Results
Characterization of pure compounds isolated from *Ocimum sanctum* and *Evolvulus alsinoides*.

1. **K-099 [OS/A]**

The IR spectrum of the compound displayed characteristic absorption bands at 3435 (OH), 1705 (CO) and (C=C) cm\(^{-1}\). Solubility of the compound in bicarbonate solution indicated for the presence of an acid group. Its EIMS depicted the presence of a molecular ion peak at m/z 456 corresponding to the molecular formula C\(_{39}\)H\(_{48}\)O\(_{3}\), in addition to diagnostically important peak at m/z (a) 438 (M-H\(_{2}\)O\(^{+}\)) (b) 393 (M-COOH-H\(_{2}\)O\(^{+}\)) (c) 378 (d) 248 (e) 207 (f) 203 and (g) 189 as shown below in mass fragmentation pattern. These fragments indicated for a triterpenoids of ursane or oleanane series having hydroxyl and carboxylic groups.

The proton \(^1\)H NMR spectrum exhibited multiplet one proton at \(\delta\) 3.24 for a carbonyl group. Signal at \(\delta\) 5.25 (1H, \(m\)) was assigned to vinylic proton. Two doublet at integrating three protons each at 0.76 \((J=4.0\) Hz) and \(\delta\) 0.84 \((J=3.0\) Hz) were due to C-29 and C-30, secondary methyls respectively. Remaining, five of the tertiary methyl responded to the signets at \(\delta\) 0.86 (3H, H-29), 0.94 (3H, H-23), 0.97(3H, H-25), 1.00 (3H, H-26) and 1.08 (3H, H-27) further suggested it was pentacyclic triterpenoids acid. It conformed to an acylable hydroxyl group by the formation of monoacetate which was evident from the FAB-MS m/z 499 [M+H]\(^{+}\).

The \(^{13}\)C NMR spectral showed the existence of 30 carbon atoms in the molecule. All the values were matched with literature report. On the basis of foregoing spectral and chemical studies was identified as ursolic acid. Final structure of the compound was established by the comparison of its spectral data with those reported in literature (Seo *et al.*, 1975, Papnov *et al.*, 1993).
**K-099**

This compound was identified as 4-allyl-1-O-β-D-glucopyranosyl-2-hydroxy benzene.

FAB-MS 313 m/z [M+H]^+ 
UV (CH$_3$CN) $\lambda_{max}$ nm: 200, 218sh ,278, 
IR $\nu_{max}$ (KBr) cm$^{-1}$: 3440, 2926, 2820, 1470, 1375, 1260, 940 cm$^{-1}$.

$^1$H NMR : (DMSO-d$_6$, 200MHz) δ 7.07 (1H, d, J= 8.2 Hz, H-6), 6.63 (1H, d, J= 8.8 Hz, H-5), 6.59 (1H, d, J= 1.6 Hz, H-3), 5.90 (1H, m, H-8), 5.09 (1H, dd, J= 16.8 Hz, 1.9 Hz H-9 trans ), 5.03 (1H, dd, J= 8.9 Hz, 1.8 Hz H-9 cis), 4.62 (1H, d, J= 6 Hz, H-1' anomeric proton ), 3.25 to 3.71 (glucopyranosyl proton), 3.24 (2H, d, J= 7 Hz, H-7).
NMR spectrum of K-116 (OS/B)
K-171 [OS/C]

This compound was identified as 3,4,5-trihydroxy-6-[5-hydroxy-2-(4-hydroxy-phenyl)-4-oxo-4H-chromen-7-yloxy]-tetrahydro-pyran-2-carboxylic acid and obtained as amorphous powdered (200mg) posses

Mass m/z 461[M+H]⁺

UV (MeOH) λmax nm: 333, 268,

IR νmax (KBr) cm⁻¹: 3404, 2929, 2824, 1743, 1665, 1498, 1351, 1239, 940 cm⁻¹,

¹H NMR : (DMSO-d₆, 200MHz) 7.92 (2H, d, J= 8.8 Hz, H-2'6'), 6.92 (2H, d, J= 8.8 Hz, H-3'5''), 6.83 (2H, s, H-3,8 ), 6.45 (1H, d,J= 1.8 Hz, H-6), 5.31 (1H, d, J= 6 Hz, H-1'' anomic proton), 4.22 (1H, d, J= 8.8 Hz, H-5''), 3.10 to 3.4 (glucopyranosyl proton).

Two bands at 1746 and 1639 cm⁻¹ were found in IR spectrum indicating for ester and enolic system in the molecule. The absorption bands at 1605 and 1412 cm⁻¹ indicated for an aromatic system. UV spectrum showed absorption band at 267 and 371 nm (MeOH). FAB-MS spectra showed molecular ion peak [M+H]⁺ at m/z 503 corresponding to molecular formula C₂₅H₂₆O₁₁. Proton NMR and Carbon NMR are same as reported. (Heidrun et al., 1992; Yoshio et al, 1985).
$^1$H NMR of K-171 [OS/C]
Characterization of compound K-117 [OS/D]

[α]_{D}^{25} : -1.06 (MeOH, c, 0.094)

IR (KBr) $\nu_{\text{max}}$ : 3436, 2921, 2852, 2365, 1631, 1589.


$^1$H NMR : (CDCl$_3$, 200 MHz): See table 1

$^{13}$C NMR : (CDCl$_3$, 50 MHz): See table 1

Compound K-117 was obtained as amorphous powder. IR spectrum showed strong absorption peaks at 3436 cm$^{-1}$ for hydroxyl group, 1631 and 1589 cm$^{-1}$ for amide functional group. FAB-MS spectrum showed molecular ion peak at the m/z 866 [M+Na]$^+$, 844 [M+H]$^+$, 826 [M+H-H$_2$O]$^+$, 682 [M+H-C$_6$H$_{11}$O$_3$]$^+$ corresponding to molecular formula C$_{48}$H$_{93}$NO$_{10}$. The $^1$H NMR spectrum (Table 3.3) showed a peak at δ 7.50 (d, J=9.3 Hz) for the NH group, δ 5.58 (m) for a double bond, δ 0.86 (m) for six protons of two methyl groups, δ 1.22 (m) for CH$_2$ protons of long chain fatty acid moiety, δ 3.05-4.90 protons of glucopyranose moiety and other characteristic signals of a sphingosine type cerebrosides possessing 2-hydroxy fatty acid as determined by D$_2$O shake and $^1$H-$^1$H COSY spectrum. The $^{13}$C NMR spectrum (Table 1) showed some of the characteristic signals at δ 173.6 for a carbonyl carbon, at δ 50.3 for CH attached to -NH, at δ 73.9- 76.9 for 6 CH attached to hydroxyl groups, at δ 61.5 for CH$_2$-6", at δ 103.9 for anomeric CH carbon of glucopyranose, at δ 14.3 for two methyl carbons and at δ 130.3, 129.7 for two methine carbons. When K-117 was methanolized with methanolic hydrochloric acid, fatty acid methyl ester (FAM) was obtained together with long chain base (LCB) and methyl glucopyranoside. On the basis of mass spectrometry analysis the FAM was characterized as methyl 2-hydroxy tetracosanoate and the LCB was characterized as 2-amino-1,3,4-trihydroxy-8-octadecene. The mass spectra of the dimethyl disulphide derivative of K-117 showed a fragment ion peak at m/z 221, due to the bond cleavage between the carbons bearing a methyl-thio group, indicating the position of double bond at C-8 in LCB residue. Further the geometry of the double bond was indicated cis on the basis of $^{13}$C NMR chemical shift of the methylene carbon adjacent to the olefinic carbon at δ 27.4 for C-7 and 27.1 for C-10 in Z isomer. The FAM
obtained by methanolysis did not exhibited unsaturation in $^1$H and $^{13}$C spectra indicating unsaturation in the LCB moiety. The FAM was found to be methyl 2-hydroxy tetracosanoate. The stereochemistry of the ceramide moiety was determined by comparison of the $^1$H NMR of the cerebrosides reported (Yoda et al., 1996). Thus based on these foregoing data K-117 was characterized as cerebrosides. Further the identity of the compound K-117 as cerebroside was also confirmed by comparing the spectral and physicochemical data with that reported data (Cateni, et al., 2003). Cerebroside was isolated first time from this plant.

\[ \text{Methanolysis of K-117} \]
Table 1. $^1$H and $^{13}$C data of compound K-117 in DMSO-d$_6$

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_H$ ($J$ in Hz)</th>
<th>$\delta_C$</th>
</tr>
</thead>
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<tr>
<td>NH</td>
<td>7.53 ($d$, 9.3)</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>4.90 ($d$, 8.1)</td>
<td>69.4</td>
</tr>
<tr>
<td>2</td>
<td>3.64 ($m$)</td>
<td>50.3</td>
</tr>
<tr>
<td>3-4</td>
<td>3.05 ($m$)</td>
<td>76.9, 74.5</td>
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<tr>
<td>5</td>
<td>1.97 ($m$)</td>
<td>32.2</td>
</tr>
<tr>
<td>6</td>
<td>1.49 ($m$)</td>
<td>26.0</td>
</tr>
<tr>
<td>7</td>
<td>1.97 ($m$)</td>
<td>27.4</td>
</tr>
<tr>
<td>8,9</td>
<td>5.58 ($m$)</td>
<td>130.3, 129.7</td>
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<tr>
<td>10</td>
<td>1.97 ($m$),</td>
<td>27.1</td>
</tr>
<tr>
<td>11-17</td>
<td>1.22 ($m$)</td>
<td>29.4-32.2</td>
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<tr>
<td>18, 24'</td>
<td>0.86 ($m$)</td>
<td>14.3</td>
</tr>
<tr>
<td>1'</td>
<td>-</td>
<td>173.6</td>
</tr>
<tr>
<td>2'</td>
<td>4.90 ($m$)</td>
<td>73.9</td>
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<td>3'</td>
<td>1.97 ($m$)</td>
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<td>4'</td>
<td>1.49 ($m$)</td>
<td>27.1</td>
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<td>5'-23'</td>
<td>1.22 ($m$)</td>
<td>22.5-34.8</td>
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<td>1&quot;</td>
<td>4.15 ($d$, 7.8)</td>
<td>103.9</td>
</tr>
<tr>
<td>5&quot;- 6&quot;</td>
<td>3.05 ($m$)</td>
<td>73.9, 61.5</td>
</tr>
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</table>
$^1$H NMR Spectrum of K-117
$^1$H-$^1$H COSY Spectrum of K-117
Characterization of K-014 (EA/A)

This is a simple molecule characterized as 3,4-dihydroxy cinnamic acid.

