5. Results

5.1. Standardization of plant materials

Microscopy of UD leaf showed straight and sharp stinging hair (A), upper epidermis (B), lower epidermis (C), spongy parenchyma (D) and vascular bundle (E) (Figure 15).

![Figure 15](image)

Figure 15: Transverse section of Stinging nettle leaf stained with safranin solution (40X).

Microscopy of St. John's wort leaf showed very thin mid rib, oil gland, upper epidermis, lower epidermis and vascular bundle (Figure 16).

![Figure 16](image)

Figure 16: Transverse section of St. John's wort (*Hypericum perforatum*) leaf stained with safranin solution and viewed at 10X (A) and 40X (B).

The powder microscopy of UD leaves revealed the presence of sharp pointed unicellular trichomes, prismatic and raphide crystals of calcium oxalate, paracytic or rubiaceous stomata,
septed fibers, elongated cork cells, epidermal cells, collenchymal cells with starch and starch granules. Powder microscopy of St. John's wort leaves revealed the presence of large oil glands, pitted and thick walled fibers, fragments of vascular bundles, collenchymal cells and starch granules. We did not observe the presence of trichomes and calcium oxalate crystals in the powder of St. John's wort leaves.

Physical constant like ash values, extractive values and moisture content are reported in Table 4 to Table 6.

**Table 4: Ash values (% w/w)**

<table>
<thead>
<tr>
<th>S No.</th>
<th>Physical constant</th>
<th>Stinging nettle</th>
<th>H. perforatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total ash</td>
<td>21.2</td>
<td>6.2</td>
</tr>
<tr>
<td>2.</td>
<td>Acid insoluble ash</td>
<td>3.04</td>
<td>2.4</td>
</tr>
<tr>
<td>3.</td>
<td>Water insoluble ash</td>
<td>3.3</td>
<td>3.2</td>
</tr>
</tbody>
</table>

**Table 5: Extractive values (% w/w)**

<table>
<thead>
<tr>
<th>S No.</th>
<th>Extracts</th>
<th>Extractive value of Stinging nettle</th>
<th>Extractive value of H. perforatum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Water soluble extractives</td>
<td>23.3</td>
<td>14.2</td>
</tr>
<tr>
<td>2.</td>
<td>Alcohol soluble extractives</td>
<td>25.3</td>
<td>14.02</td>
</tr>
</tbody>
</table>

**Table 6: Moisture content**

<table>
<thead>
<tr>
<th>S No.</th>
<th>Plant materials</th>
<th>Initial weight (gm)</th>
<th>Final weight (gm)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
</table>

(70)

Phytochemical evaluation on UD extract revealed the presence of alkaloids, carbohydrates, glycosides, phenolics, flavonoids, proteins and amino acids (Table 7).

Table 7: Phytochemical evaluation of plant extracts

<table>
<thead>
<tr>
<th>S No.</th>
<th>Phytochemical test</th>
<th>S. nettle extract</th>
<th>SJW extract</th>
<th>S No.</th>
<th>Phytochemical test</th>
<th>S. nettle extract</th>
<th>SJW extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td></td>
<td></td>
<td>5.</td>
<td>Phenolics &amp; flavonoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Mayer’s test</td>
<td>-</td>
<td>-</td>
<td>a)</td>
<td>Ferric chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b) Dragendorf’s test</td>
<td>++</td>
<td>+</td>
<td>b)</td>
<td>Gelatin test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>c) Lead acetate test</td>
<td></td>
<td></td>
<td>c)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>d) Alkaline reagent</td>
<td></td>
<td></td>
<td>d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates &amp; Glycosides</td>
<td></td>
<td></td>
<td>6.</td>
<td>Proteins &amp; Amino acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Molish test</td>
<td>+</td>
<td>+</td>
<td>a)</td>
<td>Biuret test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>b) Fehling test A</td>
<td>-</td>
<td>-</td>
<td>b)</td>
<td>Ninhydrin test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>c) Fehling test B</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>d) Barfoed test</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>e) Legal test</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>f) Kellar-Killiani test</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>g) Ferric chloride test</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Saponins</td>
<td></td>
<td></td>
<td>7.</td>
<td>Gum &amp; Mucilage</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Foam test</td>
<td>-</td>
<td>-</td>
<td>a)</td>
<td>Alcohol 95% test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Phytosteroids</td>
<td></td>
<td></td>
<td>8.</td>
<td>Fixed oils &amp; fats</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a) Liebermann-Burchards test</td>
<td>-</td>
<td>-</td>
<td>a)</td>
<td>Spot test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>b) Saponification test</td>
<td></td>
<td></td>
<td>b)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.1.1. Specific chemical tests for stinging nettle extract:

Moistened dried extract was taken into test tubes and covered with filter paper shocked in dilute NaOH and kept in water bath. After some time, filter paper was exposed to UV light. It showed yellowish-green fluorescence and indicated the presence of scopoletin in UD extract.

In our preliminary experiment, 5HT in UD extract showed an intense blue colored chromogen with Folin-Ciocalteu reagent in presence of sodium carbonate.

5.1.2. LC-MS and HPLC analysis

Mass calculated for C₁₀H₈O₄ scopoletin, exact mass: 192.04, found 191.04 (M-1); C₇H₆O₄ gentisic acid, exact mass: 154.02, found 153.02 (M-1); C₉H₆O₄ esculetin, exact mass: 178.02, found 177.02 (M-1); C₁₅H₁₀O₇ quercetin, exact mass: 302.04, found 301.04 (M-1) and C₂₇H₃₀O₁₆ rutin, exact mass: 610.51, found 609.51 (M-1) in LC-MS analysis (Figure 17).

Figure 17: Negative ion LC-MS spectrum of hydro-alcoholic extract of UD leaves showing peak at m/z 153.02 (gentisic acid), m/z 177.02 (esculetin), m/z 191.04 (scopoletin), m/z 301.04 (quercetin) and m/z 609.51 (rutin).
In HPLC analysis, the crude hydro-alcoholic extract of UD leaves showed peak ($t_R=14.254$) corresponding to standard scopoletin ($t_R=14.296$). Herein, we observed that the crude UD extract contains 6.51% of scopoletin (Figure 18 A and B).

**Figure 18:** HPLC chromatogram of standard scopoletin (A) and crude hydro-alcoholic extract of UD leaves (B).
Figure 19: Negative ion LC-MS chromatogram of hyperforin ($m/z$ 535.38) (A) and hypericin ($m/z$ 503.08) (B) in hydro-alcoholic extract of St. John’s wort.

Mass calculated for $C_{35}H_{52}O_4$ hyperforin, exact mass: 536.38, found 535.38 (M-1) and $C_{30}H_{16}O_8$ hypericin, exact mass: 504.08, found 503.08 (M-1) in LC-MS analysis (Figure 19).
Figure 20: Typical HPLC chromatograms of the standard hypericin (CAS 548-04-9) (A), hyperforin (CAS 11079-53-1) (B), 50% methanolic extract of St John’s wort (C), 75% methanolic extract of St John’s wort (D) and 25% methanolic extract of St John’s wort (E) with detection wavelength set at 200-800 nm using PDA detector.
HPLC chromatograms of standard hypericin and hyperforin were reported in Figure 20A and 20B, respectively. Quantitative HPLC analysis revealed the presence of hyperforin (6.12%) and hypericin (0.37%) in Hypericum extract (methanol: water, 1:1) (Figure 20C). 75% methanolic extract of St. John’s wort showed 6.14% of hyperforin and 0.27% of hypericin (Figure 20D). 25% methanolic extract of St. John’s wort showed 6.14% of hyperforin and 0.25% of hypericin (Figure 20E).

5.2. Assessment of depressive like behaviour in stressed mice and the effect of UD extract

Chronic stress significantly increased the duration of immobility in FST (p<0.001 vs. CTRL). Chronic UD treatment significantly improved (p<0.01 vs. CUMS) the mobility period in FST comparable to FLX (p<0.01 vs. CUMS). Duration of immobility was also significantly reduced by chronic treatment with ROSI (p<0.05 vs. CUMS) and HYP (p<0.05 vs. CUMS) in FST. There were no significant alterations in the duration of immobility between CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL) (Figure 21A).

CUMS significantly increased the duration of immobility in TST (p<0.01 vs. CTRL). Chronic UD treatment significantly improved (p<0.001 vs. CUMS) the mobility period in stressed mice subjected to TST. Duration of immobility was also significantly reduced by chronic treatment with FLX (p<0.01 vs. CUMS), ROSI (p<0.01 vs. CUMS) and HYP (p<0.05 vs. CUMS) in TST. There were no significant alterations in the mobility periods between CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL) (Figure 21B).
Figure 21: Effect of UD extract on CUMS-induced behavioural alterations in FST (A) and TST (B). Data were mean ± SEM values (n=6). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract; FST = forced swim test; TST = tail suspension test.

Before CUMS and drug treatment, the basal level of sucrose preference was not significantly altered among the groups (p>0.05) (Figure 22A). Further, one week of chronic stress or drug treatment did not modulate sucrose preference (p>0.05). In addition, there were no significant alterations in the sucrose preference between CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL) (Figure 22B) on one week of stress exposure.

