RESULTS
### 6.1 Changes in the body weight during treatment period:

Comparison between male and female: There is no significant difference in the body weight of male and female rats at all the study intervals (Day 1, 7, 21 and 28).

Hence the data of male and female rats were collapsed for each group for further statistical analysis using One way analysis of Variance (ANOVA) followed by Bonferroni multiple comparison test.

**Table – 1:** Statistical observation of two - tail P value for comparison of body weight between male and female rats.

<table>
<thead>
<tr>
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<th>Day 1</th>
<th>Day 7</th>
<th>Day 21</th>
<th>Day 28</th>
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<tbody>
<tr>
<td></td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Control</td>
<td>0.690</td>
<td>1.279</td>
<td>0.894</td>
<td>1.302</td>
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<tr>
<td>Stress</td>
<td>0.326</td>
<td>2.321</td>
<td>0.414</td>
<td>2.338</td>
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<tr>
<td>Res 10</td>
<td>0.080</td>
<td>1.022</td>
<td>0.080</td>
<td>1.133</td>
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<tr>
<td>Res 20</td>
<td>0.396</td>
<td>1.108</td>
<td>0.386</td>
<td>1.099</td>
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<tr>
<td>Res 10 + Stress</td>
<td>0.691</td>
<td>3.201</td>
<td>0.668</td>
<td>1.021</td>
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<tr>
<td>Res 20 + Stress</td>
<td>0.832</td>
<td>0.214</td>
<td>0.8480</td>
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Table – 2: Body weight of the rats (in g) during treatment period (n=20). Values are expressed as mean ± SD. For Day1: P=0.0451, F=2.352. Day 7: P=0.0667, F=2.131. Day 21: P=0.0016, F=4.170, Control Vs Others ** =p<0.01, *=p<0.05. Day 28: P<0.0001, F=5.823, Control Vs others ***=p<0.001, **=p<0.01, *=p<0.05.

<table>
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<tr>
<th>Time interval</th>
<th>Control</th>
<th>Stress</th>
<th>Res 10</th>
<th>Res 20</th>
<th>Res 10 + Stress</th>
<th>Res 20 + Stress</th>
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</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>237±23.136</td>
<td>236.35±25.44</td>
<td>224.25±22.18</td>
<td>219.35±28.07</td>
<td>222.65±15.73</td>
<td>220.65±21.82</td>
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<tr>
<td>Day 7</td>
<td>238±22.77</td>
<td>231.6±25.14</td>
<td>223.75±22.66</td>
<td>218.05±28.16</td>
<td>224.65±22.57</td>
<td>218.3±21.46</td>
</tr>
<tr>
<td>Day 21</td>
<td>243.35±23.58</td>
<td>226.7±26.04</td>
<td>221.75±22.29</td>
<td>215.1±27.55 **</td>
<td>219.65±22.612 *</td>
<td>213.05±21.47 **</td>
</tr>
<tr>
<td>Day 28</td>
<td>247.6±22.77</td>
<td>224.9±25.96 *</td>
<td>220±22.43 **</td>
<td>214.35±27.38 ***</td>
<td>218.5±22.612 **</td>
<td>212.2±21.50 ***</td>
</tr>
</tbody>
</table>
Day 1

The initial body weight of the rats (approximately 4 month old) during the beginning of the experiment were ranging from 215 – 240gms. There was no statistically significant difference in the body weight when all the animal groups were compared with each other.

Day 7

At Day 7 of the experiment the gain in the body weight was consistent among all the group as there was no significant difference between them.

Day 21

Rats who received 20mg/kg dose of resveratrol failed to gain the body weight at par with the control. There was a significant (p<0.01) difference between resveratrol (20mg/kg dose) treated and control group. Further rats who received stress and resveratrol also showed significant difference in the body weight compared to control rats. The significant level was more (p<0.01) in 20mg/kg dose of resveratrol and less (p<0.05) in 10mg/kg dose.

Day 28

Interestingly the body weight of the stressed rats showed a marginal decline (p<0.05) compared to control rats at day 28, but this difference was not observed during day 7 and 21. Resveratrol alone treatment (20mg/kg) continued to show a declined body weight at day 28 and the statistical significant was more (p<0.01) compared to the decline observed on day 21. Rats who received stress and resveratrol (both the doses) continued to show a decline in the body weight compared to control. The significant level was more (p<0.001) in 20mg/kg dose of resveratrol and less (p<0.01) in 10mg/kg dose.

Summary:

Oral resveratrol treatment for continuous 28 days in rats showed a reduced body weight on day 21 and this effect continued on day 28 also. This loss in body weight seems to be dose dependent as 20mg/kg was found to be more severe. The body weight of stressed rats who received resveratrol also showed a decline in the body weight on day 21 and this effect continued on day 28 also. These results indicate that resveratrol treatment under normal and stressed circumstances
reduce the body weight. Though stressed rats did not show any reduction in body weight on day 21 but it was significant on day 28 indicating chronic restraint stress reduces the bodyweight.
Fig.1: Change in the body weight during different interval of resveratrol treatment. Values are expressed as mean g ± SD (n=24). Comparison between Control Vs others * = p<0.05, ** = p<0.01, ***=p<0.001.
Comparision between: control vs stress *** P<0.001, control vs Res10 *** P<0.001, control vs Res20 *** P<0.001, control vs Res10+S *** P<0.001, control vs Res20+S *** P<0.001, stress vs Res10 *** P<0.001, stress vs Res20 *** P<0.001, stress vs Res10+S ns  P>0.05, stress vs Res20+S ns  P>0.05, Res10 vs Res20 ns  P>0.05, Res10 vs Res10+S *** P<0.001, Res10 vs Res20+S *** P<0.001, Res10+S vs Res20+S ns  P>0.05, (F = 177.44).
Comparison of mean body weight gain/loss after 28 days of treatment:

- Restraint stress in rats has caused a significant (p<0.001) reduction in body weight compared to their control counterparts.
- Resveratrol alone treatment (either 10 or 20mg/kg dose) also resulted in a significant loss in body weight compared to control (p<0.001).
- Restraint stress has more severe effect (p<0.001) when compared to rats who received only resveratrol (either 10 or 20mg/kg dose).
- The reduction in the body weight of rats who received stress as well as resveratrol (either 10 or 20mg/kg dose) did not differ statistically (p>0.05) when compared with rats who received only restraint stress, but it differed significantly (p<0.001) when compared with control rats.
- There was no significant difference in reduction of body weight when compared between the two doses of resveratrol treatments.