FAB M/z = 181

1H, (CD$_3$Cl) 200MHz

87.67 (H$_1$, d, J = 15.9, α-H) 6.45 (H$_1$d, J = 15.9, β-H) 7.22 (H$_1$,d,J = 1.9, H-2) 6.97 (H$_1$, d, J = 8.4 H-5) 7.19 (H$_1$, dd, J = 8.4,1.9,H-6)

C$^{13}$,CD$_3$Cl, 200MHz

8168.8 (COOH) 153.0 (C-H) 150.9 (C-3) 146.9 (C-13) 128.9 (C-1) 124.2 (C-6) 116.6 (C-α) 112.8 (C-5) 111.8 (C-2).

\[ \text{HO} \begin{array}{c} \text{C} \end{array} \text{HO} \]

\[ \text{KO14} \]
NMR Spectrum of K-014[EA/A]
Characterization of K-015 (EA/B)

The compound is characterized as 2-[(5,7-dihydroxy-2-(4-hydroxy-phenyl)-4-oxo-4H-chromen-7-yl)|glucose. By the following data:

$\text{Fab (M/z) = 433}$

$H^1$ (200 MHz) DMSO- $d_6$

813.558 (1H, s, chelated on at C-5), 9.93 (1H, s, OH at C-4') 87.42 (2H,d,J=7.8 H-2' 6')
7.012 (2H,d,J= 7.8, H-3' 5') 86.67 (1H,s, H- 6) 6.473 (1H, s, H-3) 84.6 (1H, d, J=9.4,H-1") 4.04-3.153 (6 H,m,H-2", 3", 4", 5",6") or sugar proton.

$\text{C}^{13}$

8181.9 (C-4)
8164.0 (C-2) 162.4 (C-7) 160.9 (C-4') 160.6 (C-5) 155.8 (C-S) 128.5 (C-2' 8 6') 121.8 (C-1') 116.0 (C-3', 5') 104.2 (C-8,10) 102.6 (C-3) 98.9 (C-6) 81.4 (C-5") 78.7 (C-3") 73.9 (C-1") 71.4 (C-2") 69.9 (C-4") 61.5 (C-6")

![](image-url)
NMR Spectrum of K-015[EA/B]
**Characterization of K-019 (EA/C)**

This compound was isolated in the form of its acetate.

\[ M/z = 262 \]

\[ [\alpha]_{D20} : +18.1^\circ (\text{CHCl}_3, C = 18.4 \, \text{mg}) \]

\( H^1 (\text{CDCl}_3) \): 81.23 (3H, S, test methyl) 2.02, 2.09 and 2.09 (all three hydrogen and singlet of three acetyl) 4.15 and 3.91 (AB pattern \( J_{AB} = 11.5 \text{Hz, -CH}_2\text{-OAc} \)) 4.57 (A), 4.16 (B) and 5.2 (X) ABX system, \( J_{AB} = 12.0 \), \( J_{AX} = 3.0 \) and \( J_{BX} = 7.5 \) 2.76 (S, OH, concentration dependent). The simple structure of this compound was determined by the above characteristic peaks and compared with the reported data (Anthonsen et al., 1976) and is confirmed to be Erythritol.
Standardization of stress models

Effect of stress on adrenal gland weight.

Rats were subjected to Acute stress (AS), Chronic stress (CS) and Chronic unpredictable stress (CUS). A significant (P<0.01) increase in the adrenal gland weight was observed in all the three stress models when compared to the non-stress group. The results were graphically represented in Figure: 1

**Figure: 1**

Effect of Stress on Adrenal gland weight

![Bar chart showing effect of stress on adrenal gland weight]

- **NS**
- **AS**
- **CS**
- **CUS**

*The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. The data was analyzed by One-way ANOVA followed by Newmannkeul's multiple comparision test.*
Effect of stress on Mean ulcer score

Rats were subjected to Acute stress (AS), Chronic stress (CS) and Chronic unpredictable stress (CUS). A significant (P<0.001) increase in the Mean ulcer score was observed in AS, CUS and CS (P<0.01) models when compared to non stress groups. The results were graphically represented in Figure: 2.

**Figure: 2**

Effect of stress on Mean ulcer score

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. The data was analyzed by Non-parametric ANOVA followed by Dunn's multiple comparison test.
Effect of stress on plasma biochemical changes

Rats were subjected to Acute stress (AS), Chronic stress (CS) and Chronic unpredictable stress (CUS). A significant (P<0.001) increase in the plasma glucose level was observed in AS model where as CS and CUS has not significantly effected the plasma glucose level. The plasma corticosterone level was significantly (P<0.001) increased in Acute and chronic unpredictable stress. In CS, a relatively less significant (P<0.05) Increase in plasma corticosterone when compared to other groups was observed. The resulted are graphically represented in Figure: 3.

Figure: 3

Effect of stress on plasma glucose and corticosterone levels

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. ** P<0.001 and *P<0.05 when compared to non-stress control. The data was analyzed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Rats were subjected to Acute stress (AS), Chronic stress (CS) and Chronic unpredictable stress (CUS). A significant (P<0.001, P<0.01) increase in the plasma Cretine kinase (CK) level was observed in AS and CUS models. However CS has not significantly affected the plasma CK levels. The resulted are graphically represented in Figure: 4.

**Figure: 4**

**Effect of stress on Plasma creatine kinase levels**

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. **P<0.001 , * P<0.01when compared to non-stress control. The data was analyzed by One-way ANOVA followed by Newmannkeul's multiple comparison tests.**
**Effect of stress on Free radical Homeostasis In plasma.**

Free radical damage during stress full conditions was evaluated by measuring lipid peroxidation, total anti-oxidant capacity and enzymatic defense systems. Acute stress has not produced any significant damaging effects as reflected by the unaltered status of lipid peroxidation and total anti-oxidant capacity. In Chronic unpredictable stress (CUS) condition, a significant increase in the plasma lipid peroxidation (P<0.01) and decrease (P<0.01) in total anti-oxidant capacity (TAC) was observed. Chronic stress (CS), however, has not produced any significant changes in the measured parameters. The results were graphically represented in Figure: 5.

**Figure: 5**

![Graph showing effect of stress on plasma lipid peroxidation (LPO) and Total Antioxidant Capacity (TAC)]

The stress group was compared with non-stress control group. Results were represented as mean ± S.E.M. with n=6 in each group, **P<0.001**, *P<0.01* when compared to non-stress control. The data was analyzed by One-way ANOVA followed by Newman-Keul’s multiple comparison tests.
Acute stress (AS) has not shown any significant effect on the anti-oxidant defense status as reflected by the unaltered levels of Super-oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX) enzyme levels. Chronic unpredictable (CUS) stress has significantly (P<0.05) decreased SOD and CAT levels with significant increase of glutathione peroxidase (P<0.05). Where as chronic stress (CS) was ineffective in producing any significant changes in plasma anti-oxidant enzyme status. The results were graphically represented in Figure: 6.

**Figure: 6**

![Effect of stress on Enzymatic plasma anti-oxidant defence](image)

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. * P<0.05 when compared to non-stress control and the data was analyzed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Rats were subjected to Acute stress (AS), Chronic stress (CS) and Chronic unpredictable stress (CUS) and plasma was measured for altered glutathione levels a non-enzymatic marker for free radical load. AS has not shown any significant effect on the plasma glutathione levels. CUS stress has significantly decreased ($P<0.01$) plasma glutathione levels. Where as CS was ineffective in producing any significant change. The results were graphically represented in Figure:7

**Figure:7**

Effect of stress on plasma glutathione levels

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with $n=6$ in each group. **$P<0.001$** when compared to non-stress control and the data was analyzed by One-way ANOVA followed by Newmannkeul's multiple comparision tests
Effect of stress on free-radical homeostasis in different regions of brain.

Acute stress (AS) and Chronic stress (CS) has not produced any significant change in lipid peroxidation. There is a significant (P<0.01) enhancement of lipid peroxidation in Frontal cortex (FC), Hippocampus (HP), Hypothalamus (HT) and Striatum (ST) brain regions of rats subjected to Chronic unpredictable stress (CUS). The results were graphically represented in Figure: 8

Figure: 8

Effect of stress on lipid peroxidation (LPO) in different brain regions

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. ** P<0.01 when compared to non -stress control and the data was analyzed by one-way ANOVA followed by Newmannkeul's multiple comparison test.
Acute stress (AS) has no significant effect on antioxidant enzymes in all the brain regions considered in our study. Chronic unpredictable stress (CUS) has significantly (P<0.01) reduced the levels of Superoxide dismutase (SOD), Catalase (CAT) and significantly increased (P<0.01) Glutathione peroxidase (GPX) levels in all the brain regions considered in our study. Chronic stress (CS) has not produced any significant changes of the enzyme levels in Frontal cortex (FC), Hippocampus (HP), Hypothalamus (HT) and Striatum (ST) regions of the brain. All the changes were graphically represented in Figures: 9, 10 and 11.

**Figure: 9**

**Effect of stress on Superoxide dismutase enzyme in various brain regions**

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. * P<0.01 when compared to non-stress control and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. * P<0.01 when compared to non-stress control and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparison tests.
Effect of Stress on Brain Neurotransmitter changes

Distribution of Neurotransmitters in brain regions was observed and Noradrenaline (NA) was significantly (P<0.001) higher in hypothalamus compared to other brain regions where as Dopamine was found to be higher (P<0.001) in striatum when compared to other brain regions. There is no significant distributional difference of 5-HT when compared, between the brain regions considered in our study. The results were graphically represented in Figure: 12.