Figure 22: Effect of UD extract on CUMS-induced behavioural alteration in SPT: SPT baseline (A), SPT at week one (B), SPT week at two (C) and SPT at week three (D). Data were mean ± SEM values (n=6). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001.
CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = *Hypericum perforatum* extract; UD = *Urtica dioica* extract; SPT = sucrose preference test.

Stress exposure significantly reduced the sucrose intake in mice on week two (p<0.001 vs. CTRL). Chronic UD administration significantly ameliorated sucrose preference in stressed mice on week two (p<0.01 vs. CUMS). Sucrose preference was also significantly ameliorated by chronic treatment with FLX (p<0.05 vs. CUMS) and ROSI (p<0.05 vs. CUMS). Hypericum extract did not modulate sucrose preference on week two (p>0.05 vs. CUMS). There were no significant alterations in the sucrose preference between CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL) (Figure 22C) on week two of CUMS exposure.

CUMS exposure significantly reduced the sucrose intake in mice on week three (p<0.001 vs. CTRL). Chronic UD administration significantly ameliorated sucrose preference in stressed mice on week three (p<0.001 vs. CUMS) comparable to FLX, ROSI and HYP treatment. Sucrose preference was also significantly ameliorated by chronic treatment with FLX (p<0.001 vs. CUMS), ROSI (p<0.001 vs. CUMS) and HYP (p<0.001 vs. CUMS). In addition, there were no significant alterations in the sucrose preference between CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL) at the end of CUMS exposure (Figure 22D).

**Figure 23:** Overall effects of CUMS and drug treatment on SPT. Data were mean ± SEM values (n=6). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + UD. **p < 0.01**

When combined SPT data from baseline to week three, we observed that CUMS gradually declined the sucrose preference after week one onwards and significantly reduced after week two (p<0.001 vs. CTRL) and at the end of CUMS exposure (p<0.001 vs. CTRL). Conversely, chronic UD treatment significantly induced sucrose preference after week two (p<0.01 vs. CUMS) and at the end of CUMS paradigm (p<0.001 vs. CUMS). In addition, there were no significant alteration between CTRL and CTRL treated with UD (p>0.05) (Figure 23).

5.3. Effect of UD extract on depression mediated cognitive deficit

On day 1 of training (day 22), all the animals showed similar learning behaviour and there was no significant alteration in the learning pattern between the groups (p>0.05) in Morris water maze task. The control animals showed improvement in learning between trials from day 23-25 as evident from decrease in the escape latency, while chronically stressed mice did not show any significant improvement in learning between the trials. During training trial, CUMS significantly increased the escape latency on day 3 (p<0.001 vs. CTRL) and day 4 (p<0.001 vs. CTRL). Chronic UD treatment significantly decreased the escape latency on day 3 (p<0.001 vs. CUMS) and day 4 (p<0.001 vs. CUMS) in stressed animals comparable to chronic FLX, ROSI and HYP treatment (p<0.001 vs. CUMS). In addition, there were no significant alteration between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05) (Figure 24A).

In the probe trial, number of crossings across the platform area was significantly decreased in stressed mice (p<0.01 vs. CTRL). Chronic UD treatment significantly increased the number of crossings across the platform area in stressed mice (p<0.001 vs. CUMS), and this effect was comparable to chronic ROSI treated stressed mice (p<0.001 vs. CUMS). Chronic FLX and HYP treatment also significantly increased the number of crossings across the platform area (p<0.05 vs. CUMS and p<0.01 vs. CUMS, respectively) (Figure 24B). In addition, there were no significant alteration between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).
Figure 24: Effect of UD extract on CUMS-induced behavioural alterations in Morris water maze task (A) and probe trial (number of crossings) (B). Data were mean ± SEM values (n=6). Significant differences: *CTRL vs. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

Figure 25: Effect of UD extract on CUMS-induced behavioural alteration in PA task: PA task base line (A), PA task at week one (B), PA task at week two (C) and PA task at week...
three (D). Data were mean ± SEM values (n=6). Significant differences: *CTRL vs. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD; *CTRL vs. CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract; PA task = passive avoidance step through task.

In PA task, before CUMS and drug treatment, the base line values of step through latency (STL) or transfer latency during memory retention trial was not significantly altered among the groups (p>0.05) (Figure 25A). One week of chronic stress or drug treatment did not modulate STL during memory retention trial (p>0.05). In addition, there were no significant alterations in the STL during memory retention trial between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL) after one week of stress exposure (Figure 25B).

Neither CUMS nor drugs treated stressed mice modulated STL during memory retention trial after week 2 (p>0.05). There were no significant alterations in the STL during memory retention trial between CTRL and CTRL treated with FLX, ROSI and HYP (p>0.05 vs. CTRL), while chronic UD treatment significantly increased the STL in control animals (p<0.05 vs. CTRL) after week two (Figure 25C). Chronically stressed mice showed no significant alteration (p>0.05) in STL on day 23 (memory retention trial) when compared with their respective day 22 (acquisition trial) STL at the end of CUMS exposure. During memory retention trial, the stressed animals showed significantly decreased (p<0.001 vs. CTRL) STL. During memory retention trial, chronic UD administration significantly increased the STL (p<0.001 vs. CUMS) in stressed mice, and the effect was comparable to chronic HYP treatment (p<0.001 vs. CUMS). Chronic FLX and ROSI treatment also significantly increased the STL during memory retention trial after three weeks of CUMS exposure (p<0.05 vs. CUMS and p<0.01 vs. CUMS, respectively). There were no significant alterations in the STL during memory retention trial between CTRL and CTRL treated with FLX, ROSI and HYP (p>0.05 vs. CTRL), while chronic UD administration significantly increased the STL in control animals (p<0.05 vs. CTRL) after week three (Figure 25D).
When combined PA task data of memory retention trial from base line to week three, we observed that CUMS exposure did not modulate the STL after week one and week two (p>0.05 vs. CTRL), while significantly declined the STL after week three (p<0.001 vs. CTRL). Conversely, chronic UD treatment significantly increased transfer latency after week three of CUMS paradigm (p<0.001 vs. CUMS). Chronic UD treatment did not modulate STL on stressed mice between week two and week three (p>0.05 vs. CUMS). In addition, chronic UD treatment gradually increased the STL from week one onwards, while significantly improved (p<0.05 vs. CTRL) after week two and week three in control animals (Figure 26).

5.4. Effect of UD extract on depression mediated locomotor deficit

In actophotometer test, before CUMS and drug treatment, the base line values of locomotor activity score was not significantly altered among the groups (p>0.05). One week of stress paradigm or drug treatment did not modulate locomotor activity scores (p>0.05) in mice. Further, stress paradigm or drug treatment did not modulate locomotor activity scores in mice after week two (p>0.05). However, CUMS exposure significantly reduced the locomotor activity score after week three (p<0.01 vs. CTRL). Chronic UD, FLX, ROSI and HYP treatment did not reverse locomotor activity in stressed mice (p>0.05 vs. CUMS) after week three. There were no significant alterations in the locomotor activity score between CTRL
and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL) after week three (Figure 27A).

**Figure 27:** Effect of UD extract on CUMS-induced alterations in locomotor activity in actophotometer using bar graph (A) and locomotor performance using line graph (B). Data were mean ± SEM values (n=6). Significant differences: */#CTRL vs. CUMS. **p < 0.01. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

When drawn the overall performance of locomotor activity using line graph, observed that CUMS exposure started dysregulation of locomotor activity from week one onwards, while significantly reduced after week three of CUMS exposure (p<0.01 vs. CTRL). Chronic UD treatment did not significantly reverse locomotor activity score in chronically stressed mice (p>0.05 vs. CUMS) at any point. There were no significant alterations in the locomotor activity between CTRL and CTRL treated UD (p>0.05 vs. CTRL) during the study (Figure 27B).

5.5. Depression mediated insulin resistance and hypercorticosteronemia and the effect of UD

Three weeks of CUMS exposure did not significantly increase the level of fasting blood glucose in mice (p>0.05 vs. CTRL). Further, chronic UD, FLX, ROSI and HYP administration did not modulate the level of fasting blood glucose in control and stressed mice (p>0.05) (Figure 28A).
Figure 28: Effect of UD extract on CUMS-induced alterations in the level of fasting blood glucose (A), oral glucose tolerance test (B), plasma corticosterone (C) and serum insulin (D). Data were mean ± SEM values (n=6). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

While, in oral glucose test, CUMS significantly increased the level of blood glucose after 0.5 h (p<0.01 vs. CTRL), 1.0 h (p<0.001 vs. CTRL), 1.5 h (p<0.01 vs. CTRL) and 2 hr (p<0.01 vs. CTRL) resulting in insulin resistance. Chronic UD administration significantly reduced the glucose load after 1.0 h (p<0.001 vs. CUMS), 1.5 h (p<0.05 vs. CUMS) and 2 h (p<0.05 vs. CUMS) comparable to ROSI. However, chronic FLX and HYP administration did not modulate the level of blood glucose in stressed mice (p>0.05 vs. CUMS). There were no
significant alterations in the level of blood glucose at any interval between CTRL and CTRL treated UD, FLX, ROSI and HYP (p>0.05 vs. CTRL) (Figure 28B).

