CHAPTER- 5

ANTIDEPRESSANT LIKE EFFECTS OF ETHANOLIC EXTRACT OF

Hypericum hookerianum (EEHh) AND ITS GLYCOSIDIC FLAVONOID
ENRICHED EXTRACT (GFHh) IN RESERPINE INDUCED DEPRESSION
IN SWISS ALBINO MICE

5.1. Objective

To analyze the antidepressant like effects of ethanolic extract of

H. hookerianum (EEHh) and its glycosidic flavonoid enriched extract (GFHh) in
reserpine induced depression in Swiss Albino mice by behavioral and biochemical
methods.

5.2. Introduction

Depression is denoted as Major Depressive Disorder (MDD) or mental
apprehension affecting over 120 million people globally. Epidemiological studies
conducted in general population have revealed that the lifetime prevalence of
depression is in the range of 10–15 % (Lepine and Briley, 2011). World Health
Organization (WHO) have reported that, depression may become the second cause
of illness-induced disability by the year 2020.

Depression is a general mood disorder associated with loss of attention, low
confidence, disturbed sleep, poor appetite, low energy and imposes a considerable
health burden on society (Yu et al., 2002; Nemeroff, 2007). Depression is mainly
casted by physiological stress, environmental factors and biological alterations of
neurotransmitters that are interconnected with one another. The main pathogenesis of
depression i.e., monoamine hypothesis predicted that the major neurobiochemical
process is the destruction of monoaminergic function associated with decreased
levels of serotonin, dopamine, adrenaline, nor- adrenaline and gamma amino butyric
acid (Delgado, 2006). Before the usage of Tri Cyclic Antidepressants (TCAs),
Selective Serotonin Reuptake Inhibitors (SSRIs), Monoamine Oxidase inhibitors
(MAOI’s) and specific Serotonin–Nor Adrenaline Reuptake Inhibitors (SNRIs) are used for the treatment of depression. But these antidepressant drugs were slow in their onset of action with severe side effects (Dhingra and Parle, 2012). Therefore, the compounds extracted from St. John’s wort or H. perforatum can be employed for the treatment of depression rather than incorporating the conventional medicines (Linde and Knuppel, 2005). The search for new pharmacotherapeutic agents from medicinal plants for treating depression have increased over the past decades (Zhang, 2004; Sarris et al., 2011). Prominently, it is worth mentioning that prospective treatments for depression seems to act through a mechanism which does not differ significantly with respect to that of “classical” antidepressants. The mechanism of action of herbal drugs should mimic the classical antidepressant drugs that are commercially available which may modify the monoaminergic systems (Machado et al., 2007; Sarris et al., 2011).

In the recent past, traditional based herbal drugs were mainly employed for the study of antidepressant like effects. Plant phytoconstituents, particularly flavonoids have gained much attention and it is also considered as a supplemental intervention to maintain good health and treat various disorders (Mancuso et al., 2007; Stevenson et al., 2007). The biological activity of polyphenols in neurodegenerative disorders, inflammation, cancer and cardiovascular diseases involves the regulation of cell growth and production, enzyme activity and the accent of cellular signaling cascades (Pandey et al., 2007; Darvesh et al., 2010).

Reserpine (methyl reserpate 3, 4, 5- tri methoxy cinnamic acid ester) is a sympathetic drug that leads to hypothermia (decrease in body temperature) which depletes catecholamine in peripheral nervous tissues and also in brain (Bao et al., 2006). Reserpine can permanently inhibit the vesicular uptake of monoamines, including nor - adrenaline, dopamine and 5- hydroxyl tryptamine which depletes monoamines in the brain and leads to depression-like syndrome in animals (Bourin et al., 1983; Lijian et al., 2011). These irreversible changes can be antagonized by major classes of antidepressant drugs. Different types of antidepressant drugs, like
tricyclic antidepressants and selective serotonin-reuptake inhibitors (SSRIs), as well as antidepressant herbal medicines like St. John's wort, are used to treat depression.

The genus *Hypericum* encompasses different species which are used in traditional medicine worldwide (Ferraz et al., 2005). In neuropsychopharmacology, researchers have focused mainly on *Hypericum* species due to their enormous health benefits including synergistic antioxidant activity of phytoconstituents. The aim of this experiment is to study the antidepressant-like effects of ethanolic extract of *H. hookerianum* (EEHh) and its glycosidic flavonoid enriched extract (GFHh) of reserpine induced Swiss Albino mice.

### 5.3. Materials and Methods

#### 5.3.1. (a) Preparation of plant extract

The procedure was previously described in Chapter: 3.3.6

#### 5.3.1. (b). Separation of Glycosidic Flavonoid enriched extract of *H. hookerianum* (GFHh) by acid hydrolysis method

The procedure was previously described in Chapter: 3.3.8.

#### 5.3.2. Experimental animals

The procedure was previously described in Chapter: 4.3.2.