Figure: 12

Neurotransmitter distribution in various brain regions

- NA
- DA
- HT

Results were represented as mean ± S.E.M. with n=6 in each group. ** P<0.001 when compared between the groups. The data was analysed by One-way ANOVA followed by Newmankeul's multiple comparision tests.
Rats were exposed to acute stress (AS), chronic stress (CS) and chronic unpredictable stress (CUS) regimen and measured for altered Neurotransmitter changes in Frontal cortex (FC), Hippocampus (HP) and Hypothalamus (HT) regions of brain. Acute stress has significantly (P<0.05) decreased nor-adrenaline (NA) in FC, HP and HT. Dopamine (DA) was increased (P<0.05) in FC and HT but a significant (P<0.05) decrease was observed in HP. A significant (P<0.05) increase of 5-Hydroxy tryptamine (5-HT) was observed in all the brain regions considered.

Chronic unpredictable stress (CUS) significantly (P<0.05) depleted the neurotransmitters in FC, HP and HT brain regions considered in our study.

Chronic stress (CS) has significantly (P<0.05) decreased NA and DA in FC and increased 5-HT in HP. How ever CS produced insignificant increase in the level of DA, NA in HT and HP. All the neurotransmitter changes are graphically represented in Figures: 13, 14 & 15 in the order of brain regions.

**Figure: 13**

**Effect of stress on neurotransmitters response in Cortex**

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. * P<0.05 when compared to non -stress control and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Figure: 14

Effect of stress on neurotransmitter response in Hippocampus

- Nor-adrenaline
- Dopamine
- 5-HT

Groups: NS, AS, CUS, CS

Figure: 15

Effect of stress on neurotransmitters in Hypothalamus

- Nor-adrenaline
- Dopamine
- 5-HT

Groups: NS, AS, CUS, CS

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. * P<0.05 when compared to non-stress control and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Open field behavior of rats

Normal rats were monitored further open field behaviour to exactly record the open field activity. Open field activity of rats was significantly (P<0.01) decreased with the increase in time interval. A significant decrease of general behavior was observed as reflected by number of stereotypy counts, horizontal and vertical activity. The data was represented in Table: 1

Table: 1

Time dependent profile for Open field activity in normal rats.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Horizontal activity (cms)</th>
<th>Vertical activity (cms)</th>
<th>Number of Stereotypy counts</th>
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<tbody>
<tr>
<td>15</td>
<td>1235 ± 220</td>
<td>257 ± 87</td>
<td>237 ± 120</td>
</tr>
<tr>
<td>30</td>
<td>522 ± 110*</td>
<td>115 ± 35*</td>
<td>159 ± 95*</td>
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<tr>
<td>45</td>
<td>356 ± 98*</td>
<td>49 ± 12*</td>
<td>93 ± 65*</td>
</tr>
</tbody>
</table>

Data is represented as mean ± S.E.M. with n=6 in each group. * P<0.01 when compared to initial 15 min and the data was analysed by One-way ANOVA followed by Newmann-Keul’s multiple comparision tests.

Effect of Stress on open field activity of rats.

A decrease in the overall open field activity was observed in the rats exposed to acute, chronic and chronic unpredictable stress. There is a significant (P<0.01) decrease in horizontal activity, total distance travelled and stereotypy counts. In terms of intensity, there was no any significant change in the behavior of rats when compared between the stress groups. The results were represented in Table: 2

Behavioral activity was monitored after 24hrs of the cessation of stress and it was found that in no significant difference in their behavioral activity was observed after the termination of stress. The results were represented in Table: 3
Table : 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Horizontal activity</th>
<th>Total distance (cms)</th>
<th>Stereotypy counts</th>
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<tbody>
<tr>
<td>Non-stress</td>
<td>1086 ± 243.0</td>
<td>135.0 ± 25.0</td>
<td>126 ± 21.0</td>
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<tr>
<td>Acute Stress</td>
<td>580 ± 120.0</td>
<td>78 ± 21.0*</td>
<td>85 ± 22.0*</td>
</tr>
<tr>
<td>Chronic unpredictable stress</td>
<td>432 ± 95.0*</td>
<td>66 ± 35.0 *</td>
<td>79 ± 22.0*</td>
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<tr>
<td>Chronic Stress</td>
<td>520 ± 82.0*</td>
<td>72 ± 22.0 *</td>
<td>75 ± 20.0*</td>
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</table>

Table : 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Horizontal activity</th>
<th>Total distance (cms)</th>
<th>Stereotypy counts</th>
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<tbody>
<tr>
<td>Non-stress</td>
<td>1093 ± 286.0</td>
<td>145.0 ± 22.0</td>
<td>105 ± 32.0</td>
</tr>
<tr>
<td>Acute Stress</td>
<td>1032 ± 332.0</td>
<td>138 ± 25.0</td>
<td>101 ± 32.0</td>
</tr>
<tr>
<td>Chronic unpredictable stress</td>
<td>985± 45.0</td>
<td>142 ± 15.0</td>
<td>98 ± 12.0</td>
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<tr>
<td>Chronic Stress</td>
<td>1020 ± 92.0</td>
<td>122 ± 32.0</td>
<td>101 ± 28.0</td>
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</table>

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. * P<0.01 when compared to non-stress control the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision test.
Anti-stress effect of Fresh juice of Ocimum sanctum.

Acute stress has significantly increased Mean ulcer score, adrenal gland, and Plasma glucose, corticosterone and creatine kinase levels. Fresh juice of Ocimum sanctum was significantly (P<0.01) effective in reducing acute stress elevated levels of Glucose and Corticosterone. But, was ineffective in producing any normalizing effects in acute stress induced increase in mean ulcer score, adrenal gland weight and creatine kinase levels. The data was graphically represented in Figures : 16,17 and 18.

Figure: 16

Effect of Fresh juice of Ocimum sanctum on Acute stress induced changes in Mean ulcer and Adrenal gland weight

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to Non-stress control and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests. Ulcer data was analyzed by Non-parametric ANOVA followed by Dunn's multiple comparison test.
Figure: 17

Effect of Fresh juice of Ocimum sanctum on acute stress induced changes in plasma

Figure: 18

Effect of Fresh juice of Ocimum sanctum on Acute stress induced changes in plasma creatine kinase levels

* The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to Non-stress control and * P<0.01 when compared to Acute-stress control and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Dose response study.

Graded doses of 100, 200 and 400 mg / kg body weight crude ethanol extracts of Ocimum sanctum (OS) and Evolvulus alsinoides (EA) were screened for anti-stress activity. In all the doses tested, oral administration of 200mg/kg and 400mg/kg were equally effective (P<0.01) in reducing the acute stress elevated levels of Plasma glucose and Corticosterone and 400mg/kg dose was also equally potent. In the crude extracts, however, 100mg/kg dose was ineffective. The results were graphically represented in Figure: 19

Figure: 19

Effect of graded doses of Crude extracts of Ocimum sanctum and Evolvulus alsinoides on acute stress induced increase plasma glucose and Corticosterone levels

The stress group was compared with non stress control group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to Non-stress control and * P<0.01 when compared to Acute-stress control and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests)
**Anti stress effect of Crude extracts of Ocimum sanctum in acute stress model**

Crude extracts of *Ocimum sanctum* in ethanol (EtOH/W) and dichloromethane (DCM) and fractions of these extracts in Butanol (BuOH), Ethanol (EtOH), Water (Aq/F) at a dose of 200mg/kg body weight were screened for their effects on ulceration and adrenal gland weight during stress in acute stress (AS) model. They are compared with standard drug *Panax quinquefolium* (PQ) root simultaneously treated with a dose of 100mg/kg body weigh.

Acute stress (AS) significantly (P<0.01) increased adrenal gland weight and mean ulcer score. DCM, BuOH, EtOH, EtOH/W and PQ were significantly (P<0.01) effective in reducing acute stress induced increase in adrenal gland weight and mean-ulcer score. However Aq/F was ineffective in reducing the acute stress induced changes in adrenal gland weight and mean ulcer score. The mean ± SEM of adrenal gland weight/100gm body weight and ulcer score are graphically represented in Figure: 20.

**Figure: 20**

**Effect of Crude extracts and fractions of Ocimum sanctum on Acute stress induced changes in Adrenal gland weight and Mean ulcer score**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to non-stress control and *P<0.01 when compared with acute stress group. (One-way ANOVA followed by Newmannkeul's multiple comparision tests) Ulcer data was analyzed by Non-parametric ANOVA followed by Dunn's multiple comparison test.
Crude extracts of *Ocimum sanctum* in ethanol (EtOH/W) and dichloromethane (DCM) and fractions of these extracts in Butanol (BuOH), Ethanol (EtOH), Water (Aq/F) at a dose of 200mg/kg body weight were screened for their effect on plasma glucose and corticosterone in acute stress model (AS). They were compared with standard drug *Panax quinqufolium* (PQ) treated simultaneously at a dose of 100mg/kg body weight. Acute stress significantly (P<0.01) increased plasma glucose and Corticosterone levels DCM, BuOH, EtOH, EtOH/W and PQ were significantly (P<0.01) effective in reducing acute stress induced increase in Plasma glucose and Corticosterone levels. However Aq/F was ineffective in reducing the acute stress induced increase in plasma glucose and Corticosterone activity. The mean ± SEM of plasma glucose (mg/dl) and Corticosterone (ng/ml plasma) were graphically represented in Figure: 21

**Figure: 21**

**Effect of Crude extracts and Fractions of *Ocimum sanctum* on Acute stress induced changes in plasma glucose and corticosterone.**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non -stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Crude extracts of *Ocimum sanctum* in Ethanol (EtOH/W) and Dichloromethane (DCM), fractions of these extracts in Butanol (BuOH), Ethanol (EtOH), Water (Aq/F) were screened at a dose of 200mg/kg body weight for their effect on plasma creatine kinase (CK) levels during stress in acute stress (AS) model. They are compared with standard drug *Panax quinquefolium* (PQ) simultaneously treated at a dose of 100mg/kg body weight. Acute stress significantly (P<0.001) increased plasma creatine kinase (CK) levels. EtOH/W, BuOH, DCM, EtOH and PQ were significantly (P<0.05) effective in reducing acute stress induced increase in Creatine kinase (CK) levels. However Aq/F was ineffective in reducing the acute stress induced increase in plasma Creatine kinase (CK) activity. The mean±SEM of plasma CK(IU/dl) was graphically represented in Figure: 22

**Figure: 22**

**Effect of Crude extracts and fractions of *Ocimum sanctum* on acute stress induced changes In Creatine kinase activity**

@ NS  
AS  
DCM  
BuOH  
EtOH  
EtOH(W)  
Aq/F  
PQ

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and **P<0.01 *P<0.05 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmankeul's multiple comparison tests.
Crude extracts of *Ocimum sanctum* in Ethanol (EtOH/W) and Dichloromethane (DCM), Fractions of these extracts in Butanol (BuOH), Ethanol (EtOH) and standard drug *Panax quinquefolium* (PQ) are tested in chronic un-predicatable stress (CUS) model. CUS significantly (P<0.01) Increased adrenal gland weight and Mean ulcer score.