CUMS significantly increased the level of plasma corticosterone in mice (p<0.001 vs. CTRL). Chronic UD administration significantly attenuated CUMS induced hypercorticosteronemia (p<0.01 vs. CUMS) in stressed mice comparable to chronic FLX administration (p<0.01 vs. CUMS). Chronic ROSI and HYP administration also significantly reduced the level of plasma corticosterone in stressed mice (p<0.05 vs. CUMS and p<0.001 vs. CUMS, respectively). There were no significant alterations in the level of plasma corticosterone between CTRL and CTRL treated UD, FLX, ROSI and HYP (p>0.05 vs. CTRL) (Figure 28C).

Three weeks of CUMS exposure did not significantly decreased the level of serum insulin under non-fasting condition (p>0.05 vs. CTRL). Further, chronic UD, FLX, ROSI and HYP administration did not modulate the level of serum insulin in control and stressed mice (p>0.05) (Figure 28D).

5.6. Effect of UD extract on depression mediated alteration in hippocampal insulin signaling pathway

CUMS induced depressed mice showed significant downregulation in hippocampal PPARγ mRNA expression (p<0.01 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal PPARγ mRNA expression in stressed mice (p<0.01 vs. CUMS) comparable to ROSI (p<0.01 vs. CUMS), while chronic FLX and HYP treatment did not reverse the expression level of PPARγ mRNA in stressed mice (p>0.05 vs. CUMS). Chronic UD administration significantly upregulated the mRNA expression of PPARγ in stressed mice as compared to FLX (p<0.01 vs. CUMS+FLX) and HYP administration (p<0.05 vs. CUMS+HYP) (Figure 29A). In addition, chronic UD, FLX, ROSI and HYP administration did not modulate the expression of PPARγ mRNA in control mice (p>0.05 vs. CTRL).

Hippocampal IR mRNA expression was significantly downregulated (p<0.05 vs. CTRL) in chronically stressed animals. Chronic UD treatment significantly upregulated the mRNA expression of IR in stressed mice (p<0.05 vs. CUMS) comparable to ROSI (p<0.05 vs. CUMS). However, chronic FLX and HYP administration did not modulate the mRNA expression of IR in stressed mice (p>0.05 vs. CUMS). Chronic UD administration

significantly upregulated the mRNA expression of IR in stressed mice as compared to FLX (p<0.01 vs. CUMS+FLX) and HYP administration (p<0.001 vs. CUMS+HYP) (Figure 29B). There were no significant alterations in the mRNA expression of IR between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).

Figure 29: Effect of UD extract on CUMS-induced alterations in the mRNA expression of hippocampal PPARγ (A), IR (B), ILGF 1r (C), GLP1 (D), IRS1 (E) and IRS2 (F). Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + ROSI and CUMS + UD, †CUMS + FLX vs. CUMS + UD, ‡CUMS + HYP vs. CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

Three weeks of CUMS exposure did not regulate the expression level of hippocampal ILGF 1r mRNA (p>0.05 vs. CTRL) in stressed mice. Further, chronic UD, FLX, ROSI and HYP administration did not alter the expression of ILGF 1r mRNA in control and stressed mice (p>0.05) (Figure 29C).
CUMS induced depressed mice did not show significant alteration in mRNA expression of hippocampal GLP1 (p>0.05 vs. CTRL) in stressed mice. In addition, chronic UD, FLX, ROSI and HYP administration did not alter the expression of GLP1 mRNA in control and stressed mice (p>0.05) (Figure 29D).

CUMS induced depressed mice showed significant downregulation in hippocampal IRS1 mRNA expression (p<0.05 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal IRS1 mRNA expression in stressed mice (p<0.05 vs. CUMS) comparable to ROSI (p<0.05 vs. CUMS), while chronic FLX and HYP treatment did not reverse the expression level of IRS1 mRNA in stressed mice (p>0.05 vs. CUMS) (Figure 29E). In addition, chronic UD, FLX, ROSI and HYP administration did not regulate the expression of IRS1 mRNA in control mice (p>0.05 vs. CTRL).

Hippocampal IRS2 mRNA expression was significantly downregulated (p<0.01 vs. CTRL) in chronically stressed animals. Chronic ROSI treatment significantly upregulated the mRNA expression of IRS2 in stressed mice (p<0.01 vs. CUMS), while chronic UD, FLX and HYP administration did not regulate the mRNA expression of IRS2 in stressed mice (p>0.05 vs. CUMS) (Figure 29F). There were no significant alterations in the mRNA expression of IRS2 between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).

CUMS induced depressed mice showed significant downregulation in hippocampal PI3K mRNA expression (p<0.01 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal PI3K mRNA expression in stressed mice (p<0.01 vs. CUMS) comparable to ROSI (p<0.05 vs. CUMS), while chronic FLX and HYP treatment did not reverse the expression level of PI3K mRNA in stressed mice (p>0.05 vs. CUMS). UD administration significantly upregulated the mRNA expression of PI3K in stressed mice as compared to FLX (p<0.001 vs. CUMS+FLX) and HYP administration (p<0.001 vs. CUMS+HYP) (Figure 30A). In addition, chronic UD, FLX, ROSI and HYP administration did not regulate the expression of PI3K mRNA in control mice (p>0.05 vs. CTRL).

Hippocampal PKB mRNA expression was significantly downregulated (p<0.05 vs. CTRL) in chronically stressed animals. Chronic UD treatment significantly upregulated the mRNA expression of PKB in stressed mice (p<0.05 vs. CUMS) comparable to ROSI (p<0.05 vs. CUMS), while chronic FLX and HYP administration did not regulate the mRNA expression
of PKB in stressed mice (p>0.05 vs. CUMS). UD administration significantly upregulated the mRNA expression of PKB in stressed mice as compared to HYP administration (p<0.05 vs. CUMS+HYP) (Figure 30B). There were no significant alterations in the mRNA expression of PKB between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).

CUMS induced depressed mice showed significant downregulation in hippocampal GLUT4 mRNA expression (p<0.01 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal GLUT4 mRNA expression in stressed mice (p<0.01 vs. CUMS) comparable to ROSI (p<0.001 vs. CUMS), while chronic FLX and HYP treatment did not reverse the expression level of GLUT4 mRNA in stressed mice (p>0.05 vs. CUMS). UD administration significantly upregulated the mRNA expression of GLUT4 in stressed mice as compared to FLX (p<0.01 vs. CUMS+FLX) and HYP administration (p<0.01 vs. CUMS+HYP) (Figure 30C). In addition, chronic UD, FLX, ROSI and HYP administration did not regulate the expression of GLUT4 mRNA in control mice (p>0.05 vs. CTRL).

**Figure 30**: Effect of UD extract on CUMS-induced alterations in the mRNA expression of hippocampal PI3K (A), PKB (B), GLUT4 (C), INSG1 (D) and MAPK1 (E). Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + FLX, **CUMS vs. CUMS + ROSI, ***CUMS vs. CUMS + HYP.
CUMS + ROSI, CUMS + HYP and CUMS + UD; ³CTRL vs. CTRL + HYP and CTRL + UD, ⁴CUMS + FLX vs. CUMS + UD; ⁵CUMS + HYP vs. CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

Hippocampal INSG1 mRNA expression was significantly downregulated (p<0.001 vs. CTRL) in chronically stressed animals. Chronic UD treatment significantly upregulated the mRNA expression of INSG1 in stressed mice (p<0.05 vs. CUMS) comparable to ROSI (p<0.01 vs. CUMS), while chronic FLX and HYP administration did not regulate the mRNA expression of INSG1 in stressed mice (p>0.05 vs. CUMS) (Figure 30D). There were no significant alterations in the mRNA expression of INSG1 between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).

CUMS induced depressed mice showed significant downregulation in hippocampal MAPK1 mRNA expression (p<0.001 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal MAPK1 mRNA expression in stressed mice (p<0.01 vs. CUMS) comparable to FLX (p<0.01 vs. CUMS). Chronic ROSI and HYP treatment also significantly upregulated the expression level of MAPK1 mRNA in stressed mice (p<0.001 vs. CUMS). In addition, chronic FLX and ROSI administration did not regulate the expression of MAPK1 mRNA in control mice (p>0.05 vs. CTRL), while UD and HYP treatment significantly upregulated the mRNA expression of MAPK1 in control mice (p<0.05 vs. CTRL) (Figure 30E).

Hippocampal GLUT4 membrane protein was significantly decreased (p<0.001 vs. CTRL) in chronically stressed animals. Chronic UD treatment did not significantly increased the content of GLUT4 membrane protein in stressed mice (p>0.05 vs. CUMS). There was no significant alteration in the content of GLUT4 membrane protein between CTRL and CTRL treated with UD (p>0.05 vs. CTRL). Chronic ROSI administration significantly increased the content of hippocampal GLUT4 membrane protein in stressed mice (p<0.001 vs. CUMS). There was no significant alteration in the GLUT4 membrane protein between CTRL and CTRL treated with ROSI (p>0.05 vs. CTRL) (Figure 31).
Figure 31: Effect of UD extract on CUMS-induced alteration in the content of hippocampal GLUT4 membrane protein. Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + ROSI. ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; ROSI = rosiglitazone; UD = Urtica dioica extract; GLUT4 = glucose transporter type 4.