Summary: Both restraint stress as well as resveratrol alone treatment has caused body weight loss, but this effect was more pronounced in stressed rats. Comparison of weight loss between stress alone with that of combination of stress and resveratrol did not show any significant difference. It appears that stress and resveratrol may exert different mechanism in reduction of body weight.
Neuroprotective effects of resveratrol against restraint stress

6.2 Behavioral studies:

6.2.1 Open field test:

Total number of line crossings

- The mean number of total line crossings for a duration of 5 min did not differ (p>0.05) between control and stressed rats.
- Resveratrol at 10mg/kg dose and also at 20mg/kg dose has enhanced locomotor activity (p<0.001) compared to either control or stressed rats.
- Total line crossings after resveratrol treatment of 10mg/kg in stressed rats did not differ significantly (p>0.05) when compared to control but differed significantly when compared to stressed group (p<0.01).
- Resveratrol (20mg/kg) dose in stressed rats showed a moderate significance (p<0.05) decline in total line crossings compared to control, but when it was compared to stressed group it was not significant. (Fig 2.1a)

Number of central square crossings

- The restraint stress has significantly (p<0.001) reduced the number of central square crossings.
- Treatment of resveratrol alone (either 10 or 20mg/kg dose) has not shown any difference (p>0.05) in central square crossing when compared to control.
- Resveratrol at 10 mg/kg dose (p<0.001) and at 20mg/kg dose (p<0.01) increased the number of central square crossings in stressed rats when compared to this performance by rats received only stress. (Fig.2.1b)

Rearing and grooming

- Rearing and grooming activities were reduced (p<0.001) in stressed rats compared to control.
- Resveratrol treatment in stressed rat has enhanced (p<0.001) grooming activities but not affected rearing activities.
- Resveratrol alone treatment also showed a significant increase (p<0.001) in these activities when compared to stressed rats. (Fig.2.1c)

Summary: Considering total number of line crossings in the open field test, the restraint stress has not affected locomotor activity in general. In stressed condition resveratrol at 10mg/kg dose
has enhanced locomotor activity but not at 20mg/kg dose. Resveratrol alone treatment (at both doses) in normal rats has enhanced locomotor activity. The reduced exploration in the central portion of the open field is an indicator of its anxiety-like behavior. Resveratrol treatment in stressed rats appears to minimize this anxiety-like behavior.
Fig.2.1a: Observation on open field activity by rats subjected to restraint stress and resveratrol treatment. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ***=p<0.001, *-=p<0.05, Comparison between Stress Vs others @@@ = p<0.01, @@@@ = p<0.001, Comparison between R10 Vs others $$$ = p<0.001, Comparison between R20 Vs others #### = p<0.001, Comparision between R10+S Vs others ccc=p<0.001.
Fig. 2.1b: Observation on open field activity by rats subjected to restraint stress and resveratrol treatment. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others *** = p<0.001, Comparison between Stress Vs others @@ = p<0.01, @@@@ = p<0.001.
Fig. 2.1c: Observation on open field activity by rats subjected to restraint stress and resveratrol treatment. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ***=p<0.001, Comparison between Stress Vs others @@@ = p<0.001.
6.2.2 Passive avoidance test

- Rats who received restraint stress took significantly (p<0.001) lesser time to enter the dark compartment when compared to control. This shorter latency indicates poor retrieval of learning behavior.
- Resveratrol alone treatment has enhanced the time to enter the dark compartment (p<0.01 for 10mg/kg dose and p<0.001 for 20mg/kg dose) an indication of good learning abilities.
- Resveratrol treatment in stressed rat did not show any increase in time interval to enter the dark compartment compared to control as the latency to enter the dark compartment were significantly less (p<0.001) when compared to control. This would indicate a poor retrieval of learning behavior in stressed rats even after resveratrol treatment.
- However, resveratrol at both doses has enhanced latency to enter the dark compartment in stressed rats compared to rats who received only stress. *(Fig.2.2)*

**Summary:** Chronic restraint stress resulted in poor retrieval of learning behavior. Though resveratrol has not enhanced retrieval of learning behavior to the normalcy, but it was better than the performance of stressed rats, an indication of neuroprotective effect.
Fig. 2.2: Observations of passive avoidance test by rats subjected to restraint stress and resveratrol treatment. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ** = p<0.01, ***=p<0.001. Comparison between Stress Vs others @@@ = p<0.001. Comparison between R10 Vs others $$$=p<0.001. Comparison between R20 Vs others ###=p<0.001. Comparison between R10+S Vs R20 +S ccc =p<0.001.
6.2.3 Active avoidance test (Condition avoidance/Shuttle box test)

**Mean of 5 days avoidance score**

- Restraint stress has significantly (p< 0.001) reduced mean of 5 days score comparing with control an indication of learning disability.
- Interestingly resveratrol alone at both the doses has significantly (p<0.001) increased mean of 5 days score compared to control as well as stressed rats an indication of enhancement of learning skills.
- Resveratrol at both doses has enhanced (p<0.001) mean of 5 days score in stressed rats compared to rats received only stress indicating a neuroprotective effect of resveratrol.
- The mean of 5 days score in rats who received resveratrol as well as restraint stress was significantly (p<0.001) less when compared to rats who received only resveratrol but these values did not differ from the control rats. This indicates that the mean of 5 days score (learning ability) in stressed rats after treatment with resveratrol was near to the values of control rats. *(Fig.2.3)*

**Retest score**

- The results of the retest score were similar to the mean of 5 days score. Restraint stress has significantly (p<0.001) reduced retest score compared to control as well as resveratrol alone treatment.
- Resveratrol treatment in stressed rats has significantly (p<0.001) enhanced the retest score showing a neuroprotective effect. *(Fig.2.3)*

**Retention score in percentage**

- The memory retention score was significantly (p<0.001) less in stressed rats when compared to control as well as rats who received only resveratrol (at both the doses).
- Resveratrol alone treatment (at both doses) showed a highly significant (p<0.001) increase in retention score when compared to control.
- Resveratrol in stressed rats (at both the doses) has enhanced the percentage of retention score significantly compared to rats who received only restraint stress. This indicates a neuroprotective effect of resveratrol against restraint stress induced memory dysfunction.
- The percentage of retention score in rats who received resveratrol as well as restraint stress was significantly (p<0.001) less when compared to rats who received only resveratrol but these values did not differ from the control rats. This indicates that
theretention score (ability to recall the learnt behavior) in stressed rats after treatment with resveratrol was near to the values of control rats. (Fig.2.3)

**Summary:** Chronic restraint stress has adversely affected the learning abilities and memory retention in rats. The resveratrol treatment has reversed this adverse effect in stressed rats and also interestingly it has enhanced the learning abilities and ability to retain the memory tremendously under normal circumstances also.
Fig.2.3: Observation of active avoidance test by rats subjected to restraint stress and resveratrol treatment. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ***=p<0.001, Comparison between Stress Vs others @@@ = p<0.001, Comparison between R10 Vs others $$$ = p<0.001, Comparison between R20 Vs others #### = p<0.001.
6.3 Biochemical studies:

Antioxidant studies:

6.3.1 Lipid peroxidation: MDA level is a marker of lipid peroxidation.

- A highly significant (p<0.001) elevation in the expression of lipid peroxidation in the rat brain homogenate who received stress was noticed when compared to the control rat brain homogenate.
- Resveratrol at both doses has reduced significantly (p<0.001) the lipid peroxidation level when compared with stressed rats as well as control rats brain homogenate.
- Rats who received both resveratrol and stress showed a significant (p<0.001) decrease in brain MDA level compared to control as well as rats who received only stress.
- Resveratrol at 10mg/kg dose had more profound effect compared to 20mg/kg dose.
- The decrease in MDA level of rats who received stress as well as resveratrol (10mg) is significant (p<0.001) compared to rats who received only resveratrol (at both doses). Combination of stress and 20mg/kg dose of resveratrol showed a significant (p<0.05) decrease in brain MDA level when compared to rats received only 10mg/kg dose. However the combination of stress and 20mg/kg dose of resveratrol showed a significant (p<0.001) increase in brain MDA level when compared to rats who received only 20mg/kg dose. (Fig.3.1)

