 showed better efficacy than UD50 against dexamethasone induced alteration in the level of corticosterone and GLUT4 mRNA. Consistent with our results, it was reported that rosiglitazone, a peroxisome proliferator-activated receptor-γ agonist possesses a specific antidepressant-like activity in behavioral models and the effect was mediated by reduction of plasma corticosterone level (Eissa Ahmed and Al-Rasheed 2009). Rosiglitazone modulates GLUT4 level and insulin sensitivity of GLUT4 translocation in the mature adipocytes (Martinez et al. 2010). Further, it ameliorates cognitive impairment associated with long standing diabetes (Patel and Udayabanu 2013). Herein we report, rosiglitazone significantly increased the mRNA expression of GLUT4 in hippocampus, which might improve cognitive performance. Further, it has been reported that, scopoletin a chemical constituent of UD modulates the expression of peroxisome proliferator-activated receptor-γ (Zhang et al. 2010), which is involved in the neuronal insulin receptor functioning in the hippocampus (Pipatpiboon et al. 2012) and reduction of corticosterone level during diabetes (Torres et al. 2012). The reversal of hypercortisolemia during chronic dexamethasone treatment might contribute to the antidepressant like action of UD.

Accumulated evidence suggests that, oxidative stress during dexamethasone-induced diabetes contributes to the development of neurological disorders including depression, cognitive dysfunction, parkinson's disease, diabetic neuropathy, etc. In addition, oxidative stress further contributes to inflammation mediated neurological disorders (Shukla et al. 2011). A longitudinal population-based study revealed that increased systemic levels of oxidative stress and antioxidant deficiencies may pose risk factors for cognitive decline (Berr et al. 2000). In our results, we observed that, dexamethasone induces oxidative stress as evident from elevated plasma TBARS and reduced catalase levels. Further, we observed that, chronic UD and rosiglitazone treatment significantly reduced TBARS and increased catalase levels, which could be beneficial in counteracting the dexamethasone/hyperglycemia induced oxidative stress. On the other hand, dexamethasone showed a slight but insignificant increase in the level of systemic NO, which was reduced by chronic UD and rosiglitazone treatment. UD100 showed better efficacy than UD50 against dexamethasone mediated alteration in the level of plasma catalase and NO, whereas UD50 was more effective against TBARS.

In conclusion, UD significantly reversed the corticosteroid mediated depressive and diabetic like state. Further, it improved associative memory impairment and GLUT4 expression in the hippocampus. UD100 showed better efficacy than UD50 and is comparable to rosiglitazone. UD could be used as an adjuvant therapy for long term corticosteroids treatment regime.

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