5.7. The effect of UD extract on depression mediated impairment in Smo-Gli pathway and synaptic plasticity

Three weeks of CUMS exposure did not regulate the expression level of hippocampal Shh mRNA (p>0.05 vs. CTRL) in stressed mice. Further, chronic UD, FLX, ROSI and HYP administration did not alter the expression of Shh mRNA in control and stressed mice (p>0.05) (Figure 32A).

CUMS induced depressed mice showed no significant alteration in hippocampal Ptch1 mRNA expression (p>0.05 vs. CTRL). Chronic UD, FLX, ROSI and HYP administration did not modulate the hippocampal Ptch1 mRNA expression in stressed mice (p>0.05 vs. CUMS). Chronic FLX and ROSI administration did not modulate the expression of Ptch1 mRNA in control mice (p>0.05 vs. CTRL), while UD treatment significantly upregulated the mRNA expression of Ptch1 in control mice (p<0.01 vs. CTRL, CTRL + FLX and CTRL + ROSI). Chronic HYP administration significantly increased the mRNA expression of Ptch1 in control mice (p<0.05 vs. CTRL) (Figure 32B).

CUMS induced depressed mice showed significant downregulation in hippocampal Smo mRNA expression (p<0.05 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal Smo mRNA expression in stressed mice (p<0.05 vs. CUMS) comparable to HYP (p<0.05 vs. CUMS). Chronic FLX and ROSI treatment did not modulate the expression level of Smo mRNA in stressed mice (p>0.05 vs. CUMS). Chronic FLX and ROSI administration did not modulate the expression of Smo mRNA in control mice (p>0.05 vs. CTRL), while UD and HYP treatment significantly upregulated the mRNA expression of Smo in control mice (p<0.05 vs. CTRL and p<0.01 vs. CTRL, respectively). UD treatment significantly increased the mRNA expression of Smo in control mice (p<0.05 vs. CTRL + FLX and CTRL + ROSI) (Figure 32C).

Figure 32: Effect of UD on CUMS-induced alterations in hippocampal Shh mRNA (A), Ptch1 mRNA (B), Smo mRNA (C) and Gli1 mRNA (D) expression. Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + HYP and CUMS + UD; ΨCTRL vs. CTRL + HYP and CTRL + UD, ³CTRL + FLX vs. CTRL + UD; βCTRL + ROSI vs. CTRL + UD; aCUMS + FLX vs. CUMS + UD; bCUMS + ROSI vs. CTRL + ROSI.
CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

CUMS induced depressed mice showed significant downregulation in hippocampal Gli1 mRNA expression (p<0.05 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal Gli1 mRNA expression in stressed mice (p<0.01 vs. CUMS) comparable to HYP (p<0.05 vs. CUMS). Chronic UD administration significantly increased the level of Gli1 in stressed mice as compared to FLX and ROSI treated stressed mice (p<0.01 vs. CUMS + FLX and CUMS + ROSI). Chronic FLX and ROSI treatment did not modulate the expression level of Gli1 mRNA in stressed mice (p>0.05 vs. CUMS). Chronic FLX and ROSI administration did not modulate the expression of Gli1 mRNA in control mice (p>0.05 vs. CTRL), while UD treatment significantly upregulated the mRNA expression of Gli1 in control mice (p<0.01 vs. CTRL) (p<0.001 vs. CTRL + FLX and CTRL + ROSI). Chronic HYP treatment significantly increased the mRNA expression of Gli1 in control mice (p<0.01 vs. CTRL) (Figure 32D).

Three weeks of CUMS exposure did not regulate the expression level of hippocampal Hhip mRNA (p>0.05 vs. CTRL) in stressed mice. Further, chronic UD, FLX and ROSI administration did not alter the expression of Hhip mRNA in control and stressed mice (p>0.05). Chronic HYP administration significantly downregulated the mRNA expression of Hhip in stressed mice (p<0.01 vs. CTRL and p<0.05 vs. CUMS) (Figure 33A).

CUMS induced depressed mice showed significant downregulation in hippocampal cyclin D1 mRNA expression (p<0.05 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal cyclin D1 mRNA expression in stressed mice (p<0.05 vs. CUMS) comparable to HYP (p<0.01 vs. CUMS). Chronic FLX and ROSI treatment did not regulate the expression level of cyclin D1 mRNA in stressed mice (p>0.05 vs. CUMS). Chronic UD, FLX, ROSI and HYP administration did not regulate the expression of cyclin D1 mRNA in control mice (p>0.05 vs. CTRL) (Figure 33B).

Hippocampal BDNF mRNA expression was significantly downregulated (p<0.01 vs. CTRL) in chronically stressed animals. Chronic UD and ROSI treatment did not modulate the mRNA expression of BDNF in stressed mice (p>0.05 vs. CUMS), while chronic FLX and HYP

(92)
administration significantly upregulated the mRNA expression of BDNF in stressed mice (p<0.01 vs. CUMS and p<0.001 vs. CUMS, respectively) (Figure 33C). There were no significant alterations in the mRNA expression of BDNF between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).

CUMS induced depressed mice showed significant downregulation in hippocampal TrkB mRNA expression (p<0.01 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal TrkB mRNA expression in stressed mice (p<0.05 vs. CUMS). Also, chronic FLX, ROSI and HYP treatment significantly upregulated the expression level of TrkB mRNA in stressed mice (p<0.01 vs. CUMS). Further, chronic FLX, ROSI, HYP and UD administration did not regulate the expression of TrkB mRNA in control mice (p>0.05 vs. CTRL) (Figure 33D).

Figure 33: Effect of UD on CUMS-induced alterations in hippocampal Hhip mRNA (A), cyclin D1 mRNA (B), BDNF mRNA (C) and TrkB mRNA (D) expression. Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + FLX,
CUMS + ROSI, CUMS + HYP and CUMS + UD; *CTRL vs. CUMS + HYP. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

Cyclopamine (Cyc) 2.5µM did not alter the mRNA expression of Gli1 (p>0.05 vs. CTRL), while Cyc 5µM significantly downregulated (p<0.001 vs. CTRL) the mRNA expression of Gli1 in hippocampal slices. Acute purmorphamine (Pur) 1µM significantly upregulated the mRNA expression of Gli1 in hippocampal slices (p<0.001 vs. CTRL), while co-treatment with Cyc 5µM the level of Gli1 mRNA was significantly downregulated (p<0.001 vs. Pur 1µM). Both dosage (50 and 100µg) of HYP significantly upregulated the mRNA expression of Gli1 in hippocampal slices (p<0.001 vs. CTRL), while this effect was significantly blocked in presence of Cyc 5µM (p<0.001 vs. Pur 1µM) and (p<0.001 vs. HYP 50 and 100µg). UD 125µg treatment did not modulate the Gli1 mRNA expression in hippocampal slices (p>0.05 vs. CTRL). UD 250µg treatment significantly upregulated the mRNA expression of Gli1 in hippocampal slices (p<0.001 vs. CTRL), while pre-administration with Cyc 5µM blocked the effect of UD 125µg and UD 250µg on Gli1 mRNA expression (p<0.001 vs. Pur 1µM) and (p<0.001 vs. UD125 and 250µg) (Figure 34A).

**Figure 34:** Effect of UD on Gli1 (A) and Ptc1 (B) mRNA expression in hippocampal slices pre-treated with Smo antagonist cyclopamine. Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. Cyc 5µM; *CTRL vs. Pur 1µM, HYP 50µg, HYP 100µg, (94)
UD 250µg; \( ^{4}\)HYP 50µg and UD 125µg vs. HYP 50µg + Cyc 5µM and UD 125µg + Cyc 5µM; \( ^{5}\)HYP 100µg and UD 250µg vs. HYP 100µg + Cyc 5µM and UD 250µg + Cyc 5µM; \( ^{6}\)Pur 1µM vs. Cyc 5µM+ Pur 1µM, HYP 50µg + Cyc 5µM, HYP 100µg + Cyc 5µM, UD 125µg + Cyc 5µM and UD 250µg + Cyc 5µM. ***p < 0.001. CTRL = control; Cyc = cyclopamine; Pur = purmorphamine; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

5µM of Cyc treatment significantly downregulated \((p<0.001\ vs.\ CTRL)\) the mRNA expression of Ptc1 in hippocampal slices. Acute Pur 1µM significantly upregulated the mRNA expression of Ptc1 in hippocampal slices \((p<0.001\ vs.\ CTRL)\), while pre-treatment with Cyc 5µM the level of Ptc1 mRNA was significantly downregulated \((p<0.001\ vs.\ Pur 1µM)\). Both dosage (50 and 100µg) of HYP significantly upregulated the mRNA expression of Ptc1 in hippocampal slices \((p<0.001\ vs.\ CTRL)\), while this effect was significantly blocked in presence of Cyc 5µM \((p<0.001\ vs.\ Pur 1µM)\) and \((p<0.001\ vs.\ HYP 50\ and\ 100µg)\). UD 125µg treatment did not modulate the Ptc1 mRNA expression in hippocampal slices \((p>0.05\ vs.\ CTRL)\). UD 250µg treatment significantly upregulated the mRNA expression of Ptc1 in hippocampal slices \((p<0.001\ vs.\ CTRL)\), while pre-administration with Cyc 5µM blocked the effect of UD 125µg and UD 250µg on Ptc1 mRNA expression \((p<0.001\ vs.\ Pur 1µM)\) and \((p<0.001\ vs.\ UD125\ and\ 250µg)\) (Figure 34B).