Summary: Chronic restraint stress expressed an elevated brain MDA level an indication of increased free radical generation (oxidative stress). The antioxidant resveratrol has lowered the elevated MDA level to the normalcy indicating antioxidant potential of resveratrol against restraint stress induced oxidative damage.
Fig. 3.1: Expression of lipid peroxidation (MDA levels) in rat brain homogenate subjected to restraint stress and resveratrol treatment. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others *** = p<0.001. Comparison between Stress Vs others @@@ = p<0.001. Comparison between R10 Vs others $ = p<0.05, $$$$=p<0.001. Comparison between R20 Vs others ###=p<0.001. Comparison between R10+S Vs R20 +S ccc =p<0.001.
6.3.2 Glutathione reductase (GSSG-Rd) activity in brain.

- The activity of brain glutathione reductase was significantly (p<0.001) decreased in stressed rats compared to control as well as resveratrol alone treatment.
- Resveratrol alone treatment at 20mg/kg dose enhanced (p<0.001) the glutathione reductase level compared to control, but not at 10mg/kg dose.
- Resveratrol treatment (both doses) in stressed rats has significantly (p<0.001) increased glutathione reductase activity compared to rats who received only stress claiming the antioxidant potential of resveratrol in stressed conditions.
- Resveratrol treatment at 10mg/kg dose in stressed rats has shown an increase in the glutathione reductase level at par with control (as there is no statistical significant difference between their values). However resveratrol at 20mg/kg dose in stressed rats differed significantly with control group in expression of glutathione reductase. *(Fig.3.2)*

**Summary:** Chronic restraint stress expressed a severe oxidative damage in rat brain. Resveratrol at 10mg/kg dose has protected this oxidative damage by bringing the glutathione reductase level to the normalcy. Though resveratrol treatment in stressed rats has elevated the glutathione reductase level, but not to the extent of antioxidant potential exerted by resveratrol alone treatment.
Fig. 3.2: Brain Glutathione reductase activity (nmol NADPH oxidized/min/mg protein) in rats. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ***=p<0.001. Comparison between Stress Vs others @@@ = p<0.001. Comparison between R10 Vs others $ = p<0.05, $$$=p<0.001. Comparison between R20 Vs others ### = p<0.001. Comparison between R10+S Vs R20+S CCC =p<0.001.
6.3.3 Reduced glutathione (GSH) level in brain.

• There is a significant (p<0.001) reduction in reduced glutathione level (GSH) in stressed rat brain homogenate compared to the control as well as rats who received resveratrol alone.

• Resveratrol at both doses enhanced (p<0.001) the reduced glutathione levels compared to control.

• Resveratrol treatment (both doses) in stressed rats reversed the oxidative damage by elevating reduced glutathione level significantly (p<0.001).

• Resveratrol treatment at 10mg/kg dose in stressed rats has shown an increase in the reduced glutathione level at par with control. (as there is no statistical significant difference between their values)

• Though resveratrol treatment in stressed rats has elevated the reduced glutathione level, but not to the extent of antioxidant potential exerted by resveratrol alone treatment. (Fig.3.3)

Summary: Chronic restraint stress has caused a decline in reduced glutathione level in rat brain homogenate and this effect was reversed by resveratrol treatment. This is an indication of free radical scavenging properties of resveratrol.
Fig.3.3: Brain reduced Glutathione level (mg/gm protein) in rats. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ** = p<0.01, *** = p<0.001. Comparison between Stress Vs others @@@ = p<0.001. Comparison between R10 Vs others $$$ = p<0.001. Comparison between R20 Vs others ### = p<0.001.
6.3.4 Total antioxidants in the brain.

- The total antioxidants (TAO) level in the brain decreased significantly ($p<0.001$) in stressed rats when compared to control group.
- Rats exposed to stress along with resveratrol treatment showed a significant increase ($p<0.001$ for R10+S and $p<0.01$ for R20+S) in total antioxidants level compared to rats who received only stress.
- Resveratrol alone treatment has showed a highly significant ($p<0.001$) increase in brain TAO level compared to control as well as stressed rats.
- Though resveratrol treatment has enhanced brain TAO level in stressed rats, but did not elevate to the values expressed by control rats as well as rats who received only resveratrol (at both doses).
- There was also a significant ($p<0.001$) difference between the two doses of resveratrol treatment. (Fig.3.4)

Summary: Chronic restraint stress has adversely affected the antioxidant defence system in the rat brain. The antioxidant resveratrol has reversed this toxic effect by enhancing brain TAO activity. Resveratrol under normal circumstances also showed an antioxidant effect by expressing elevated TAO activity in rat brain.
Fig.3.4: Effects of restraint stress and resveratrol administration on the total antioxidant level (mmoles/lit) in the brain. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ** = p<0.01, ***=p<0.001. Comparison between Stress Vs others @@ = p<0.01, @@@ = p<0.001. Comparison between R10 Vs others $$$=p<0.001. Comparison between R20 Vs others ### = p<0.001. Comparison between R10+S Vs R20 +S ccc =p<0.001.
6.4 Expression of BDNF

6.4.1 Comparison of BDNF expression in rats treated with various doses of resveratrol.

- Immobilization stress in rats resulted in significant (p<0.01) decline in BDNF level in whole brain homogenate compared to control rats.
- Resveratrol alone treatment at both the doses (10mg and 20mg/kg body weight) has not affected the BDNF level (p>0.05) when compared to control.
- Rats who received resveratrol alone (at both doses) has shown a significant (p<0.001) increase in the BDNF level when compared to rats who received only stress.
- Resveratrol at 10mg/kg dose has enhanced the BDNF level in stressed rats compared to rats who received only stress, but not at 20mg/kg dose.
- Resveratrol treatment (10mg/kg dose) has enhanced BDNF expression which is at par with control (as the values between these two groups are statistically not significant). However such effect was not observed at 20mg/kg dose of resveratrol (as their values differed significantly p<0.01)
- Resveratrol at 20mg/kg dose in stressed rats showed a highly significant decrease in BDNF expression compared to rats who received either 10 or 20mg/kg dose of resveratrol alone.
- A comparison between rats who received stress as well as resveratrol showed that, resveratrol at 10mg/kg dose is more effective compared to 20mg/kg dose (as their values showed a highly significant (p<0.001) difference between them). (Fig.4.1)

Summary: Chronic restraint stress has reduced BDNF level in rat brain homogenate. Resveratrol treatment at 10mg/kg dose has reversed this effect by elevating BDNF level expression. However, the neuroprotective effect of resveratrol on BDNF expression against restraint stress seems to be dose specific as 10mg/kg dose found to be more effective.
Fig. 4.1: Comparison of BDNF expression in rats treated with various doses of resveratrol. Values expressed as mean ± SD (n=6). Control Vs others ** = p<0.01. Comparison between Stress Vs others $$$ = p<0.001. Comparison between R10 Vs others aaa = p<0.001. Comparison between R20 Vs others £££ = p<0.001. Comparison between R10+S Vs others ### = p<0.001.
6.4.2 Comparison of BDNF expression in brain homogenates of various groups of rats treated with Vitamin C.