EtOH/W, BuOH, DCM, EtOH and PQ were significantly (P<0.05) effective in reducing CUS induced increase in mean ulcer score and adrenal gland weight. The mean±SEM of adrenal gland weight/100gm body weight and ulcer score was graphically represented in Figure: 23

**Figure: 23**

**Effect of Crude extracts and Fractions of *Ocimum sanctum* on CUS induced changes in Mean ulcer score and Adrenal gland weight**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non -stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests. Ulcer data was analyzed by Non-parametric ANOVA followed by Dunn's multiple comparison test.
Crude extracts of *Ocimum sanctum* in Ethanol (EtOH/W) and Dichloromethane (DCM), Fractions of these extracts in Butanol (BuOH), Ethanol (EtOH) and standard drug *Panax quinquefolium* (PQ) root powder are tested in chronic un-predicatable stress (CUS) model. CUS significantly (P<0.01) Increased plasma corticosterone levels but has no effect on plasma glucose levels. EtOH/W, BuOH, DCM, EtOH and PQ were significantly (P<0.05) effective in reducing CUS induced increase in plasma corticosterone levels. The mean±SEM of plasma glucose (mg/dl) and corticosterone (ngm/ml) were graphically represented in Figure: 24

**Figure: 24**

**Effect of crude extracts and fractions of Ocimum sanctum on CUS induced changes in plasma glucose and corticosterone**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparison tests.
Ethanol (EtOH/W) and Dichloromethane (DCM) crude extracts of *Ocimum sanctum* and their fractions in Butanol (BuOH) and Ethanol (EtOH) at a dose of 200mg/kg body weight and standard drug *Panax quinquisfolium* (PQ) root powder at a dose of 100mg/kg body weight were tested for effect on creatine kinase levels (CK) in chronic unpredictable stress (CUS) model. CUS significantly (P<0.01) increased Plasma CK. EtOH /W, BuOH, DCM, EtOH and PQ were significantly (P<0.01) effective in reducing CUS induced increase in plasma CK. The mean ± SEM of CK (IU/dl) was graphically represented in Figure: 25

**Figure: 25**

*Effect of *Ocimum sanctum* on CUS induced changes in Plasma creatine kinase levels*

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmann-Keul’s multiple comparison tests.
Anti-stress effects of Ethanolic and Aqueous Crude extracts of *Evolvulus alsinoides*

Crude ethanolic (EtOH) and aqueous (Aq) extracts of *Evolvulus alsinoides* at a dose of 200mg/kg body weight and standard drug *Panax quiniquifolium* (PQ) root powder at a dose of 100mg/kg body weight were screened for their effect on plasma glucose levels during stress in acute stress (AS) and chronic unpredictable stress (CUS) models. AS significantly (P<0.001) increased plasma glucose levels where as CUS has no effect on plasma glucose levels. EtOH, Aq and PQ were significantly (P<0.001) effective in reducing AS induced increase in Plasma glucose levels. No significant effect on plasma glucose levels in treated group was produced in rats subjected to CUS. The mean ± SEM of Plasma glucose mg/dl is graphically represented in **Figure: 26.**

**Figure: 26**

Effect of crude Ethanolic and Aqueous extracts of *Evolvulus alsinoides* on stress induced changes in plasma glucose levels

![Graph showing plasma glucose levels](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and **P<0.001** when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Crude ethanolic (EtOH) and aqueous (Aq) extracts of *Evolvulus alsinoides* at a dose of 200mg/kg body weight and standard drug *Panax quiniquifolium* (PQ) at a dose of 100mg/kg body weight were screened for their effect on plasma Cretine kinase (CK) levels during stress in acute stress (AS) and chronic unpredictable stress (CUS) models. AS and CUS significantly (P<0.001) increased plasma creatine kinase levels. Aq, EtOH and PQ were significantly (P<0.05, P<0.001) effective in reducing AS and CUS induced increase in Plasma CK levels. The mean±SEM of Plasma glucose mg/dl is graphically represented in **Figure: 27**.

**Figure: 27**

![Graph showing the effect of Crude extracts of *Evolvulus alsinoides* on stress induced changes in plasma creatinine kinase levels](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 and ** P<0.001 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Crude ethanolic (EtOH) and aqueous (Aq) extracts of *Evolvulus alsinoides* at a dose of 200mg/kg body weight and standard drug *Panax quinquefolium* (PQ) at a dose of 100mg/kg body weight were screened for their effect on adrenal gland weight during stress in acute stress (AS) and chronic unpredictable stress (CUS) models. AS and CUS significantly (P<0.05) increased adrenal gland weight. Treatment with Aq, EtOH and PQ were significantly (P<0.05) effective in reducing AS and CUS induced increase in adrenal gland weight. The mean±SEM of adrenal gland weight/100gm body weight is graphically represented in Figure: 28.

**Figure: 28**

Effect of Crude extracts of *Evolvulus alsinoides* on stress induced changes in adrenal gland weight

![Graph depicting the effect of different treatments on adrenal gland weight.](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.001 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Crude Ethanolic (EtOH), Aqueous (Aq) extracts of *Evolvulus alsinoides* at a dose of 200mg/kg body weight and standard drug *Panax quiniquifolium* (PQ) at a dose of 100mg/kg body weight were screened for their effect on gastric ulceration during stress in acute stress (AS) and Chronic unpredictable stress (CUS) model. AS and CUS significantly (P<0.001) increased Mean ulcer severity. EtOH, and PQ were effective significantly (P<0.001) in reducing AS and CUS induced increase in mean ulcer severity. But, Aq extract was significantly (p<0.001) effective in acute stress model and however ineffective in normalizing CUS induced mean ulcer score. The mean±SEM of ulcer score was graphically represented in Figure: 29.

**Figure: 29**

![Effect of Crude extracts of *Evolvulus alsinoides* on stress induced mean ulcer score](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparison tests. Ulcer data was analyzed by Non-parametric ANOVA followed by Dunn's multiple comparison test.
Crude Ethanollic (EtOH), Aqueous (Aq) extracts of *Evolvulus alsinoides at a dose of 200 mg/kg body weight* and standard drug *Panax quinquefolium* (PQ) root powder at a dose of 100mg/kg body weight were screened for their effect on stress induced changes of plasma corticosterone levels in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS significantly (P<0.001) increased plasma corticosterone levels. EtOH, and PQ were effective significantly (P<0.01) in reducing AS and CUS induced increase in plasma corticosterone levels. Aq extract was significantly (P<0.01) effective in AS model and was ineffective in reducing the CUS induced increase in plasma corticosterone. The mean±SEM of plasma corticosterone (ng/ml/plasma) was graphically represented in Figure: 30

**Figure: 30**

Effect of Crude extracts of *Evolvulus alsinoides* on stress induced changes in plasma corticosterone levels

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non stress control and *P<0.01 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Crude ethanolic extract of *Evolvulus alsinoides* were fractionated in Dichloromethane (DCM), Butanol (BuOH) and Water (Aq) and standard drug *Panax quiniquifolium* (PQ) root powder at a dose of 200mg/kg body weight and 100mg/kg body weight were screened for effect of stress induced changes in plasma Glucose levels in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS significantly (P<0.001) increased plasma glucose levels and CUS did not show any significant effect. All the fractions screened and PQ were significantly effective (P<0.01) in reducing AS induced increase in plasma No significant effect was produced in drug treated and control rats subjected to CUS. The mean±SEM of Plasma glucose mg/dl was graphically represented in Figure: 31

**Figure: 31**

*Effect of *Evolvulus alsinoides* fractions on Acute and Chronic unpredictable stress induced changes in plasma glucose levels.*

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Crude ethanolic extract of Evolulus alsinoides fractionated in Dichloromethane (DCM), Butanol (BuOH) and Water (Aq) was at a dose of 200mg/kg body weight were screened for effect on stress induced changes of plasma Creatine kinase levels (CK) in acute stress (AS) and Chronic unpredictable stress (CUS) models. They are compared with standard drug Panax quiniquifolium (PQ) root powder treated simultaneously at a dose of 100mg/kg body weight. AS and CUS significantly (P<0.001) increased plasma CK levels. BuOH fraction and PQ were significantly effective (P<0.01) in reducing stress induced increase in plasma CK. DCM and Aq fractions were ineffective in both AS and CUS models to reduce stress induced increase in Plasma CK levels. The mean±SEM of Plasma CK IU/dl was graphically represented in Figure: 32.