5.8. Depression mediated alteration in hippocampal and striatal cholinergic system and the effect of UD and FLX

CUMS induced depressed mice showed significant downregulation in hippocampal mACHR1 mRNA expression \((p<0.01\ vs.\ CTRL)\). Chronic UD administration significantly upregulated the hippocampal mACHR1 mRNA expression in stressed mice \((p<0.05\ vs.\ CUMS)\) comparable to FLX \((p<0.05\ vs.\ CUMS)\). Chronic ROSI and HYP treatment did not modulate the expression level of mACHR1 mRNA in stressed mice \((p>0.05\ vs.\ CUMS)\). In addition, chronic FLX, ROSI, UD and HYP administration did not regulate the expression of mACHR1 mRNA in control mice \((p>0.05\ vs.\ CTRL)\) (Figure 35A).

Three weeks of CUMS exposure did not regulate the expression level of hippocampal mACHR4 mRNA \((p>0.05\ vs.\ CTRL)\) in stressed mice. Further, chronic UD, FLX, ROSI and
HYP administration did not alter the expression of hippocampal mAChR4 mRNA in control and stressed mice (p>0.05) (Figure 35B).

CUMS induced depressed mice did not show significant alteration in mRNA expression of striatal mAChR1 (p>0.05 vs. CTRL) in stressed mice. In addition, chronic UD, FLX, ROSI and HYP administration did not alter the expression of striatal mAChR1 mRNA in control and stressed mice (p>0.05) (Figure 35C).

CUMS induced depressed mice showed significant upregulation in striatal mAChR4 mRNA expression (p<0.05 vs. CTRL). Besides, chronic UD, FLX, ROSI and HYP administration did not significantly alter the expression of striatal mAChR4 mRNA in control and stressed mice (p>0.05) (Figure 35D).

Figure 35: The effect of UD on CUMS-induced alterations mAChRs expression: mAChR1 mRNA in hippocampus (A), mAChR4 mRNA in hippocampus (B), mAChR1 mRNA in striatum (C) and mAChR4 mRNA in striatum (D). Data were mean ± SEM values (n=4).
Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + FLX and CUMS + UD; *p < 0.05, **p < 0.01. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

Herein, we observed that CUMS differentially alters the protein expression of mAChRs in hippocampus and striatum. Hippocampal mAChR1 protein expression was significantly downregulated (p<0.01 vs. CTRL) in chronically stressed animals. UD treatment significantly upregulated the protein expression of mAChR1 in stressed mice (p<0.05 vs. CUMS). There was no significant alteration in protein expression between control and control treated with UD (p>0.05 vs. CTRL). Chronic FLX treatment significantly upregulated the protein expression of mAChR1 in stressed mice (p<0.05 vs. CUMS). There was no significant alteration in protein expression between control and control treated with FLX (p>0.05 vs. CTRL) (Figure 36A).

**Figure 36:** The effect of UD on CUMS-induced alterations in the expression of mAChR1 protein in hippocampus (A), mAChR4 protein in hippocampus (B), mAChR4 protein in striatum (C), AChE protein in hippocampus (D) and ChAT protein in hippocampus (E). Data
were mean ± SEM values (n=4). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + UD and CUMS vs. CUMS + FLX. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; UD = *Urtica dioica* extract; FLX = fluoxetine.

CUMS showed insignificant alteration in protein expression of mAChR4 in hippocampus as compared to control animals (p>0.05 vs. CTRL). Chronic UD and FLX treatment did not modulate the level of hippocampal mAChR4 protein in stressed and non-stressed animals (Figure 36B).

CUMS induced depressed mice showed significant upregulation in striatal mAChR4 protein expression (p<0.05 vs. CTRL). Chronic UD administration did not significantly alter the expression of striatal mAChR4 protein in stressed mice and non-stressed mice (p>0.05 vs. CUMS). Chronic FLX administration did not modulate the expression of striatal mAChR4 protein in stressed and control mice (p>0.05 vs. CUMS) (Figure 36C).

Chronically stressed mice showed significant upregulation in hippocampal AChE protein expression (p<0.001 vs. CTRL). Chronic UD administration significantly downregulated the hippocampal AChE expression in stressed mice (p<0.05 vs. CUMS). Chronic UD administration did not modulate the hippocampal AChE expression in control mice (p>0.05 vs. CTRL). Chronic FLX administration significantly downregulated the hippocampal AChE expression in stressed mice (p<0.01 vs. CUMS). Chronic FLX administration did not modulate the hippocampal AChE expression in control mice (p>0.05 vs. CTRL) (Figure 36D).

Hippocampal ChAT protein expression was significantly downregulated (p<0.01 vs. CTRL) in chronically stressed mice. UD treatment significantly upregulated the protein expression of ChAT in stressed mice (p<0.05 vs. CUMS). There was no significant alteration in ChAT protein expression between control and control treated with UD (p>0.05 vs. CTRL). FLX treatment insignificantly upregulated the protein expression of ChAT in stressed mice (p>0.05 vs. CUMS). There was no significant alteration in ChAT protein expression between control and control treated with FLX (p>0.05 vs. CTRL) (Figure 36E).
5.9. Effect of UD on depression mediated impairment in ATG and neuronal survival

CUMS induced depressed mice showed significant downregulation in hippocampal BCL2 mRNA expression (p<0.01 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal BCL2 mRNA expression in stressed mice (p<0.05 vs. CUMS) comparable to ROSI and HYP (p<0.05 vs. CUMS). Chronic FLX treatment also significantly upregulated the expression level of BCL2 mRNA in stressed mice (p<0.001 vs. CUMS) (Figure 37A). In addition, chronic UD, FLX, ROSI and HYP administration did not regulate the expression of BCL2 mRNA in control mice (p>0.05 vs. CTRL).

![Figure 37](image-url) Effect of UD on CUMS-induced alterations in the mRNA expression of hippocampal BCL2 (A), AIP2 (B), ATG5 (C) and ATG7 (D). Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL =

control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = *Hypericum perforatum* extract; UD = *Urtica dioica* extract.

Hippocampal AIP2 mRNA expression was significantly downregulated (p<0.01 vs. CTRL) in chronically stressed animals. Chronic UD and ROSI treatment did not modulate the mRNA expression of AIP2 in stressed mice (p>0.05 vs. CUMS), while chronic FLX and HYP administration significantly upregulated the mRNA expression of AIP2 in stressed mice (p<0.05 vs. CUMS and p<0.001 vs. CUMS, respectively) (Figure 37B). There were no significant alterations in the mRNA expression of AIP2 between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).

CUMS induced depressed mice showed significant downregulation in hippocampal ATG5 mRNA expression (p<0.01 vs. CTRL). Chronic UD administration significantly upregulated the hippocampal ATG5 mRNA expression in stressed mice (p<0.05 vs. CUMS) comparable to FLX and HYP (p<0.05 vs. CUMS), while chronic ROSI administration did not modulate the expression level of ATG5 in stressed mice (p>0.05 vs. CUMS) (Figure 37C). In addition, chronic UD, FLX, ROSI and HYP administration did not regulate the expression of ATG5 mRNA in control mice (p>0.05 vs. CTRL).

Hippocampal ATG7 mRNA expression was significantly downregulated (p<0.01 vs. CTRL) in chronically stressed mice. Chronic ROSI treatment did not modulate the mRNA expression of ATG7 in stressed mice (p>0.05 vs. CUMS), while chronic FLX, UD and HYP administration significantly upregulated the mRNA expression of ATG7 in stressed mice (p<0.01 vs. CUMS, p<0.01 vs. CUMS and p<0.001 vs. CUMS, respectively) (Figure 37D). There were no significant alterations in the mRNA expression of ATG7 between CTRL and CTRL treated with FLX, ROSI, HYP and UD (p>0.05 vs. CTRL).

**5.10. Effect of UD extract on CUMS-induced oxidative and nitrative stress**

CUMS induced depressed mice showed significant upregulation in hippocampal iNOS mRNA expression (p<0.001 vs. CTRL). Chronic UD administration significantly downregulated the hippocampal iNOS mRNA expression in stressed mice (p<0.05 vs. CUMS) comparable to FLX (p<0.05 vs. CUMS) and ROSI (p<0.05 vs. CUMS). Also, chronic HYP treatment significantly downregulated the expression level of iNOS mRNA in stressed mice (p<0.01 vs. CUMS). Further, chronic FLX, ROSI, HYP and UD administration
did not regulate the expression of iNOS mRNA in control mice (p>0.05 vs. CTRL) (Figure 38A).