- Immobilization stress in rats has resulted in significant (p<0.001) decline in BDNF level in whole brain homogenate compared to control rats.
- Interestingly Vitamin C administration at both the doses (p<0.001) to the rats which have not received the stress also showed a significant decline in BDNF expression compared to control.
- Vitamin C alone treatment at 200mg/kg dose has shown a highly significant (p<0.001) decrease in brain BDNF expression compared to rats who received only stress.
- Vitamin C at 200mg/kg dose had more severe effect (decline in BDNF level, p<0.05) when compared to 100mg/kg dose of vitamin C.
- Vitamin C at 100mg/kg dose in stressed rats showed a marginal significant decrease in brain BDNF level (p<0.05) compared to control, but this effect was not observed in Vitamin C 200mg/kg dose. This shows a restoration of BDNF level in stressed rats, as its value did not differ significantly (p>0.05) from control group.

Vitamin C at 200mg/kg dose in stressed rats has significantly enhanced (p<0.01) BDNF level when compared to rats who received only stress. This effect was not observed in rats who received stress as well as 100mg/kg dose of vitamin C.\textbf{(Fig.4.2)}

\textbf{Summary:} Vitamin C is found to have an adverse effect on BDNF expression in rat brain homogenate. Interestingly vitamin C at higher dose in stressed condition has elevated BDNF level near to the normalcy.
Fig. 4.2: Comparison of BDNF expression in brain homogenates of various groups of rats treated with Vitamin C. Values expressed as mean ± SD (n=6). Control vs others * = p<0.05, *** = p<0.001. Comparison between Stress vs others $$ = p<0.01, $$$ = p<0.001. Comparison between VitC100 vs others § = p<0.05, §§§ = p<0.001. Comparison between VitC200 vs others @@@ = p<0.001.
6.4.3 Comparison of BDNF expression values between Resveratrol and Vitamin C treatment regime.

- The BDNF expression in rat brain homogenate was enhanced (p<0.001) in rats who received both resveratrol (10mg/kg dose) and stress compared to rats who received only stress, but vitamin C treatment showed a reverse effect. (Fig 4.1 and 4.2)
- Resveratrol alone treatment (at both doses) showed a highly significant (p<0.001) increase in BDNF expression compared to rats who received vitamin C (at both doses).
- Comparison between two doses of vitamin C alone treatment revealed that the higher dose of vitamin C (200mg/kg dose) is more detrimental for BDNF expression.
- Interestingly the comparison of resveratrol treatment with vitamin C treatment in stressed rats did not show any statistical significant difference (p>0.05), however there was a difference (p<0.01) between two doses of resveratrol treatment in stressed rats, the 10mg/kg dose proving to be more beneficial. (Fig.4.3)

**Summary:** The Vitamin C treatment has an adverse effect on expression of BDNF level in rat brain homogenate under normal circumstances but resveratrol did not show such effect. Resveratrol is found to be beneficial compared to vitamin C as far as BDNF expression in rat brain homogenate.
Fig.4.3: Comparison of BDNF expression between resveratrol and vitamin C treatment. Values expressed as mean ± SD (n=6). Comparison between R10 Vs VitC100 & VitC200 aaa = p<0.001. Comparison between R20 Vs VitC100 & VitC200 £££ = p<0.001. Comparison between VitC100 Vs VitC200 § = p<0.05. Comparison between R10+S Vs R20+S ## = p<0.01.
6.5 Histomorphological studies: (Cresyl violet staining)

6.5.1 Neuronal assay of Dentate Gyrus (DG):

- The expression of number of healthy neurons in DG has reduced significantly (p<0.001) in stressed rats comparing to control indicating a reduction in neuronal loss.
- Resveratrol alone treatment has not affected (p>0.05) the neuronal expression when compared to control
- Resveratrol at both doses in stressed group has enhanced (p<0.001) the number of neurons compared to stress alone.
- Resveratrol alone treatment (both the doses) showed a higher (p<0.001) number of neuronal expression compared to rat who received only stress.
- The expression of number of healthy neurons in rats who received resveratrol 10mg/kg dose as well as stress did not differ (p>0.05) with that of the control, but this numerical expression differed (p<0.05) at 20mg/kg dose of resveratrol. This indicates 10mg/kg dose of resveratrol is more beneficial.
- It is further proved, when rats who received stress and resveratrol 10mg/kg dose expressed higher (p<0.01) number of neurons compared to rats who received stress and 20mg/kg dose of resveratrol. (Fig.5.1)

Summary: Chronic restraint stress has shown neuronal loss in the dentate gyrus where there is an active neurogenesis even in adulthood. Resveratrol in stressed rats has reversed this neurotoxic effect.
Fig: 5.1: Resveratrol and their combination induced changes in the neuronal numbers in 240sqµm² area in Dentate Gyrus (DG). Values are expressed as mean ± SD (n=24 slides/group). Comparison between Control Vs others *** = p<0.001, * = p<0.05. Comparison between Stress Vs others @@ @ = p<0.001. Comparison between R10 Vs others $ = p<0.05. Comparison between R20 Vs others ## = p<0.01. Comparison between R10+S Vs R20 +S cc = p<0.01.
6.5.2 Neuronal assay of various regions of the Hippocampus.

CA4 neurons

- The expression of number of healthy neurons in CA4 region of hippocampus did not show any statistical significant difference (p>0.05) when compared among all the groups, except for a marginal difference (p<0.01) when rat received resveratrol (20mg/kg dose) and stress was compared with rats who received only resveratrol (10mg/kg dose). (Fig.5.2)

CA3 neurons

- The expression of number of healthy neurons in CA3 has reduced significantly (p<0.001) in stressed rats comparing to control.
- Resveratrol alone treatment (at both the doses) has not differed (p>0.05) the neuronal expression when compared to control.
- Resveratrol at (10mg/kg dose) in stressed rats enhanced (p<0.001) the expression of neurons compared to rats who received only stress. Resveratrol at 20mg/kg dose in stressed rats also expressed a higher (p<0.01) number of neurons comparing to rats who received only stress.
- Resveratrol alone treatment (both the doses) showed a higher (p<0.001) number of neuronal expression compared to rats who received only stress.
- The expression of number of healthy neurons in rats who received resveratrol (at both doses) as well as stress did not differ (p>0.05) with that of the control.
- Resveratrol at 20mg/kg dose in stressed rats showed a lesser (p<0.01) number of neurons when compared with rats who received only 20mg/kg dose of resveratrol.
- Resveratrol at 20mg/kg dose in stressed rats showed a lesser (p<0.05) number of neurons when compared with rats who received only 10mg/kg dose of resveratrol. (Fig.5.2)