Figure: 32

Effect of fractions of Evolulus alsinoides on Acute and Chronic unpredictable stress induced changes in plasma creatine kinase levels

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non -stress control and *P<0.01 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Crude ethanolic extract of *Evolvulus alsinoides* fractionated in Dichloromethane (DCM), Butanol (BuOH) and Water (Aq) was screened at a dose of 200mg/kg body weight for its effect on adrenal gland weight during stress in Acute stress (AS) and Chronic unpredictable stress (CUS) model. They were compared with standard drug *Panax quinquefolium* (PQ) treated simultaneously with a dose of 100mg/kg body weight. AS and CUS significantly (P<0.001) increased adrenal gland weight. BuOH fraction and PQ were significantly effective (P<0.01) in reducing Stress induced increase in adrenal gland weight. DCM and AQ fractions were ineffective in both AS and CUS models to reduce stress induced increase in adrenal gland weight. The mean±SEM of adrenal gland weight/100gm body weight was graphically represented in Figure: 33

**Figure: 33**

*Effect of Evolvulus alsinoides* fractions on acute and Chronic unpredictable stress induced changes in adrenal gland weight.

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Crude ethanolic extract of *Evolvulus alsinoides* fractionated in Dichloromethane (DCM), Butanol (BuOH) and Water (Aq) were tested at a dose of 200mg/kg body weight for their effect on stress induced ulceration in Acute stress (AS) and Chronic unpredictable stress (CUS) models. The results are compared with standard drug *Panax quinongifolium* (PQ) treated simultaneously at a dose of 100mg/kg body weight. AS and CUS significantly (P<0.001) increased mean ulcer severity. BuOH, DCM fractions and PQ were significantly effective (P<0.01) in reducing Stress induced increase in mean ulcer score. AQ fractions were ineffective in both AS and CUS models to reduce stress induced ulceration. The mean±SEM of ulcer score are graphically represented in Figure: 34

**Figure: 34**

**Effect of Fraction of Evolvulus alsinoides on Acute and Chronic unpredictable induced changes in Mean ulcer Score**

![Graph showing mean ulcer score](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparison tests. Ulcer data was analyzed by Non-parametric ANOVA followed by Dunn's multiple comparison test.
Crude ethanolic extract of *Evolvulus alsinoides* fractionated in Dichloromethane (DCM), Butanol (BuOH) and Water (Aq) at a dose of 200mg/kg body weight was screened for its effect on plasma corticosterone level during stress in acute stress (AS) and Chronic unpredictable stress (CUS) models. They were compared with standard drug *Panax quiniquifolium* (PQ) treated simultaneously at a dose of 100mg/kg body weight. AS and CUS significantly (P<0.001) increased plasma corticosterone levels. BuOH fraction and PQ were significantly effective (P<0.01) in reducing stress induced increase in Plasma corticosterone levels. DCM and AQ fraction were ineffective in both AS and CUS models to reduce stress induced elevation of plasma corticosterone. The mean±SEM of plasma corticosterone are graphically represented in Figure: 35

**Figure: 35**

Effect of fractions of *Evolvulus alsinoides* on Acute and Chronic unpredictable stress induced changes in Plasma Corticosterone levels

The stress group was compared with non-stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with acute stress group and the data was analysed by One-way ANOVA followed by Newmann-Keul's multiple comparision tests.
**Figure: 36**

**Effect of Crude extracts and Fractions in learning Behavior.**

**Ocimum sanctum.**

Crude extracts of Ocimum sanctum in Ethanol –EtOH/W, EtOH, DCM and fractions in Butanol (BuOH) and Water (Aq) were tested for their learning behavior in Morris water maze test at a dose of 100mg/kg body weight. All the extracts and fractions were ineffective in producing any significant learning behavior in rats. The resulted are graphically represented in **Figure: 36**.

**Figure: 36.**

![Effect of Crude extracts and fractions of Ocimum sanctum on learning behavior in morris water maze test](image)

**Evolvulus alsinoides**

Crude Ethanolic (EtOH) and Aqueous extracts (AQ) of Evolvulus alsinoides along with the fractions in Butanol (BuOH/F), Dichloromethane (DCM/F) and Water (AQ/F) were tested for learning performance at a dose of 100mg/kg body weight in as a measure of Mean latency time in rats subjected to Morris water maze test. Crude EtOH extract was effective in decreasing the mean latency time significantly (p<0.01) when compared directly with the untreated /vehicle treated control. However Crude Aqueous extract and other fractions were ineffective in increasing the learning performance. The Mean ± SEM was graphically represented in **Figure: 37**.
The Drug Treated group was compared with vehicle treated control. Results were represented as mean ± S.E.M. with n=6 in each group. *P<0.01 when compared with vehicle treated group. (Non-parametric Dunn’s multiple comparisons Test)

Crude ethanolic (EtOH) extract of *Evolvulus alsinoides* for its memory enhancing effects in Scopolamine induced amnesia and examined in passive avoidance task. In passive avoidance task vehicle treated group has shown significant increase in TL (Transfer latency) on second trial compared to first trial (P <0.001). Scopolamine treatment (amnesia) could not produce a significant increase in TL on second trial as compared to first trial. EtOH treated group exhibited a significant increase in TL on 2nd trial in comparison to first trial as well as compared to amnesia group (P <0.05). The mean±SEM of transfer latency time between different groups was graphically represented in **Figure: 38**
**Figure: 38**

Effect of crude ethanolic extract of *Evolvulus alsinoides* on scopolamine induced amnesia (Dementia) in passive avoidance test in mice

The Drug Treated group was compared with vehicle treated control. Results were represented as mean $\pm$ S.E.M. with n=6 in each group. @ When compared to first trial in vehicle treated group. *P<0.05 when compared with first trial in drug treated group (Non-parametric Dunn's multiple comparisons Test).
Effect of Crude extracts of *Ocimum sanctum* (OS) and *Evolvulus alsinoides* (EA) on free radical homeostasis in Plasma of rats subjected to chronic unpredictable stress (CUS).

Chronic unpredictable stress (CUS) regimen in rats for 7 days has significantly (p<0.01) increased lipid per oxidation in plasma. There is a significant (P<0.01) decrease of Super oxide dismutase (SOD), Catalase (CAT), and Glutathione levels with a significant (P<0.01) increase of Glutathione peroxidase (GPX) levels. The entire perturbed free radical defense status was significantly reflected in decreased (P<0.01) plasma Total Anti-oxidant Capacity (TAC).

Crude ethanolic (EtOH) and aqueous (AQ) extracts of OS and EA at a dose of 200mg/kg body weight and Panax *quinquisfolium* at a dose of 100mg/kg body weight were tested for their effect on CUS induced free radical load. EtOH extract of EA and OS were significantly (P<0.05) effective in reducing CUS induced plasma lipid per oxidation and significantly (P<0.05) normalized depleted levels of SOD, CAT and glutathione levels. EtOH of OS and EA were also effective in normalizing significantly (P<0.05) the CUS elevated levels of glutathione peroxidase enzyme levels. The normalization of free radical defense status was well associated by the increase (P<0.05) of the Plasma total anti-oxidant capacity in comparison to stress control. The anti-oxidant capacity of the EtOH extract were comparable to the standard *panax quinquisfolium* which is significantly effective in reducing the CUS induced free radical load as shown by its significantly (P<0.05) normalizing effects for all the observed parameters. Crude aqueous extracts of *Ocimum sanctum* and *Evolvulus alsinoides* were however found ineffective in this study for anti-oxidant potential under stressful conditions. The results were tabulated in Tables 1 and 2.
Table: 1

Anti-oxidant profile of Crude extracts in plasma of rats subjected to Chronic unpredictable stress (CUS)

<table>
<thead>
<tr>
<th>Group (mg/kg body weight, p.o, n = 6)</th>
<th>Lipid peroxidation (nmole/mg protein)</th>
<th>Super oxide dismutase (U/mg protein)</th>
<th>Catalase (U/mg protein)</th>
<th>Glutathione peroxidase (U/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>Non stress</td>
<td>0.85 ± 0.03</td>
<td>123.49 ± 21.67</td>
<td>0.069 ± 0.02</td>
<td>34.5 ± 11.36</td>
</tr>
<tr>
<td>Chronic unpredictable stress control</td>
<td>2.83 ± 0.048</td>
<td>72.64 ± 1.94</td>
<td>0.035 ± 0.014</td>
<td>58.11 ± 18.74</td>
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Ocimum sanctum

<table>
<thead>
<tr>
<th></th>
<th>EtOH (200)</th>
<th>AQ (200)</th>
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<tbody>
<tr>
<td></td>
<td>0.92 ± 0.009*</td>
<td>114.2 ± 23.37*</td>
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<tr>
<td>EtOH (200)</td>
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</tr>
<tr>
<td>AQ (200)</td>
<td>1.39 ± 0.024</td>
<td>95.8 ± 22.02</td>
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</table>

Evolvulus alsinoides

<table>
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<th></th>
<th>EtOH (200)</th>
<th>AQ (200)</th>
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<tbody>
<tr>
<td></td>
<td>0.95 ± 0.028*</td>
<td>118.43 ± 31.13*</td>
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<tr>
<td>EtOH (200)</td>
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<td></td>
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<tr>
<td>AQ (200)</td>
<td>1.06 ± 0.03</td>
<td>93.045 ± 28.16</td>
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Panax quinqufolium (100)

<table>
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<th>EtOH (200)</th>
<th>AQ (200)</th>
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<tbody>
<tr>
<td></td>
<td>0.96 ± 0.019*</td>
<td>118.5 ± 20.39*</td>
</tr>
</tbody>
</table>

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to non-stress control and *P<0.05 when compared with Chronic unpredictable stress group. (One-way ANOVA followed by Newmannkeul’s multiple comparision tests)
Table: 2

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glutathione (mg/ml of plasma)</th>
<th>Total Anti-oxidant capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non stress</td>
<td>56.6 ± 15.36</td>
<td>1.32 ± 0.050</td>
</tr>
<tr>
<td>Chronic unpredictable stress</td>
<td>22.36 ± 10.35(^\circ)</td>
<td>0.53 ± 0.042(^\circ)</td>
</tr>
<tr>
<td><em>Ocimum sanctum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EtOH (200)</td>
<td>51.36 ± 14.38(^*)</td>
<td>1.28 ± 0.03(^*)</td>
</tr>
<tr>
<td>AQ (200)</td>
<td>28.30 ± 15.32</td>
<td>0.65 ± 0.02</td>
</tr>
<tr>
<td><em>Evolvulus alsinoides</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EtOH (200)</td>
<td>48.56 ± 12.54(^*)</td>
<td>1.16 ± 0.01(^*)</td>
</tr>
<tr>
<td>AQ (200)</td>
<td>30.32 ± 9.86</td>
<td>0.82 ± 0.03</td>
</tr>
<tr>
<td><em>Panax quinquefolium</em> (100)</td>
<td>50.36 ± 14.32(^*)</td>
<td>1.20 ± 0.02(^*)</td>
</tr>
</tbody>
</table>

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to non-stress control and *P<0.05 when compared with Chronic unpredictable stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Effect of Crude extracts of *Ocimum sanctum* (OS) and *Evolvulus alsinoides* (EA) on free radical homeostasis in Brian region of rats subjected to chronic unpredictable stress (CUS).