**Figure 38:** Effect of UD on CUMS-induced alterations in the mRNA expression of hippocampal iNOS (A), IL6 (B) and TNFα (C). Data were mean ± SEM values (n=4). Significant differences: *CTRL vs. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = *Hypericum perforatum* extract; UD = *Urtica dioica* extract.

Hippocampal IL6 mRNA expression was significantly upregulated (p<0.01 vs. CTRL) in chronically stressed mice. Chronic UD administration significantly downregulated the hippocampal IL6 mRNA expression in stressed mice (p<0.05 vs. CUMS). Also, chronic HYP, FLX and ROSI treatment significantly downregulated the expression level of IL6 mRNA in stressed mice (p<0.01 vs. CUMS). Further, chronic FLX, ROSI, HYP and UD
administration did not regulate the expression of IL6 mRNA in control mice (p>0.05 vs. CTRL) (Figure 38B).

CUMS induced depressed mice showed significant upregulation in hippocampal TNFα mRNA expression (p<0.01 vs. CTRL). Chronic UD administration significantly downregulated the hippocampal TNFα mRNA expression in stressed mice (p<0.05 vs. CUMS) comparable to FLX (p<0.05 vs. CUMS) and HYP (p<0.05 vs. CUMS). Also, chronic ROSI treatment significantly downregulated the expression level of TNFα mRNA in stressed mice (p<0.01 vs. CUMS). Further, chronic FLX, ROSI, HYP and UD administration did not modulate the expression of TNFα mRNA in control mice (p>0.05 vs. CTRL) (Figure 38C).

CUMS significantly increased the level of plasma thiobarbituric acid-reactive substances (TBARS) in mice (p<0.01 vs. CTRL). Chronic UD administration insignificantly attenuated CUMS induced elevated level of TBARS (p>0.05 vs. CUMS) in stressed mice. Chronic FLX (p<0.05 vs. CUMS) and ROSI (p<0.05 vs. CUMS) administration reduced the level of TBARS in stressed mice. Chronic HYP administration also significantly reduced the level of plasma TBARS in stressed mice (p<0.001 vs. CUMS). There were no significant alterations in the level of plasma TBARS between CTRL and CTRL treated UD, FLX, ROSI and HYP (p>0.05 vs. CTRL) (Figure 39A).

Level of plasma nitric oxide (NO) was significantly increased in chronically stressed mice (p<0.001 vs. CTRL). Chronic UD administration significantly attenuated CUMS induced elevated level of NO (p<0.05 vs. CUMS) in stressed mice comparable to chronic ROSI (p<0.05 vs. CUMS) administration. Chronic HYP and FLX administration also significantly reduced the level of plasma NO in stressed mice (p<0.001 vs. CUMS and p<0.01 vs. CUMS, respectively). There were no significant alterations in the level of plasma NO between CTRL and CTRL treated UD, FLX, ROSI and HYP (p>0.05 vs. CTRL) (Figure 39B).

CUMS significantly decreased the level of plasma catalase in mice (p<0.01 vs. CTRL). Chronic UD administration significantly increased the level of plasma catalase (p<0.05 vs. CUMS) in stressed mice comparable to chronic FLX (p<0.05 vs. CUMS) administration. Chronic ROSI and HYP administration also significantly increased the level of plasma catalase in stressed mice (p<0.01 vs. CUMS and p<0.001 vs. CUMS). There were no
significant alterations in the level of plasma catalase between CTRL and CTRL treated UD, FLX, ROSI and HYP (p>0.05 vs. CTRL) (Figure 39C).

**Figure 39**: Effect of UD on CUMS-induced 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. CUMS; *CUMS vs. CUMS + FLX, CUMS + ROSI, CUMS + HYP and CUMS + UD. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract; TBARS = thiobarbituric acid-reactive substances; NO = nitric oxide.

Level of plasma total thiol was significantly decreased in chronically stressed mice (p<0.01 vs. CTRL). Chronic UD administration significantly increased the level of plasma total thiol (p<0.05 vs. CUMS) in stressed mice comparable to chronic ROSI (p<0.05 vs. CUMS), FLX.
(p<0.05 vs. CUMS) and HYP (p<0.05 vs. CUMS) administration. There were no significant alterations in the level of plasma total thiol between CTRL and CTRL treated with UD, FLX, ROSI and HYP (p>0.05 vs. CTRL) (Figure 39D).

5.11. Accumulation of stinging nettle and St. John's wort constituents on whole brain after CUMS paradigm

Figure 40: HPLC chromatogram of standard scopoletin (A) as well as whole brain homogenate of chronically stressed mice treated with Urtica dioica extract (B).
Figure 41: HPLC chromatogram of standard hyperforin (A), hypericin (B) as well as whole brain homogenate of chronically stressed mice treated with St. John's wort extract (C).

Orally administered Stinging nettle extract accumulated as its constituent scopoletin (0.002%) in the brain tissue of mice after the last dose followed by 12 hr fasting (Figure 40).

Orally administered Hypericum extract accumulated as hyperforin-0.18% & hypericin-0.06% in the brain tissue of mice after the last dose followed by 12 hr fasting (Figure 41).
5.12. Effect of UD extract on STZ induced hyperglycemia, hypoinsulinemia and insulin resistance

STZ significantly elevated the level of fasting blood glucose (≥210 mg/dl) (p<0.001 vs. CTRL) and decreased circulating insulin level (p<0.001 vs. CTRL) in experimental animals. Treatment with UD to diabetic mice significantly (p<0.05 vs. STZ) reduced the blood glucose level comparable to ROSI (p<0.05 vs. STZ) (Figure 42A). Further, there was a significant increase in circulating insulin level in UD (p<0.01 vs. STZ) and ROSI (p<0.05 vs. STZ) treated diabetic mice as compared to diabetic animals (Figure 42B).

Figure 42: Effect of UD extract on STZ induced alteration in fasting blood glucose level (A), serum insulin level (B) and oral glucose tolerance test (C). Data were mean ± SEM values (n=6). Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI. *p
< 0.05, **p < 0.01, ***p < 0.001. CTRL = control; STZ = streptozotocin; UD50 = *Urtica dioica* extract 50 mg/kg; ROSI = rosiglitazone.

In oral glucose tolerance test, the blood glucose level at 0 time was different among the groups like fasting blood glucose. Glucose challenge dramatically raised the blood glucose level of STZ group compared with control group at 0.5-2.0 h intervals indicating insulin resistance. Chronic UD and ROSI treatment significantly reduced the level of blood glucose (p<0.05 vs. STZ and p<0.01 vs. STZ, respectively) at 2 h as compared to STZ group (Figure 42C).

5.13. Effect of UD extract on diabetes mediated alteration in body weight and water intake

After STZ injection, animals exhibited decreased body weight (p<0.01 vs. CTRL) as compared with control mice. Chronic treatment with UD and ROSI to diabetic mice after 5th–60th day significantly (p<0.01 vs. STZ) ameliorated body weight loss as compared with STZ treated diabetic mice (Figure 43A).

![Figure 43](image)

**Figure 43:** Effect of UD extract on STZ induced alteration in body weight (A) and water intake (B). Data were mean ± SEM values (n=6). Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI. **p < 0.01, ***p < 0.001. CTRL = control; STZ = streptozotocin; UD50 = *Urtica dioica* extract 50 mg/kg; ROSI = rosiglitazone.
In our study, long standing diabetes significantly (p<0.001 vs. CTRL) increased the water intake as compared to normal control mice, which is a classic sign of diabetes. Chronic administration of UD to diabetic mice significantly (p<0.001 vs. STZ) reduced the water intake when compared with STZ-diabetic mice. ROSI also reversed the water intake in STZ induced diabetic mice significantly (p<0.001 vs. STZ) (Figure 43B).

### 5.14. Diabetes mediated depressive like behaviour and motor function deficit and the effect of UD extract

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). Also, chronic ROSI treatment significantly reduced the duration of immobility in diabetic mice (p<0.001 vs. STZ) (Figure 44A).

**Figure 44:** Effect of UD extract on STZ induced depressive like behaviour in forced swim (A) (B) (C)
test (A), depressive like behaviour in tail suspension test (B) and locomotor deficit in actophotometer test (C). Data were mean ± SEM values (n=6). Significant differences: *CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; STZ = streptozotocin; UD50 = Urtica dioica extract 50 mg/kg; ROSI = rosiglitazone.

Chronically 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). Also, chronic ROSI treatment significantly reduced the duration of immobility in diabetic mice (p<0.01 vs. STZ) (Figure 44B).

STZ induced diabetes significantly reduced the locomotor activity in mice during actophotometer test (p<0.001 vs. CTRL). Chronic UD treatment did not modulate the locomotor activity in diabetic mice (p>0.05 vs. STZ). Chronic ROSI administration also did not revere diabetes induced hypolocomotion (p>0.05 vs. STZ) (Figure 44C).