CA2 neurons

- The expression of number of healthy neurons in CA2 has reduced significantly (p<0.01) in stressed rats comparing to control.
• Resveratrol alone treatment (at both the doses) has not differed (p>0.05) the neuronal expression when compared to control.
• Resveratrol at (10mg/kg dose) in stressed rats enhanced (p<0.01) the expression of neurons compared to rats who received only stress. Resveratrol at 20mg/kg dose in stressed rats did not show any statistical significant difference (p>0.05) in number of neurons comparing to rats who received only stress.
• Resveratrol alone treatment (both the doses) showed a higher (p<0.001) number of neuronal expression compared to rats who received only stress.
• The expression of number of healthy neurons in rats who received resveratrol (at both doses) as well as stress did not differ (p>0.05) with that of the control.
• Resveratrol at 20mg/kg dose in stressed rats showed a lesser (p<0.05) number of neurons when compared with rats who received either 10 or 20mg/kg dose of resveratrol. (Fig.5.2)

CA1 neurons

• The expression of number of healthy neurons in CA1 region of hippocampus did not show any statistical significant difference (p>0.05) when compared among all the groups, except for a marginal difference (p<0.05) when rat received resveratrol (10mg/kg dose) and stress was compared with rats who received only stress. (Fig.5.2)

Summary: The chronic restraint stress-induced neuronal loss in the hippocampus was region specific. The CA3 region was severely affected, followed by CA2 region. The neurons of CA4 and CA1 regions have not been affected by restraint stress. Resveratrol treatment in stressed rats has reversed this neurotoxic effect.
Fig. 5.2: Resveratrol and their combination induced changes in the neuronal numbers in 250µm length area in hippocampal Cornu Ammonis (CA4, CA3, CA2, CA1). Values are expressed as mean ± SD (n=6). Comparison between Control Vs others ** = p<0.01, *** = p<0.001. Comparison between Stress Vs others @@ = p<0.01, @@@ = p<0.001. Comparison between R10 Vs others $ = p<0.05, $$ = p<0.01. Comparison between R20 Vs others # = p<0.05.
- **Neuronal assay of medial prefrontal cortex**
  - Restraint stress has expressed significantly (p<0.001) lesser number of neurons in stressed rats compared to control as well as rats who received only resveratrol.
  - Resveratrol at both doses in stressed rats showed a significant (p<0.001) higher number of neurons compared to rats who received stress alone.
  - The expression of number of healthy neurons in rats who received both stress and resveratrol (both the doses) did not differ (p>0.05) from that of the control as well as the rats who received only resveratrol (both the doses) as their numbers are nearly equal. (Fig.5.3)

**Summary:** Chronic restraint stress in rats has resulted in loss of neurons in medial prefrontal cortex, and this adverse effect was reversed by resveratrol.
Fig. 5.3: Resveratrol and their combination induced changes in the neuronal numbers in 300 µm² area in medial prefrontal cortex. Values are expressed as mean ± SD (n=6). Comparison between Control Vs others *** = p<0.001. Comparison between Stress Vs others @@@ = p<0.001.
Fig.6: Histomorphological pictures of various regions of the rat brain studied

Fig.6.1: Coronal section through cerebrum showing dentate gyrus and various parts of the hippocampus stained with cresyl violet under 10X
Fig. 6.2: Dentate gyrus - Under 40X, Control Vs others
**Fig. 6.3:** CA2 region of the Hippocampus – Under 40X, Control Vs others

**Fig. 6.4:** CA3 region of the Hippocampus – under 40X, Control Vs others
**Fig. 6.5a:** Prefrontal cortex (medial prefrontal) area stained with cresyl violet under 10X: Picture showing the area selected

![Image showing the area selected](image_url)
Fig. 6.5b: Prefrontal cortex – under 40X, Control Vs others
DISCUSSION
Resveratrol and bodyweight loss/weight gain:

The results of the present study demonstrate that resveratrol treatment (for 28 consecutive days) under normal circumstances prevented the rats from gaining weight compared to those which did not receive resveratrol. Further resveratrol treatment in stressed rats also prevented the weight gain. This beneficial factor can be credited to the phytochemicals present in resveratrol. The mechanism of action of phytochemicals in reducing the fat mass includes enhancing lipolysis, apoptosis of adipocytes (Andersen et al., 2010). Six weeks of resveratrol treatment has reduced white adipose depots in rats (Macarulla et al., 2009). There are studies claiming that resveratrol treatment prevented accumulation of abdominal white adipose tissue (Nagao et al., 2013). A study by Baile et al., (2011) indicates decreased lipogenesis and increased lipolysis and thereby preventing weight gain even after high fat diet with treatment of resveratrol. Body fat reducing effect of resveratrol was reported by Alberdi et al., (2011) and this effect was attributed to a reduction in fatty acid uptake from circulating triacylglycerols and also to denovo lipogenesis. Resveratrol is also found to be beneficial in antipsychotic drug induced obesity and its adverse effects. Antipsychotic drug olanzapine has enhanced body weight and resveratrol treatment in these rats has reduced the weight gain, though the food intake was not differed among the treatment groups (More et al., 2012). Through these preliminary data resveratrol can be considered for obesity and obesity induced disorders. Resveratrol is known to improve metabolic functions in rodents and humans with abnormal metabolic function (Yashino et al., 2012).

The present study also showed a moderate body weight loss after 28 consecutive days of chronic restraint stress in rats, but this loss was not observed on day 21. Chronic restraint stress is also known to suppress the body weight with temporary hypophagia due to release of corticotrophin releasing factor (Harris et al., 2002). Restraint stress induced loss of body weight was reported in many experiments (Jeong et al., 2013). It is also likely that restraint stress induced depletion in brain serotonin level alter gluco-regulation in the hypothalamus which is said to regulate blood glucose level in highly complex manner (Tuomsti and Mannisto, 1985). Though the present study does not focus on brain serotonin and gluco-regulation, studies addressing the role of resveratrol in this regard will be interesting. Resveratrol treatment in stressed rats has prevented the weight gain at par with their control match in the present study. To summarize, the weight loss in stressed rats, as well as resveratrol treated rats, in this study, is consistent with the literature.

Behavioural studies:
Observation on anxiety, emotional activities, locomotor and exploratory activities, of rats subjected to restraint stress and resveratrol treatment:

The present study clearly demonstrates that chronic restraint stress has induced an anxiety like behaviour in rats which was reversed by resveratrol treatment. It has been well established that chronic restraint stress cause anxiety like behaviour in rats which is mainly due to reduced glucocorticoids receptors expression (Chiba et al., 2012). A study by Kondam et al., (2013) claim an anxiety like behaviour and declined locomotor activity after restraint stress, but in the present study locomotor activity was not affected by restraint stress. Anxiety like behaviour is associated with an increased response of HPA axis to stress with higher level of corticosterone expression (Cratty et al., 1995). Of late antioxidants are used as nutritional supplement is gaining its popularity to combat these adverse effects. An experiment by Patki et al., (2013) demonstrates treatment of grape powder in ovariectomized rats has reversed anxiety like behaviour and cognitive dysfunction in rats. Our results are consistent with the above mentioned studies.