Effect of Crude extracts of *Ocimum sanctum* (OS) and *Evolvulus alsinoides* (EA) on free radical homeostasis in the brain regions of rats subjected to chronic unpredictable stress (CUS).

**Crude Ethanolic** (EtOH) and Aqueous (Aq) extracts of *Ocimum sanctum*, *Evolvulus alsinoides* and *Panax quinquefolium* (PQ) were evaluated for their anti-oxidant potential and observed for their effect in Frontal cortex (FC), Hippocampus (HP), Hypothalamus (HT) and Striatum (ST) brain regions of rats subjected to Chronic unpredictable stress (CUS). CUS has significantly (P<0.01) increased lipid per oxidation and there is a significant (P<0.01) decrease in superoxide dismutase (SOD) and Catalase (CAT) with corresponding increase (P<0.01) in Glutathione peroxidase (GPx) levels.

Crude Ethanolic extracts of *Ocimum sanctum* and *Evolvulus alsinoides* were significantly (P<0.05) Effective in reducing CUS induced lipid peroxidation in FC, HP, HT and ST regions of brain. Anti-Oxidant defense enzymes SOD, CAT were significantly (P<0.05) increased in the brain regions observed for changes when compared to stress control rats. The enzyme levels were normalized and were comparable to the non-stress group. GPX that was increased in stressed rats was decreased significantly (P<0.05) in treated groups indicating the decreased free radical load. The anti-oxidant potential of crude extracts considered in our study were comparable to the standard drug *Panax quinquefolium*

In our study for anti-oxidant potential during stress full conditions Aqueous extracts of both the plants did not show any significant protective effects.

Which has shown a potential anti-oxidant property as was observed by its significant (P<0.05) decrease in lipid peroxidation, GPx levels and an increased (P<0.05) SOD and CAT enzyme levels.

The Mean ± SEM was represented in Tables 4 & 5.
Table 4

Effect of Crude extracts of Ocimum sanctum on CUS induced changes in brain antioxidant defense.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid Peroxidation (nmol/mg protein)</th>
<th>Super oxide dismutase (U/mg protein)</th>
<th>Catalase (U/mg protein)</th>
<th>Glutathione peroxidase (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FC</td>
<td>HP</td>
<td>HT</td>
<td>ST</td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.27 ± 0.32</td>
<td>1.83 ± 0.52</td>
<td>1.88 ± 0.08</td>
<td>1.71 ± 0.36</td>
</tr>
<tr>
<td>Cus</td>
<td>3.66 ± 0.86*</td>
<td>3.82 ± 0.99*</td>
<td>3.63 ± 0.94*</td>
<td>2.59 ± 0.58*</td>
</tr>
<tr>
<td>EtOH 200</td>
<td>2.19 ± 0.12</td>
<td>2.21 ± 0.52</td>
<td>1.96 ± 0.13</td>
<td>1.92 ± 0.21</td>
</tr>
<tr>
<td>Aq 200</td>
<td>2.86 ± 0.35</td>
<td>2.4 ± 0.36</td>
<td>2.96 ± 0.28</td>
<td>2.23 ± 0.41</td>
</tr>
<tr>
<td>Cus</td>
<td>28.3 ± 8.96*</td>
<td>19.3 ± 5.26*</td>
<td>20.99 ± 5.83*</td>
<td>28.38 ± 10.52*</td>
</tr>
<tr>
<td>EtOH (200)</td>
<td>49.17 ± 11.85</td>
<td>55.36 ± 12.32</td>
<td>45.27 ± 15.62</td>
<td>50.31 ± 15.35</td>
</tr>
<tr>
<td>Aq 200</td>
<td>26.32 ± 9.32</td>
<td>29.0 ± 10.12</td>
<td>25.32 ± 9.86</td>
<td>37.63 ± 10.18</td>
</tr>
</tbody>
</table>

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. *P<0.01 when compared to non-stress control and *P<0.05 when compared with Chronic unpredictable stress group and the data was analysed by One-way ANOVA followed by Newmankeul’s multiple comparision tests.
### Table 5

**Effect of Crude extracts of *Evolvulus alsinoides* and *Panax quinquefolium* (PQ) CUS induced changes in brain anti-oxidant defense.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>FC</th>
<th>HP</th>
<th>HT</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Stress</td>
<td>1.27 ± 0.32</td>
<td>1.83 ± 0.52</td>
<td>1.88 ± 0.08</td>
<td>1.71 ± 0.36</td>
</tr>
<tr>
<td>CUS</td>
<td>3.66 ± 0.86*</td>
<td>3.82 ± 0.99*</td>
<td>3.63 ± 0.94*</td>
<td>2.59 ± 0.58*</td>
</tr>
<tr>
<td>EtOH (200)</td>
<td>2.32 ± 0.5*</td>
<td>2.21 ± 0.91*</td>
<td>2.15 ± 0.92*</td>
<td>1.62 ± 0.32*</td>
</tr>
<tr>
<td>Aq (200)</td>
<td>3.58 ± 0.84</td>
<td>3.65 ± 0.85</td>
<td>3.32 ± 0.84</td>
<td>2.83 ± 0.95</td>
</tr>
<tr>
<td>PQ (100)</td>
<td>1.83 ± 0.72*</td>
<td>1.98 ± 0.88*</td>
<td>2.13 ± 0.95*</td>
<td>1.22 ± 0.09*</td>
</tr>
</tbody>
</table>

| Non-stress | 51.72 ± 14.62 | 61.92 ± 12.41 | 48.83 ± 11.36 | 54.5 ± 12.36 |
| CUS      | 28.3 ± 8.96* | 19.3 ± 5.26* | 20.99 ± 5.83* | 28.38 ± 10.52* |
| EtOH (200) | 45.62 ± 10.53* | 52.32 ± 13.62* | 43.36 ± 11.27* | 44.36 ± 14.30* |
| Aq (200)  | 26.36 ± 11.35 | 30.32 ± 11.25 | 28.36 ± 5.32 | 20.35 ± 11.36 |
| PQ (100)  | 46.36 ± 12.64 | 53.35 ± 16.30 | 36.41 ± 4.85 | 42.53 ± 11.82 |

| Non-Stress | 22.25 ± 5.32 | 14.22 ± 3.25 | 13.11 ± 2.3 | 18.78 ± 5.38 |
| CUS      | 9.09 ± 1.86* | 8.34 ± 1.53* | 9.53 ± 2.32* | 9.82 ± 1.26* |
| EtOH (200) | 20.62 ± 5.65* | 12.57 ± 2.35* | 11.32 ± 2.8* | 16.84 ± 3.05* |
| Aq (200)  | 10.56 ± 3.32 | 9.85 ± 2.52 | 10.87 ± 2.1 | 11.63 ± 2.36 |
| PQ (100)  | 19.65 ± 2.65* | 13.32 ± 1.86* | 12.51 ± 2.3* | 16.65 ± 2.42* |

| Non-stress | 21.88 ± 9.65 | 34.31 ± 12.52 | 32.4 ± 12.54 | 30.43 ± 11.36 |
| CUS      | 44.91 ± 10.37* | 52.54 ± 16.27* | 52.59 ± 11.62* | 58.6 ± 12.53* |
| EtOH (200) | 28.96 ± 7.52* | 37.37 ± 11.47* | 38.52 ± 10.84* | 26.53 ± 11.23* |
| Aq (200)  | 42.58 ± 9.85 | 49.53 ± 14.22 | 49.65 ± 14.12 | 51.67 ± 12.24 |
| PQ (100)  | 28.62 ± 11.36* | 37.84 ± 12.63* | 37.51 ± 11.32* | 38.36 ± 11.23* |
Anti-stress effects of the pure compounds isolated from *Ocimum sanctum*.

Anti-stress effect of pure compounds OS/A, OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were tested at a dose of 40mg/kg body weight for their effect on adrenal gland weight in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS significantly increased (P<0.01) the adrenal gland weight. OS/A, OS/B and OS/C were significantly (P<0.05) effective in reducing acute stress induced increase in adrenal gland weight. In CUS model, OS/A and OS/B were significantly (P<0.05) effective in reducing adrenal hypertrophy and OS/C was ineffective. However OS/D were ineffective in both the models. The effect of the active compounds was comparable to the standard drug *Panax ginseng*. Results are graphically represented in Figure: 39

**Figure: 39**

Effect of pure compounds isolated from *Ocimum sanctum* on stress induced increase in adrenal gland weight.