5.15. Effect of UD extract on diabetes mediated cognitive deficit

In Morris water maze task, diabetic mice showed significantly increased escape latency on day 57 (p<0.05 vs. CTRL), 58 (p<0.01 vs. CTRL) and 59 (p<0.001 vs. CTRL) as compared to the control animals (Figure 45A). The control animals showed significant improvement in learning between trials from day 57 to day 59 as evident from the decrease in the escape latency. The diabetic animals did not show any significant alteration in learning between the trials and the number of crossings across the platform area was also significantly decreased (p<0.001 vs. CTRL) as compared to normal animals during the probe trial (on day 60). UD and ROSI treatment significantly decreased the escape latency on day 58 (p<0.05 vs. STZ) and 59 (p<0.001 vs. STZ), whereas improved the number of crossings (p<0.05 vs. STZ) across the platform area (probe trial) as compared to STZ induced diabetic mice (Figure 45B).

In passive avoidance step through task, diabetic mice showed no alteration in STL on day 2 (memory retention trial) when compared with their respective day 1 (acquisition trail) STL (p>0.05). During memory retention trial on day 2 the diabetic animals showed decreased STL as compared to normal animals (p<0.01 vs. CTRL Day 2). Further, chronic treatment with
UD and ROSI significantly increased (p<0.05 vs. STZ Day 2) the STL on day 2 as compared with STZ induced diabetic mice (Figure 45C).

![Figure 45](image)

**Figure 45**: Effect of UD extract on STZ induced behavioural alteration in Morris water maze task (A), probe trial (B) and passive avoidance step through task (C). Data were mean ± SEM values (n=6). Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI. *p < 0.05, **p < 0.01, ***p < 0.001. CTRL = control; STZ = streptozotocin; UD50 = *Urtica dioica* extract 50 mg/kg; ROSI = rosiglitazone.

5.16. **Effect of UD extract on hippocampal insulin signaling pathway**

PPARγ mRNA expression was not significantly altered in the STZ induced diabetic mice as compared to control mice (p>0.05 vs. CTRL). Chronic ROSI and UD administration significantly increased (p<0.05 vs. CTRL) the mRNA expression of hippocampal PPARγ in STZ induced diabetic mice (Figure 46A).
STZ induced diabetic mice showed significant decrease (p<0.05 vs. CTRL) in the IR mRNA expression as compared to control mice. Chronic UD and ROSI treatment significantly increased (p<0.01 vs. STZ) the mRNA expression of IR in diabetic mice (Figure 46B).

Hippocampal ILGF 1r mRNA expression was not significantly altered in chronically diabetic mice (p>0.05 vs. CTRL). Chronic UD and ROSI treatment did not regulate the mRNA expression of hippocampal ILGF 1r mRNA expression in diabetic mice (p>0.05 vs. STZ) (Figure 46C).

Figure 46: 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) and IRS2 (F). Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI; ^CTRL vs. STZ + UD50 and STZ + ROSI. *p < 0.05, **p < 0.01. CTRL = control; STZ = streptozotocin; UD50 = Urtica dioica extract 50 mg/kg; ROSI = rosiglitazone.

STZ induced diabetic mice showed no significant alteration in mRNA expression of hippocampal GLP1 (p>0.05 vs. CTRL). Chronic UD and ROSI treatment did not modulate the mRNA expression of hippocampal GLP1 in diabetic mice (p>0.05 vs. STZ) (Figure 46D).
In the present study, STZ induced diabetic mice showed significant decrease (p<0.05 vs. CTRL) in the hippocampal IRS1 mRNA expression as compared to control animals. Chronic UD treatment did not regulate (p>0.05 vs. STZ) the mRNA expression of hippocampal IRS1 in diabetic mice. Chronic ROSI treatment significantly (p<0.05 vs. STZ) increased the mRNA expression of hippocampal IRS1 in diabetic mice (Figure 46E).

IRS2 mRNA expression was significantly downregulated (p<0.05 vs. CTRL) in the hippocampus of diabetic mice as compared to control mice. Further, chronic UD and ROSI administration significantly increased the mRNA expression of hippocampal IRS2 in diabetic mice (p<0.05 vs. STZ and p<0.01 vs. STZ, respectively) (Figure 46F).

PI3K mRNA expression was significantly downregulated (p<0.05 vs. CTRL) in the hippocampus of diabetic mice as compared to control mice. Further, chronic UD or ROSI treatment significantly increased (p<0.01 vs. STZ) the mRNA expression of hippocampal PI3K in diabetic mice (Figure 47A).

Figure 47: Effect of UD extract on diabetes-mediated alterations in the mRNA expression of hippocampal PI3K (A), PKB (B), GLUT4 (C), INSG1 (D) and MAPK1 (E). Data were mean ± SEM values (n=4). Significant differences: *CTRL vs. STZ; *STZ vs. STZ + UD50 and (112)
PKB mRNA expression was significantly downregulated (p<0.01 vs. CTRL) in STZ treated mice as compared to control mice. Chronic UD and ROSI administration significantly increased (p<0.05 vs. STZ and p<0.01 vs. STZ, respectively) the mRNA expression of PKB in hippocampus as compared to STZ induced diabetic mice (Figure 47B).

GLUT4 mRNA expression was significantly downregulated (p<0.05 vs. CTRL) in STZ treated animals as compared to control animals. Chronic UD and ROSI treatment significantly increased the mRNA expression of GLUT4 (p<0.05 vs. STZ and p<0.01 vs. STZ, respectively) in the hippocampus as compared to STZ treated animals (Figure 47C).

STZ induced diabetic mice showed significant downregulation (p<0.01 vs. CTRL) in hippocampal INSG1 mRNA expression as compared to control animals. Chronic UD and ROSI administration significantly increased (p<0.01 vs. STZ and p<0.001 vs. STZ, respectively) the mRNA expression of hippocampal INSG1 in diabetic animals (Figure 47D).

STZ induced diabetic mice showed significant downregulation (p<0.05 vs. CTRL) in the MAPK1 mRNA expression as compared to control mice. Chronic UD and ROSI treatment significantly increased (p<0.01 vs. STZ and p<0.05 vs. STZ, respectively) the mRNA expression of hippocampal MAPK1 in diabetic mice (Figure 47E).

5.17. Effect of UD extract on diabetes mediated impairment in hippocampal GLUT4 membrane translocation

Figure 48: Effect of UD extract on STZ-induced alterations in the content of hippocampal GLUT4 protein in cytosol (A) and plasma membrane by immunoblot (B). 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, ***p < 0.001. CTRL = control; STZ = streptozotocin; UD50 = Urtica dioica extract 50 mg/kg; ROSI = rosiglitazone.
STZ induced diabetic mice showed significant downregulation (p<0.05 vs. CTRL) in the level of cytosolic GLUT4 protein as compared to control animals. Besides, chronic UD and ROSI treatment significantly increased the expression of cytosolic GLUT4 protein in diabetic animals (p<0.05 vs. STZ) (Figure 48A). The relative abundance of plasma membrane GLUT4 protein was significantly reduced (p<0.05 vs. CTRL) in STZ induced diabetic mice when compared to control mice. Chronic UD and ROSI treatment significantly increased (p<0.05 vs. STZ) the abundance of plasma membrane GLUT4 protein in diabetic mice (Figure 48B).

Hippocampal slices were prepared and treated with vehicle (artificial cerebrospinal fluid), insulin or UD plus LY294002 (PI3K inhibitor). Insulin, UD 125µg and UD 250µg treatment increased hippocampal GLUT4 plasma membrane association, an effect that was blocked by the acute treatment with PI3kinase inhibitor LY294002 (Figure 49).

**Figure 49:** *In vitro* stimulation of hippocampal slices by UD and insulin increases the association of GLUT4 membrane protein. Data were mean ± SEM values (n=4). Significant differences: #Veh vs. Ins; *Ins vs. Ins + LY294002; +UD vs. UD + LY294002. Veh = vehicle; Ins = insulin; UD125 & UD250 = *Urtica dioica* extract 125 µg & 250 µg.

### 5.18. Effect of UD extract on hippocampal cholinergic system

STZ induced diabetic mice showed significant downregulation (p<0.05 vs. CTRL) in the mAChR1 mRNA expression in hippocampus. Chronic UD and ROSI treatment significantly
increased (p<0.01 vs. STZ) the mRNA expression of hippocampal mAChR1 in diabetic mice (Figure 50A).

Chronic diabetes did not regulate the mRNA expression of mAChR4 in hippocampus (p>0.05 vs. CTRL). Further, chronic UD and ROSI treatment did not modulate hippocampal mAChR4 mRNA expression in diabetic mice (p>0.05 vs. STZ) (Figure 50B).

Chronic diabetes did not regulate the mRNA expression of mAChR1 in striatum (p>0.05 vs. CTRL). Further, chronic UD and ROSI treatment did not modulate striatal mAChR1 mRNA expression in diabetic mice (p>0.05 vs. STZ) (Figure 50C).

Striatal mAChR4 mRNA expression was significantly upregulated (p<0.05 vs. CTRL) in STZ treated animals as compared to control animals. Chronic UD and ROSI treatment did not reverse striatal mAChR4 mRNA expression in diabetic mice (Figure 50D).