Open field test is most reliable unconditional indicator of emotionality in rodents. Self-grooming is an important behaviour observed in rodents which primarily serves to maintain the body hygiene caring for body surface, thermoregulation, chemo-communication and importantly reduction of stress (Spruijt et al., 1992). Chronic restraint stress disturbing emotional activity is well documented (Wood et al., 2003) and results of our study are consistent with these earlier well established data. Free radical generation in the CNS during restraint stress might be responsible for such stress induced suppressed behaviour. Hence variety of antioxidants were used in the past to combat the restraint stress-induced suppressed behaviour. In the present study resveratrol treatment has enhanced the emotional activities (grooming and rearing) which were suppressed due to restraint stress.

Observation on learning abilities and memory retention of rats subjected to restraint stress and resveratrol treatment:

Chronic restraint stress induced cognitive dysfunction involving hippocampus (Bodnoff et al., 1995, Mizoguchi et al., 1982) and prefrontal cortex (McEwen, 2007, Cook & Wellman, 2004, Radley et al., 2006) in humans and animal models are well established (Stillman et al., 1998, Rao et al., 2000, McLeod TM et al., 2001, Caso et al., 2008). There are number of hypotheses addressing the mechanism of stress induced cognitive dysfunction. It includes oxidative damage (Liu et al., 2011), altered glucocorticoid receptor expression (Lupien and McEwen, 1997), altered neurotransmitter and synaptic proteins (Khurana & Devaud, 2007, Zaretskii et
al., 1999), neuronal Ca\(^{2+}\) homeostatis disturbance (Augustine et al., 2003), altered dendritic morphology (Manikandan et al., 2006) and many more. The results of the present study revealed poor retrieval of learning behaviour in passive avoidance task and also declined learning abilities and memory retention in active avoidance task. Though the restraint stress-induced cognitive dysfunction was reported in many animal studies most of them assessed hippocampal dependent spatial memory using variety of maze tests. The present study was designed to evaluate the cognitive function in conditioned learning environment and our results further demonstrates the involvement of hippocampal as well as prefrontal cortex neurons in it. Among the various factors affecting the stress-induced cognitive dysfunction, the involvement of in built antioxidant defence system in brain is gaining lot of focus in the recent years. It is because of the fact that in age related dementia and in Alzheimer’s disease the neuronal loss was mainly due to loss of antioxidant defence system (Eghwrudjakpor et al., 2010) in the brain specially in hippocampus (Padurariu et al., 2012) and prefrontal cortex (Ansari and Scheff, 2010). For this reason natural antioxidants crossing blood brain barrier like resveratrol was used as therapeutic strategy. Resveratrol has exerted its antioxidant potential in enhancing cognitive function against various neuronal insults. Resveratrol has enhanced learning and memory abilities by reducing the OS after vascular dementia (Ma et al., 2013), against ethanol exposure (Tiwari and Chopra, 2011), colchicine induced AD model (Kumar et al., 2007), scopolamine-induced cognitive impairment (Pushpalatha et al., 2013), pentylentetrazole induced cognitive dysfunction (Meng et al., 2014) and age related decline in cognition (Pilsakova et al., 2010). In the current study the neuroprotective role of resveratrol against restraint stress-induced cognitive dysfunction involving various antioxidant molecule and enzymes are further discussed below.

**Observation of antioxidant molecule and enzymes in rat brain homogenate after subjecting to restraint stress and resveratrol treatment:**

ROS generated by chronic restraint stress significantly compromises the in-built antioxidant system in rat brain (Zaidi and Banu 2004, Zaidi et al., 2005). The results of the present study clearly demonstrate that restraint stress results in oxidative damage in rat brain as evidenced by significant rise in MDA level (an end product of lipid peroxidation), decreased brain reduced glutathione level, reduced glutathione reductase enzyme and also significant reduction in TAO activities. These results are consistent with some of the earlier studies. Repeated restraint stress caused oxidative damage in rat hippocampus (Fontella et al., 2005,
Sahim and Gumuslu, 2007). Restraint stress is also known to cause oxidative damage to lipid, protein and DNA in rat brains (Liu et al., 1996).

The impact of oxidative stress to nervous tissue is numerous, as nervous tissue is particularly vulnerable to oxidative stress due to its high rate of oxygen consumption. Stress-induced oxidative damage involves mitochondrial dysfunction, dysregulation of Ca^{2+} homeostasis (Amoroso et al., 2000), disruption of energy pathway (Papadopolos et al., 1997), damage to neuronal stem cells (defective neurogenesis) (Kroemer, 1997), induction of signaling events in apoptotic cell death (Cregan et al., 2002). Oxidative stress eventually leads to morphological changes and finally neuronal atrophy/death (Bremner, 1999 and Sapolsky, 2000). In the present study there was a region specific neuronal loss which could be attributed to this oxidative damage. In vitro studies focusing the probable mechanism in nerve cell damage demonstrated that corticosterone released from suprarenal cortex during stress either induces the formation of ROS (Lin et al., 2004) or decreased antioxidant enzyme activities (Brooke et al., 2002). Oxidative stress is known to add to the development of wide range of conditions like age related dementia, and even diseases like Alzheimer’s or Parkinson’s. The brain is highly susceptible to oxidative injury due to high metabolic rate and high levels of poly unsaturated lipids, the target of lipid peroxidation (Gilgun-Sherky et al., 2002). Since stress is an integral part of current day life, and aging being inevitable process, antioxidants therapy to combat these effects gains much attention.

In the present study resveratrol at both doses significantly reduced brain MDA levels and at 10mg/kg dose elevated reduced glutathione level and also elevated activity of glutathione reductase enzyme. Further TAO activity was also elevated after resveratrol treatment in stressed rats. Resveratrol has decreased cortical and hippocampal MDA level after vascular dementia in rat model (Ma et al., 2013). The same study also showed an increased glutathione level in cortical and hippocampal regions after resveratrol treatment. Similar results were also published by Sinha et al., (2002) in a stroke model of middle cerebral artery occlusion. Of late resveratrol proved to be a highly potent antioxidant and thereby inhibit free radical generation in brain, spinal cord, kidney and liver (Tadolini et al., 2000, Chander et al., 2005). Resveratrol treatment immediately after traumatic brain injury is known to reduce oxidative damage and volume of the tissue lesion (Ates et al., 2007). Though these studies demonstrate antioxidant potential of resveratrol in brain tissue as well as serum in various models/conditions, but not against stress induced oxidative damage. Our study demonstrate similar antioxidant potential of resveratrol against restraint stress.
LP is often assayed by measuring thiobarbituric acid reactive substances. The end products of lipid peroxidation like MDA assessment has been widely used to indicate oxidative stress in many studies (Greilberger et al., 2008). LP is a process whereby free radicals “steal” electrons from the lipids in cell membrane resulting in cell damage. This process is followed by a free radical chain reaction mechanism. It often affects polyunsaturated fatty acids in brain. Further the end products of LP may be mutagenic and carcinogenic. For example, the end product MDA reacts with deoxyadenosine and deoxyguanosine in DNA forming DNA adducts to them. Oxidative damage to lipids and protein is an initial event in inducing neurodegenerative diseases. Restraint stress is well known to act as prooxidant by enhancing brain lipid peroxidation (Derin et al., 2006, Ahmeda et al., 2012, Zaidi et al., 2014). Resveratrol possesses a wide range of biological effects including antioxidation. Using MDA as a marker for OS, the present study found that MDA level in brain homogenate was reduced after resveratrol treatment in stressed rats.