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmann Keul's multiple comparision tests.
Anti-stress effect of pure compounds OS/A, OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were tested at a dose of 40mg/kg body weight for their effect on mean-ulcer severity in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS induced ulceration. OS/A, OS/B and OS/C were significantly (P<0.05) effective in reducing ulceration in AS and CUS models. However OS/D was ineffective in both the stress model. The results were comparable to the standard drug *Panax quinquefolium* tested simultaneously at a dose of 100mg/kg body weight. **Figure:40**

**Figure:40**

**Effect of pure compounds isolated from *Ocimum sanctum* on stress induced changes in mean ulcer score**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group *P<0.01* when compared with stress group and the data was analysed by Non parametric One-way ANOVA followed by Dunn's multiple comparison test.
Anti-stress effect of pure compounds OS/A, OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were tested at a dose of 40mg/kg body weight for their effect on Plasma glucose in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS significantly (P<0.05) increased Plasma glucose levels and no significant change was observed in CUS model. OS/A, OS/B and OS/C were significantly (P<0.01) effective in reducing AS induced hyperglycemia and OS/D was ineffective in producing any change in plasma glucose levels. No significant change in plasma glucose of treated and control groups were observed in CUS model. The effective compounds were comparable to the standard drug *Panax quinquefolium* tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 41.

**Figure: 41**

*Effect of pure compounds isolated from *Ocimum sanctum* on stress induced increase in plasma glucose.*

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.01 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests*)
Anti-stress effect of pure compounds OS/A, OS/B, OS/C and OS/D isolated from *ocimum sanctum* were tested at a dose of 40mg/kg body weight for their effect on Plasma creatine kinase in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS significantly (P<0.01) increased Plasma creatine kinase levels. Compound OS/A was significantly (P<0.01) effective in AS model and was ineffective in CUS model. Compounds OS/B and OS/C were significantly (P<0.05) effective in reducing the AS and CUS induced increase in plasma creatine kinase level. How ever OS/D was ineffective in reducing CUS increased increase in creatine kinase levels. The results were comparable to the standard drug *Panax quiniquifolium* (PQ) tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 42

**Figure: 42**

![Bar chart showing effect of pure compounds isolated from Ocimum sanctum on stress induced increase in plasma creatine kinase level](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Effect of pure compounds OS/A, OS/B, OS/C and OS/D isolated from *ocimum sanctum* were tested at a dose of 40mg/kg body weight for their effect on Plasma corticosterone in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS significantly (P<0.01) increased Plasma corticosterone levels. Compound OS/A was significantly (P<0.01) effective in Acute stress model but has no effect in CUS model. OS/B, OS/C were significantly (P<0.05) effective in reducing AS and CUS induced increase in plasma corticosterone levels. How ever OS/D was ineffective in normalizing AS and CUS induced increase in Plasma corticosterone levels. The results were comparable to the standard drug *Panax quiniquifolium* (PQ) tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 43.

**Figure: 43**

<p>| Effect of Pure compounds isolated from <em>Ocimum sanctum</em> on stress induced changes in plasma corticosterone levels. |</p>
<table>
<thead>
<tr>
<th>NS</th>
<th>stress</th>
<th>OS/A</th>
<th>OS/B</th>
<th>OS/C</th>
<th>OS/D</th>
<th>PQ</th>
</tr>
</thead>
</table>
| ![Graph](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non -stress control. ** P<0.001 *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Effect of pure compounds EA/A, EA/B, and EA/C isolated from *Evolvulus alsinoides* were tested for their effect on adrenal gland weight during stress in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS significantly increased (P<0.01) the adrenal gland weight. EA/A and EA/B were significantly (P<0.05) effective in reducing acute stress induced increase in adrenal gland weight. EA/A and EA/B were unable to revert back the CUS induced adrenal hypertrophy. However, OS/C was ineffective in both the models considered in our study. The results were comparable to the standard drug *Panax quinquefolium* (PQ) tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 44

**Figure: 44**

**Effect of pure compounds isolated from *Evolvulus alsinoides* on stress induced increase in adrenal gland weight**

The stress group was compared with non-stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmann-Keul’s multiple comparision tests.
Anti-stress effect of pure compounds EA/A, EA/B, and EA/C isolated from *Evolvulus alsinoides* were tested at a dose of 40mg/kg body weight for their effect on ulcer severity during stress in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS induced ulceration. EA/A, EA/B were significantly (P<0.05) effective in reducing stress induced ulceration in AS and CUS models. Whereas, EA/3 was ineffective in protecting against stress induced ulceration. The results were comparable to the standard drug *Panax quinquifolium* (PQ) tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 45

**Figure: 45**

**Effect of Pure compounds isolated from *Evolvulus alsinoides* on Stress induced Mean ulcer severity**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. *P<0.05 when compared with stress group. Non-Parametric ANOVA followed by Dunn’s multiple comparison tests.
Anti-stress effect of pure compounds EA/A, EA/B, and EA/C isolated from *Evolvulus alsinoides* were tested for their effect on Plasma glucose in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS significantly (P<0.01) increased Plasma glucose levels and no significant change was observed in CUS model. EA/A and EA/B were significantly (P<0.05) effective in reducing AS induced hyperglycemia. No significant change in plasma glucose levels in stress control and treated groups was observed in CUS model. However EA/C was ineffective in reducing stress induced glucose levels. The results were comparable to the standard drug *Panax quinquifolium* (PQ) tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 46

**Figure: 46**

**Effect of purecompounds isolated from *Evolvulus alsinoides* on stress induced changes in plasma glucose levels**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Anti-stress effect of pure compounds EA/A, EA/B and EA/C isolated from *Evolvulus alsinoides* at a dose of 40mg/kg weight were tested for their effect on plasma creatine kinase in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS significantly (P<0.01) increased plasma creatine kinase levels. EA/A and EA/B were significantly (P<0.05) effective in reducing AS induced increase in plasma creatine kinase level. In CUS model compounds EA/A and EA/B were significantly (P<0.05) effective. However EA/C was ineffective in reducing CUS induced increased increase of creatine kinase levels in AS and CUS models. The results were comparable to the standard drug *Panax quiniquifolium* (PQ) tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 47

**Figure: 47**

Effect of Pure compounds isolated from *Evolvulus alsinoides* on stress induced changes in Plasma creatine kinase levels

![Bar graph showing the effect of different compounds on plasma creatine kinase levels](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non -stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Anti-stress effect of pure compounds EA/A, EA/B and EA/C isolated from *Evolvulus alsinoides* were tested at a dose of 40mg/kg body weight for their effect on Plasma corticosterone in acute stress (AS) and Chronic unpredictable stress (CUS) models. AS and CUS significantly (P<0.01) increased Plasma corticosterone levels. EA/A was significantly (P<0.05) effective in reducing AS and CUS induced increase in plasma corticosterone levels. EA/B was effective (P<0.05) only in AS and failed to produced any effect in CUS model. Where as EA/C was not active in both models tested. The results were comparable to the standard drug *Panax quinquelobium* (PQ) tested simultaneously at a dose of 100mg/kg body weight. The results are graphically presented in Figure: 48

**Figure: 48**

![Graph showing the effect of pure compounds](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmann-Keul’s multiple comparision tests.
Effect of pure compounds isolated from *Ocimum sanctum* on Acute stress induced changes in neurotransmitter levels of various brain regions.

Pure compounds OS/A, OS/B, OS/C and OS/D isolated from *ocimum sanctum* were tested at 40mg/kg body weight for their effect on acute stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in cortex region of rats subjected to acute stress (AS). AS significantly \( P<0.01 \) increased DA and 5-HT with a simultaneous decrease of NA levels. OS/A, OS/B, OS/C were effective significantly \( P<0.01 \) in reducing acute stress elevated levels of DA and 5-HT but, were ineffective in normalizing the NA levels. Compound OS/D was ineffective in normalizing the stress altered levels of monoamines. Whereas Panax quinquinolium at a dose of 100mg/kg body weight significantly \( P<0.05 \) increased stress depleted levels of NA and significantly \( P<0.05 \) decreased the DA and 5-HT levels. The results are graphically represented in Figure: 49

**Figure: 49**

Effect of pure compounds Isolated from *Ocimum sanctum* on Acute stress induced Neurotransmitter changes in Cortex

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with \( n=6 \) in each group. @ \( P<0.01 \) when compared to non -stress control and *\( P<0.05 \) when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Pure compounds OS/A, OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were screened at a dose of 40mg/kg body weight for their effect on acute stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in hippocampus region of rats subjected to acute stress (AS). AS significantly (P<0.01) decreased NA and DA with a concomitant increase in 5-HT levels. OS/A, OS/B, OS/C were effective significantly (P<0.01) in reducing acute stress elevated levels of 5-HT but, were ineffective in normalizing the NA and DA levels. Compound C was ineffective in normalizing NA, DA and 5-HT in all the three brain regions. Whereas *Panax quiniquifolium* at a dose of 100mg/kg body weight significantly (P<0.05) increased stress depleted levels of NA and significantly (P<0.05) decreased 5-HT levels and have not shown any significant effect of acute stress induced depleted DA levels.