**Figure 50:** The effect of UD on diabetes mediated alterations in mAChR4s expression: mAChR1 mRNA in hippocampus (A), mAChR4 mRNA in hippocampus (B), mAChR1 in (115)

mRNA in striatum (C) and mAChR4 mRNA in striatum (D). Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI. *p < 0.05, **p < 0.01. CTRL = control; STZ = streptozotocin; UD50 = Urtica dioica extract 50 mg/kg; ROSI = rosiglitazone.

STZ induced diabetic mice significantly downregulated the protein (p<0.01 vs. CTRL) expression of mAChR1 in hippocampus as compared to control mice. Chronic UD and ROSI administration significantly increased the protein (p<0.05 vs. STZ) expression of hippocampal mAChR1 in diabetic mice (Figure 51A).

Striatal mAChR4 protein expression was significantly upregulated (p<0.05 vs. CTRL) in STZ treated diabetic animals as compared to control animals. Chronic UD and ROSI treatment did not modulate striatal mAChR4 protein expression in diabetic mice (Figure 51B).

Figure 51: The effect of UD on diabetes mediated alterations in protein expression of mAChR1 in hippocampus (A), mAChR4 in striatum (B), AChE in hippocampus (C) and ChAT in hippocampus (D). Data were mean ± SEM values (n=4). Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI. *p < 0.05, **p < 0.01, ***p <
0.001. CTRL = control; STZ = streptozotocin; UD50 = *Urtica dioica* extract 50 mg/kg; ROSI = rosiglitazone.

STZ induced diabetic mice significantly upregulated the protein (p<0.001 vs. CTRL) expression of AChE in hippocampus as compared to control mice. Chronic UD administration significantly decreased (p<0.001 vs. STZ), while chronic ROSI administration did not modulate (p>0.05 vs. STZ) the protein expression of hippocampal AChE in diabetic mice (Figure 51C).

ChAT protein expression was significantly downregulated in the hippocampus of chronically diabetic mice (p<0.01 vs. CTRL). Chronic UD and ROSI administration significantly increased the protein expression of ChAT in diabetic mice (p>0.05 vs. STZ) (Figure 51D).

### 5.19. Effect of UD extract on oxidative stress, inflammation and neuronal survival

**Figure 52:** The effect of UD on diabetes mediated alterations in the mRNA expression of BDNF (A), TrkB (B) and cyclin D1 (C) in hippocampus. Data were mean ± SEM values

Significant differences: #CTRL vs. STZ; *STZ vs. STZ + UD50 and STZ + ROSI. **p < 0.01, ***p < 0.001. CTRL = control; STZ = streptozotocin; UD50 = *Urtica dioica* extract 50 mg/kg; ROSI = rosiglitazone.

BDNF mRNA expression was significantly downregulated in the hippocampus of chronically diabetic mice (p<0.001 vs. CTRL). Chronic UD and ROSI administration significantly increased the mRNA expression of BDNF in diabetic mice (p<0.01 vs. STZ and p<0.001 vs. STZ) (Figure 52A).

STZ induced diabetic mice significantly downregulated the mRNA (p<0.001 vs. CTRL) expression of TrkB in hippocampus as compared to control mice. Chronic UD and ROSI administration significantly increased the mRNA (p<0.001 vs. STZ) expression of hippocampal TrkB in diabetic mice (Figure 52B).

Cyclin D1 mRNA expression was significantly downregulated in the hippocampus of chronically diabetic mice (p<0.001 vs. CTRL). Chronic UD and ROSI administration significantly increased the mRNA expression of cyclin D1 in diabetic mice (p<0.001 vs. STZ) (Figure 52C).

![Figure 53: The effect of UD on diabetes mediated alterations in the mRNA expression of BCL2 (A), AIP2 (B), ATG5 (C), ATG7 (D), iNOS (E), IL6 (F) and TNFα (G) in](image-url)
BCL2 mRNA expression was significantly downregulated in the hippocampus of chronically diabetic mice (p<0.05 vs. CTRL). Chronic UD and ROSI administration significantly increased the mRNA expression of BCL2 in diabetic mice (p<0.01 vs. STZ and p<0.05 vs. STZ) (Figure 53A).

Chronic diabetes did not alter the mRNA expression of AIP2 in hippocampus (p>0.05 vs. CTRL). Chronic UD and ROSI treatment had no effect on the level of AIP2 in hippocampus of diabetic mice (p>0.05 vs. STZ) (Figure 53B).

STZ induced diabetic mice insignificantly modulated the mRNA (p>0.05 vs. CTRL) expression of ATG5 in hippocampus as compared to control mice. Chronic UD and ROSI administration significantly increased the mRNA (p<0.01 vs. STZ) expression of hippocampal ATG5 in diabetic mice (Figure 53C).

ATG7 mRNA expression was significantly downregulated in the hippocampus of chronically diabetic mice (p<0.001 vs. CTRL). Chronic UD and ROSI administration significantly increased the mRNA expression of ATG7 in diabetic mice (p<0.001 vs. STZ) (Figure 53D).

STZ induced diabetic mice significantly upregulated the mRNA (p<0.05 vs. CTRL) expression of iNOS in hippocampus as compared to control mice. Chronic UD and ROSI administration significantly decreased the mRNA expression of hippocampal iNOS in diabetic mice (p<0.05 vs. STZ and p<0.01 vs. STZ) (Figure 53E).

Chronic diabetes did not alter the mRNA expression of IL6 in hippocampus (p>0.05 vs. CTRL). Chronic UD and ROSI treatment had no effect on the level of IL6 in hippocampus of diabetic mice (p>0.05 vs. STZ) (Figure 53F).

STZ induced diabetic mice significantly upregulated the mRNA (p<0.05 vs. CTRL) expression of TNFα in hippocampus as compared to control mice. Chronic UD and ROSI administration significantly decreased the mRNA expression of hippocampal TNFα in diabetic mice (p<0.05 vs. STZ) (Figure 53G).
Figure 54: 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 + UD50 and STZ + ROSI. *p < 0.05, **p < 0.01. CTRL = control; STZ = streptozotocin; UD50 = *Urtica dioica* extract 50 mg/kg; ROSI = rosiglitazone.

The level of plasma TBARS was significantly increased (p<0.01 vs. CTRL) in diabetic animals as compared to control animals. Chronic treatment with UD and ROSI significantly decreased (p<0.05 vs. STZ and p<0.01 vs. STZ, respectively) the elevated level of TBARS in diabetic animals (Figure 54A).

STZ-induced long standing diabetes significantly elevated (p<0.01 vs. CTRL) the level of plasma NO as compared to control animals. Chronic UD and ROSI administration significantly attenuated (p<0.05 vs. STZ) the elevated level of plasma NO in diabetic mice (Figure 54B).
STZ-induced diabetes significantly decreased the level of catalase (p<0.05 vs. CTRL) in plasma as compared to control animals. UD and ROSI treatment significantly increased (p<0.05 vs. STZ) the level of plasma catalase in diabetic mice (Figure 54C).

STZ-induced diabetic mice showed significant decrease in the level of total thiol (p<0.01 vs. CTRL) in plasma as compared to control animals. Chronic UD and ROSI treatment significantly improved (p<0.01 vs. STZ) the level of plasma total thiol in diabetic mice (Figure 54D).

### 5.20. Immunofluorescence study of TNFα on hippocampal section of chronically stressed mice

Control mice showed normal nucleus on DAPI staining which were altered in CUMS group. Chronic stress increased the fluorescence of TNF-α whereas FLX, ROSI, HYP and UD treatment reversed it (Figure 55).

**Figure 55:** Effect of UD on CUMS mediated alterations in TNF-α expression using immunofluorescence study. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = *Hypericum perforatum* extract; UD = *Urtica dioica* extract.

### 5.21. Histopathology study on hippocampal section of chronically stressed mice

Control mice showed normal cellular morphology which were altered in CUMS group. Chronic stress increased the neuronal damage as indicated by arrow whereas FLX, ROSI, HYP and UD treatment reversed it (Figure 56).
Figure 56: Effect of UD on CUMS mediated neuronal damage using histopathology study. CTRL = control; CUMS = chronic unpredictable mild stress; FLX = fluoxetine; ROSI = rosiglitazone; HYP = Hypericum perforatum extract; UD = Urtica dioica extract.

5.22. Immunofluorescence study of TNFα on hippocampal section of STZ induced diabetic mice

Control mice showed normal nucleus on DAPI staining which were altered in STZ group. Chronic diabetes increased the expression of TNF-α whereas UD and ROSI treatment reversed it (Figure 57).

Figure 57: Effect of UD on diabetes induced alterations in TNF-α expression using immunofluorescence study. CTRL = control; STZ = streptozotocin; UD50 = Urtica dioica extract 50 mg/kg; ROSI = rosiglitazone.

5.23. Histopathology study on hippocampal section of STZ induced diabetic mice

Control mice showed normal cellular morphology which were altered in diabetic group. Chronic diabetes increased the neuronal damage as indicated by arrow whereas ROSI and UD treatment reversed it (Figure 58).
**Figure 58**: Effect of UD on diabetes mediated neuronal damage using histopathology study. CTRL = control; STZ = streptozotocin; UD50 = *Urtica dioica* extract 50 mg/kg; ROSI = rosiglitazone.