The function of glutathione reductase enzyme is to produce reduced glutathione from oxidised glutathione, maintaining high ratio of reduce to oxidised form intracellularly. Assay of this enzyme gives the extent of oxidative stress in terms of formation of reduced glutathione in the cell. In the present study chronic restraint stress expressed a severe oxidative damage in rat brain. Resveratrol at 10mg/kg dose has protected this oxidative damage by bringing the glutathione reductase level to the normalcy. Resveratrol has exerted its antioxidant potential in obese mice associated with increase in cerebral oxidative stress (Rege et al., 2013).

GSH contains glutamic acid, cysteine and glycine. Its mechanism of action is by reducing inactive disulphide linkages of enzymes to active sulfhydryl group while the sulfhydryl group of GSH becomes oxidized. In this way GSH exerts an important function in defence against membrane peroxidation and also by reducing hydrogen peroxide with glutathione peroxidase. In cells GSH is retained in their reduced form by the glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants. The reduced glutathione content in the present study can be explained by the higher levels of free radicals that convert more reduced glutathione to its oxidised form (Ansari et al., 2008). Reduced glutathione is essential for the cellular detoxification of ROS in brain cells (Dringen and Hirrlinger, 2003). Restraint stress has reduced brain GSH levels by 36.7% as compared with control rats (Madrigal et al., 2001). Exposure to immobilization stress resulted in a decrease in the brain levels of glutathione (Zaidi et al.,
In the present study resveratrol has significantly elevated the brain glutathione level in stressed rats. Similar protective effect of resveratrol was observed after traumatic brain injury by expressing elevated reduced glutathione level (Ateş et al., 2007). This study attribute the effect of resveratrol on elevating glutathione level to free-radical scavenging properties of resveratrol. The study by Ma et al., (2013) and Tiwari & Chopra, (2011) also show elevated brain glutathione level after resveratrol treatment against vascular dementia and alcohol induced toxicity models respectively.

The major advantage of evaluating TAO is to evaluate the antioxidant potential of all antioxidants in a given tissue and not just antioxidant capacity of a single component (Kusano and Ferrari, 2008). In the present study the TAO activity was severely affected after restraint stress and this effect was reversed by resveratrol treatment.

To summarize, resveratrol treatment after restraint stress has reduced brain MDA level, whereas elevated GSH, GSH-Rd and TAO levels. Elevation in antioxidant levels observed can be attributed to free radical scavenging properties of resveratrol. Being a well-known antioxidant resveratrol could inhibit free radical generation in brain and spinal cord (Yang & Piao, 2003, Inoue et al., 2003). It is known to hinder the lipid peroxidation (Tadolini et al., 2000) and inhibits apoptotic cell death produced by oxidative stress (Chanvitayapongs et al., 1997). It has been well claimed that resveratrol could inhibit mitochondria-induced production of ROS in rat brain (Zini et al., 1999), protect DNA from oxidative damage in stroke-prone hypertensive rats (Mizutani et al., 2001) and could prevent neuronal loss after ischemia/reperfusion injury.

**Observation on expression of BDNF in rat brain subjected to restraint stress, resveratrol or Vitamin C treatment:**

BDNF is a stress-responsive intercellular messenger known to modify HPA axis activity (Scule et al., 2006). Evidence indicates that immobilization stress can reduce BDNF expression in the hippocampus (Ueyama et al., 1997). There are other reports claiming similar results (Shi et al., 2010, Jayatisa et al., 2006). However a study by Tagliari et al, (2011) claim that various chronic stress models used in rats has not affected BDNF expression in the hippocampus. The present study clearly demonstrates that immobilization stress in rats resulted in decline in BDNF level in whole brain homogenate.
Neuroprotective effects of resveratrol against restraint stress

It is well known that BDNF is a neuroprotective factor in CNS (Li et al., 2007) and it is strongly expressed in hippocampus where it has been strongly associated with memory function. Stress induced modulation of BDNF expression in the hippocampus has been observed in depression. Though the decline in the BDNF expression was observed in whole brain homogenate, neuronal loss in hippocampus with memory dysfunction can be correlated from the results of the present study. The protective mechanism of BDNF involves following processes: a) modulating Ca\textsuperscript{2+} homeostasis (Tyler et al., 2002)  b) reducing the functions of NMDA receptors and inhibiting the toxicity of glutamate in neurons (Vanhoutte and Badling, 2003) c) resisting toxicity of nitric oxide d) protecting the nerve cells from injury by free radicals (Moreno-Lopez and Ganzalez-Forero, 2006). In the present study enhanced BDNF expression after resveratrol treatment in stressed rats which can be attributed to its free radical scavenging activity. Regarding the other protective mechanisms mentioned above was proved with respect to resveratrol treatment in variety of other animal model studies. In an in vivo study model, resveratrol is proven to enhance hippocampal BDNF mRNA, demonstrating the neuroprotective effect of it (Mostafa et al., 2011).

To combat the stress-induced neurotoxic effects involving BDNF, Vitamin C was tested for its neuroprotective effects. Although the experimental group of rats subjected to both stress and Vitamin C (200mg/kg dose), have not shown any beneficiary effect since no significant changes were seen when compared to control group. Vitamin C at 200mg/kg dose in stressed rats has enhanced BDNF level when compared to rats who received only stress. Therefore these results do not prove the neuroprotective effect of Vitamin C via enhancing the BDNF expression. Tagliari et al., (2011) evaluated the neuroprotective effects of Vitamin C and E against stress induced cognitive deficits. The results of their study claim partial restoration of cognitive dysfunction after Vitamin C as well as Vitamin E therapy suggesting the role of oxidative damage. However their study does not focus on BDNF expression after Vitamin C or E treatments. In the present study, Vitamin C treatment in rats who have not received stress has an adverse effect on BDNF expression; however in stressed rats they exerted a protective effect by enhancing BDNF expression. A study by El-Sokkary et al., (2011) showed administration of Vitamin C attenuated the oxidative damage and morphological changes in rat brain neurons. There is also a study by Coskun et al., (2005) wherein Vitamin C supplementation failed to protect the brain tissue against exercise-induced oxidative damage and behaving as pro-oxidant. In the present study Vitamin C treatment resulted in reduced BDNF expression in rats who have not received stress suggesting that Vitamin C
acted as pro-oxidant, linking the connectivity between the oxidative damage and BDNF expression. Wu et al., (2006) showed BDNF and oxidative stress can interrelate to affect synaptic plasticity and cognitive function. These studies suggest that the expression of BDNF is also linked to the status of oxidative damage in brain tissue. Numakawa et al., (2011) in their study stated that low levels of reactive oxygen species and reactive nitrogen species are important for maintenance of neuronal function, though elevated levels lead to neuronal cell death. A complex series of events including excitotoxicity, Ca\(^2+\) overload, and mitochondrial dysfunction contributes to oxidative stress-mediated neurodegeneration. Oxidative stress-mediated toxicity may be closely related to the pathogenesis of neurodegenerative diseases (Andersen, 2004). Although the antioxidiant nature \textit{in vivo} of Vitamin C has been questioned (Podmore et al., 1998) it is nonetheless marketed as supplements in doses of 500 mg or more per day as an ‘antioxidant’. The present study demonstrates that restraint stress and Vitamin C individually suppressed BDNF expression in rats whereas resveratrol treatment enhanced the level of BDNF. Hence this study indicates the use of resveratrol as a therapeutic agent to combat the stress-induced neuronal dysfunctions.