The results were graphically represented in Figure: 50

**Figure: 50**

**Effect of active compounds from *Ocimum sanctum* on acute stress induced neurotransmitter changes in Hippocampus**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Pure compounds OS/A, OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were tested at a dose of 40mg/kg body weight for their effect on acute stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in hypothalamus region of brain of rats subjected to acute stress (AS). Acute stress significantly (P<0.01) decreased NA levels where as increased significantly (P<0.01) DA and 5-HT levels in hypothalamus. OS/A, OS/B, OS/C were effective significantly (P<0.01) in reducing acute stress elevated levels of 5-HT and decreased the DA levels. However all the compounds are ineffective in normalizing the NA levels. *Panax quiniquifolium* significantly (P<0.01) decreased stress elevated levels of DA and 5-HT and have not shown any significant effect of acute stress induced depleted NA levels. The results are graphically represented in Figure: 51

**Figure: 51**

Effect of active compounds from *Ocimum sanctum* on Acute stress induced Neurotransmitter changes in Hypothalamus

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.01 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
EA/A, EA/B, and EA/C isolated from *Evolvulus alsinoides* were tested for their effect on acute stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in cortex region of rats subjected to acute stress (AS). Acute stress significantly (P<0.01) increased DA and 5-HT with a concomitant decrease in NA levels. EA/A, EA/B, were effective significantly (P<0.01) in reducing acute stress elevated levels of DA and 5-HT but, were ineffective in normalizing the NA levels. EA/C was ineffective in producing any significant changes. Whereas *Panax quiniquifolium* (PQ) has no effect on NA but significantly (P<0.01) decreased the AS elevated DA and 5-HT levels. The resulted are graphically represented in **Figure: 52.**

**Figure: 52.**

*Effect of pure compounds isolated from *Evolvulus alsinoides* on acute stress induced neurotransmitter changes in cortex.*

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
EA/A, EA/B, and EA/C were isolated from *Evolvulus alsinoides* were tested for their effect on acute stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in Hippocampus region of rats subjected to acute stress (AS). Acute stress significantly (P<0.01) increased 5-HT with a concomitant decrease in NA and DA levels. EA/A and EA/B were effective significantly (P<0.01) in reducing acute stress elevated levels of DA and 5-HT. However compound EA/C was ineffective producing any changes in the dopamine and 5-HT levels. But, all the compounds were ineffective in normalizing the NA levels. Whereas *Panax quiniquifolium* (PQ) significantly (P<0.05) increased stress depleted levels of NA and significantly (P<0.01) decreased the 5-HT levels without having any effect on stress induced change in DA levels. The resulted are graphically represented in **Figure: 53.**

**Figure 53**

Effect of pure compounds isolated from *Evolvulus alsinoides* on acute stress induced neurotransmitter changes in hippocampus

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmann-Keul’s multiple comparison tests.
EA/A, EA/B, and EA/C isolated from *Evolvulus alsinoides* were tested for their effect on acute stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in Hypothalamus region of rats subjected to acute stress (AS). Acute stress significantly (P<0.01) increased DA and 5-HT with a concomitant decrease in NA levels. EA/A, EA/B were effective significantly (P<0.01) in reducing acute stress elevated levels of 5-HT and significantly (P<0.01) effective in reducing the AS elevated DA levels. But, all the compounds were ineffective in normalizing the NA levels. Whereas *Panax quinquisfolium* (PQ) significantly (P<0.05) increased 5-HT and significantly (P<0.01) decreased the DA levels with out having any effect on stress induced change in NA levels. The resulted are graphically represented in

**Figure: 54.**

**Effect of pure compounds isolated from *Evolvulus alsinoides* on Acute stress induced Neurotransmitter changes in Hypothalamus**

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Pure compounds OS/A, OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were tested for their effect on stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in cortex region of rats subjected to Chronic unpredictable stress (CUS). CUS significantly (P<0.01) depleted NA, DA and SHT levels. Compounds OS/C and OS/D significantly (P<0.05) elevated CUS depleted levels of DA where as compounds OS/A, OS/B and *Panax quinquefolium* (PQ) were ineffective in elevating DA. Additionally compound OS/C and PQ were effective (P<0.05) in increasing the 5-HT levels. How ever all the Compounds and PQ were ineffective in normalizing stress depleted levels of NA The resulted are graphically represented in Figure: 55.

**Figure: 55**

Effect of pure compounds isolated from *Ocimum sanctum* on chronic unpredictable stress (CUS) induced neurotransmitter changes in Cortex

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non -stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Pure compounds OS/A, OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were tested for their effect on stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in Hippocampus region of rats subjected to Chronic unpredictable stress (CUS). CUS significantly (P<0.01) depleted NA, DA and 5HT levels. Compounds OS/C, OS/B and *Panax quinquefolium* (PQ) significantly (P<0.01) elevated CUS depleted levels of DA and 5-HT where as compounds OS/A and OS/D were ineffective to normalize DA and 5-HT levels when compared to stress control. However all the Compounds and PQ were ineffective in normalizing stress depleted levels of NA. The resulted are graphically represented in **Figure: 56**.

**Figure: 56**

*Effect of pure compounds isolated from *Ocimum sanctum* on Chronic unpredictable stress induced Neurotransmitter changes in Hippocampus*

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparision tests.
Pure compounds (OS /A), OS/B, OS/C and OS/D isolated from *Ocimum sanctum* were tested for their effect on stress induced changes in Nor adrenalin (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in Hypothalamus region of rats subjected to Chronic unpredictable stress (CUS). CUS significantly (P<0.01) depleted NA, DA and 5HT levels. Compound OS/B was significantly (P<0.01) in elevating the stress depleted levels of NA, DA and 5-HT. Where as *Panax quinquefolium* (PQ) significantly (P<0.01) elevated CUS depleted levels of DA and 5-HT but was has no effect on stress induced decrease in NA. However compounds OS/A, OS/C and OS/D did not show any significant effect on NA, DA and 5-HT when compared with the stress control. The resulted are graphically represented in Figure: 57.

**Figure: 57**

Effect of purecompounds isolated from *Ocimum sanctum* on Chronic unpredictable stress induced neurotransmitter changes in Hypothalamus.

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Pure compounds EA/A, EA/B and EA/C isolated from *Evolvulus alsinoides* were tested at a dose of 40mg/kg body weight for their effect on stress induced changes in Noradrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in cortex region of rats subjected to Chronic unpredictable stress (CUS). CUS significantly (P<0.01) depleted NA, DA and 5HT levels. Compounds EA/A, EA/B and EA/C has no effect on the depleted neurotransmitter levels. *Panax quinquelatum* (PQ) were ineffective in elevating DA and was effective (P<0.05) in increasing the 5-HT levels. However all the Compounds and PQ were ineffective in normalizing stress-depleted levels of NA. The resulted are graphically represented in Figure: 58.

**Figure: 58**

Effect of Pure compounds isolated from *Evolvulus alsinoides* on chronic unpredictable stress induced Neurotransmitter changes in cortex.

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non -stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
Pure compounds EA/A, EA/B and EA/C isolated from *Evolvulus alsinoides* were tested at a dose of 40mg/kg body weight for their effect on stress induced changes in Nor adrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in Hippocampus region of rats subjected to Chronic unpredictable stress (CUS). CUS significantly (P<0.01) depleted NA, DA and 5HT levels. Compounds EA/A, EA/B and EA/C has no effect on the depleted neurotransmitter levels. *Panax quinifolium* (PQ) was ineffective in elevating DA and was effective (P<0.05) in increasing the NA and 5-HT levels. However all the Compounds were ineffective in normalizing stress-depleted levels of NA. The resulted are graphically represented in Figure: 59.

**Figure: 59**

Effect of pure compounds isolated from *Evolvulus alsinoides* on chronic unpredictable stress induced neurotransmitter changes in Hippocampus

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul’s multiple comparison tests.
Pure compounds EA/A, EA/B and EA/C isolated from *Evolvulus alsinoides* were tested at a dose of 40mg/kg body weight for their effect on stress induced changes in Noradrenaline (NA), Dopamine (DA) and 5-Hydroxy tryptamine (5-HT) levels in Hypothalamus region of rats subjected to Chronic unpredictable stress (CUS). CUS significantly (P<0.01) depleted NA, DA and 5HT levels. Compounds EA/A, EA/B and EA/C has no effect on the depleted neurotransmitter levels. *Panax quinquisfolium* (PQ) was ineffective in elevating DA and was effective (P<0.05) in increasing the NA and 5-HT levels. The resulted are graphically represented in **Figure: 60.**

**Figure: 60**

**Effect of pure compounds isolated from *Evolvulus alsinoides* on chronic unpredictable stress induced Neurotransmitter changes in Hypothalamus**

![Graph showing neurotransmitter levels](image)

The stress group was compared with non stress control group and the drug treated groups were compared with their respective stress group. Results were represented as mean ± S.E.M. with n=6 in each group. @ P<0.001 when compared to non-stress control and *P<0.05 when compared with stress group and the data was analysed by One-way ANOVA followed by Newmannkeul's multiple comparision tests.
**Effect of Stress on Glucocorticoid receptor expression**

**Blott: 1**

**Blott: 2**

**Blott: 3**

- 97Kda
- 94Kda

**Blott: 4**

**Blott: 5**

**Effect of active compounds on CUS induced changes in Hippocampus**
Regional difference in glucocorticoid receptor expression profile was studied in brain regions of rats subjected to acute (AS) chronic (CS) and chronic unpredictable stress (CUS). The desired protein band of 94 Kda was identified by comparing with the molecular weight marker 97Kda subjected to electrophoresis along with the samples. The protein concentration and integrity was ensured by simultaneous estimation of constitutive protein β-actin (Blott-4).

In acute stress model there is a 40.0% decrease of GR expression in cortex (C), 44.32% in Hippocampus (HP), 39.3% in Hypothalamus (HT) and 43.8% decrease in striatum (ST) regions of brain. (Blott-1) Where as no difference was observed in chronic stress model (Blott-2). In CUS model no change in protein concentration was observed in Cortex, Hypothalamus and striatum regions of brain but in hippocampus there a 50.3% of decrease in GR receptor levels (Blott-3). The percentage change in the glucocorticoids receptor density when compared to control was graphically represented in Figure 61:

**Figure: 61.**

Percentage change of Glucocorticoid receptor (GR) density in different brain regions of rats subjected to stress

![Diagram showing percentage change in GR density](image-url)

Effect of pure compounds OS/A, OS/B, OS/C and OS/D from Ocimum sanctum and EA/A, EA/B and EA/C were evaluated for their effects on GR expression in Hippocampus of rats subjected to Chronic Unpredictable stress (CUS). (Blott-5) When compared to control there is a 46.8.0% of decrease when compared to normal. The receptor expression is normal and of comparable intensity with that of control. OS/A has a decrease of 52.0% when compared to control there is a slight increase of about 16% and 13% in the receptor density of groups treated with OS/B and OS/C. There is a decrease of 39.3% with compound OS/D. In groups treated with compounds EA/A, EA/B the decrease is 39.3% and 32% and in EA/C treated group, the decrease was observed to be 52%. The resulted are graphically represented in Figure: 62.

Figure: 62

[Diagram showing percentage change in GR density across different groups]