**Changes in the neuronal numbers of dentate gyrus, various regions of the hippocampus, and medial prefrontal cortex of rats subjected to restraint stress and resveratrol treatment:**

The present study reveals a selective and quantitative neuronal loss affecting DG, CA3, CA2 and MPFC. Restraint stress induced neuronal loss in animal models is an established fact. Further the specific regional involvement is also addressed by many neurobiologists. Oxidative stress induced neuronal loss in the brain is well explained (Satoh et al., 1998). Altered expression of glucocorticoids receptors (Landfield, 1987) and NMDA receptor (McEwen et al., 1995) in the brain is also correlated with neuronal loss and concomitant cognitive decline. Altered expression of stress proteins, neurotransmitters, and also altered BDNF expression were linked to neuronal loss with cognitive decline. Studies indicate that CA1 neurons is the most vulnerable region of the hippocampus for oxidative stress as they contain significantly higher levels of super oxide anion (Wang et al. 2005). Mitochondria isolated from the CA1 region of hippocampus release more ROS showing its vulnerability (Mattiasson et al., 2003). Further the transcription studies also show that CA1 neurons express both antioxidant and ROS producing genes at significantly higher levels (Wang et al., 2005). Although the present study does not demonstrate region specific oxidative damage in the brain but histomorphological results indicate to neuronal loss in CA3 and CA2 region of
Results of the present study showed that restraint stress is known to enhance the lipid peroxidation activity in the brain. Further declined glutathione, reduced glutathione, and TAO activity also attributed to neuronal loss. There was a strong association of BDNF as a member of the neurotrophin family, to be a strong survival-promoting factor against various neuronal insults (Numakawa et al., 2011). Brain neurons are vulnerable to OS (Satoh et al., 1998) and has been shown to decrease the expression of BDNF in rats (Schaaf et al., 1997) leading to an eventual atrophy of the hippocampus (Lee et al., 2008). Hence neuronal loss can be linked to both oxidative damage and declined BDNF expression.

Dentate gyrus is of immense significance for research as well as functional point of view. It is an area where active neurogenesis continues throughout the adulthood in humans as well as animal models. Functionally the projection of axons of granule cells of DG into CA3 region of hippocampus is a primary circuit for spatial memory. The turnover of granule cells of the DG in adult life is required for hippocampal function in spatial memory (Sherry et al., 1992). Chronic restraint stress has suppressed neurogenesis in DG (Pham et al., 2003). Long lasting stress inhibits proliferation and survival capacity of newly born neuronal stem cells (Joels et al., 2007, Torner et al., 2009). Chronic stress is also known to deregulate apoptosis in DG (Lucassen et al., 2006). The neuronal stem cells arising from sub ventricular zone differentiates then express mature neuronal markers and begin to migrate to the granule cell layer. This neurogenesis and survival are regulated negatively by glucocorticoids (as in stress), excitatory amino acids and opioids. Defective neurogenesis (Lagace et al., 2010) or loss of adult neurons (Sapolsky et al., 1985) are always associated with decline in learning abilities as well as memory retention. Further immunohistochemistry studies addressing the neurogenesis after resveratrol treatment in stressed rat can be considered in future. Nevertheless the chronic restraint stress induced cognitive decline in the present study can be attributed to break down of antioxidants defence, declined BDNF expression and region
specific neuronal loss. CA3 region is especially needed for learning and recall (Rolls, 2013). CA3 network also seems essential in supporting the retrieval of information from memory (Kesner et al., 2007). Restraint stress induced hippocampal dependent learning impairment involves loss of dendrites in CA3 region (Luine et al., 1994).

There are also many other interesting factors emerging regarding the mechanism of resveratrol in inducing neuronal protection. Pretreatment of resveratrol in an ischemic model of rats has prevented neuronal death by P13-K/Akt signaling pathway (Simao et al., 2012). Resveratrol administration in ischemic animal models enhanced NO production which lead to cerebral vasodilatation and also enhanced free radical scavenging activities and minimized neuronal loss (Lu et al., 2006).

The MPFC is involved in the integration of cognitive and emotionally relevant information and also been implicated in the modulation of attention in humans (Mac Donald et al., 2000, Kerns et al., 2004). Clinical evidence suggest that MPFC dysfunction is a feature of post-traumatic stress disorder (Rauch et al., 2003) and depression (Drevets et al., 1997). Despite these evidences indicting role of MPFC and stress-induced mental illness, nothing much is reported about its morphological changes, except for a study by Radley et al., (2004) reporting a 20% decrease in apical dendrite length of pyramidal neurons after restraint stress. Similar morphological changes were also observed by Vyas et al., (2002). Though the present study does not focus on dendritic morphology, loss of neurons in MPFC is evident.

MPFC is involved in both memory and decision making. But its functions like learning and memory consolidation depends on its connection with hippocampus (Euston et al., 2012). Quinn et al., (2008) concluded that MPFC is necessary for both recent and remote memory. Given the major role of hippocampus in memory it is not surprised that the hippocampus and MPFC are anatomically related. The connections from anterior portion of the hippocampus to the MPFC are strong compared to its connection with rest of the cortical areas (Cenquizea and Swanson, 2007). Both MPFC and hippocampus is necessary for consolidation of memory after learning. It is also known that MFC is involved in retrieval of memory after a task for subsequent days (Euston et al., 2012). In the present study model the foot shock escaping task were continued for 5 days, observing the animal’s ability to retrieve the memory during 5 days and also after a week. Loss of neurons in the MPFC with declined retrieval capability was observed after chronic stress which was reversed by resveratrol. The loss of neurons in the hippocampal region is often associated with impairment of spatial memory. From the earlier studies the interaction between hippocampus and MPFC is
necessary for consolidation of memory after learning. Quantitative loss of neurons in both the regions can be attributed to cognitive decline.

Though resveratrol is found to be neuroprotective preventing or delaying neuronal loss in various conditions and disease model, there are no studies focusing upon its effect on neurons of MPFC. In the present study resveratrol has reversed adverse effect which was in the form of neuronal loss after chronic restraint stress. The mechanism of resveratrol in exerting neuroprotection in MPFC could be similar to the ones identified in ischemic model of rat brain. Further antioxidant estimation and BDNF estimation in specific regions of the brain (hippocampus and MPFC) would have provided more insight into this mechanism. Further considering the role of neurotransmitters, Ca$^{2+}$ homeostasis, glucose metabolism, and more importantly the recent finding SIRT1 expression would have been more appropriate.