#### 5.3.3. Experimental groups

For this purpose, Swiss Albino mice were divided into six groups (n=6), first group served as Control, second group as Reserpine induced (RI), while the third and fourth groups was reserpine induced and treated with EEHh (200 and 400 mg/kg) respectively. Fifth group was reserpine induced and treated with GFHh (100 mg/kg). Finally, sixth group was treated with reference drug imipiramine (tricyclic antidepressant 10 mg/kg) which was administered intraperitoneally (i.p) to the reserpine induced groups.
5.3.3.1. Forced Swim Test (FST)

Forced swim test was used as one of the models for antidepressant activity (Fig.5.1 (a) and (b)). Here, mice were forced to swim individually for 15 min in glass cylinder (30 cm high, 22.5 cm in diameter) containing 15 cm water at room temperature which constituted the “pre-test session”. After the completion of induction and treatment period, each animals were forced to swim in a similar environment for a period of 6 min in a “test session” and immobility time of mice was recorded (Porsolt et al., 1978).

5.3.3.2. Tail Suspension Test (TST)

The total duration of immobility of the tail suspension test was measured according to the method previously described by Steru et al (1982). Briefly, both acoustically and visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period before and after treatment (Fig.5.2.).

5.3.3.3. Locomotor Activity (LMA)

The photocell activity cage was utilized to determine the degree of depression (Fig.5.3.). Spontaneous locomotor activities (photocell beam
counts) of all groups of mice were assessed individually for 10 min by Actophotometer (Medicraft, model series: 600-40, S. No: PA-0149, India). The units of the activity counts were arbitrary and based on the beam breaks by movement of the mice (Kulkarni et al., 2004).

5.3.3.4. Rota Rod Test (RRT)

For this experiment, a custom build rota rod (Medicraft Rota Rod, Model series.519/E-2C, S.NO: MRA-036, Medicraft electro medicals (P) Ltd., Lucknow) was used which consisted of a rotating spindle (diameter 7.3 cm) and individual compartments for each mouse with varying rotational speeds (Fig.5.4.). Initially, the animals were trained for four training sessions on successive days (each constituted by a maximum of 10 trials) to achieve the maximum performance. The animals were exposed individually on a rotating rod (5-25 rpm) at 5 min intervals with a cut off time of 180 sec (Rozas et al., 1995).

5.3.3.5. Reserpine induced Hypothermia

This test was performed according to the method previously described by Bourin et al (1983). The rectal temperature of animal was measured in all groups by inserting the thermister thermometer into the rectum of mice after 2 hr injection of reserpine.

5.3.4. Antioxidant assays

5.3.4.1. Estimation of enzymic antioxidants

The activities of enzymic antioxidants Superoxide Dismutase-SOD (Kakkar eta l., 1984), Catalase-CAT (Sinha, 1972), Glutathione Peroxidase –GPx (Rotruck, 1973), Glutathione-S-Transferase – GST (Habig et al., 1974) were studied using
standard method. The protein concentration present in the tissues was determined by Lowry's method (1951).

5.3.4. Estimation of lipid peroxidation

Lipid peroxidation (LPO) of brain tissue homogenate was estimated spectrophotometrically by measuring thiobarbituric acid reactive substances (TBARS) (Ohkawa et al., 1979).

5.3.4.3. Estimation of non-enzymic antioxidants

Reduced glutathione content-GSH (Ellman, 1959), levels of vitamin C (Omaye et al., 1994) and vitamin E (Desai, 1984) were studied using standard methods.

5.3.5. Estimation of brain neurotransmitters

Serotonin, dopamine, adrenaline and nor-adrenaline (Schlumf et al., 1974) were studied using standard methods.

5.3.6. Estimation of MAO A and B activity

The mouse brain mitochondrial fraction was organized following the method of Schurr and Livne (1976). The MAO activity was determined using standard methods (Yu et al., 2002).

5.4. Statistical analysis

All the data were expressed as mean ± S.D. The results were analyzed with the help of analysis of variance (ANOVA) followed by Bonferroni’s test (multiple comparison). For all the experiments, first the comparisons were made between the control and reserpine induced group and also between reserpine induced and treated groups. Statistical difference were considered significant when the ‘p’ value was <0.005.
5.5. Results

5.5.1. Effect of EEHh and GFHh on behavioral analysis

5.5.1.1. Effect of EEHh and GFHh on Forced Swimming Test (FST) in reserpine treated mice

Fig 5.5. Effect of EEHh and GFHh on Forced Swimming Test (A) Climbing (B) Swimming in reserpine-treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and reserpine induced group ; # p<0.001 when compared with reserpine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
In FST, all the groups showed normal climbing behavior during training session. But on the 6th day, reserpine induced group showed significant decrease in climbing (p<0.001) and swimming activities when compared with control group. The reserpine induced groups that were treated with EEHh (200 and 400 mg/kg) and GFHh (100 mg/kg) showed increased climbing and swimming activities when compared with reserpine induced group (p<0.001). Similar results were also observed on the 12th day of treatment. Upon comparison with all other extracts, GFHh showed high climbing and swimming activities which was comparatively similar to standard drug imipiramine (Fig.5.5. (A) and (B))

5.5.1.2. Effect of EEHh and GFHh on Tail Suspension Test (TST) in reserpine treated mice

![Graph showing effect of EEHh and GFHh on TST](image)

Fig 5.6. Effect of EEHh and GFHh on Tail Suspension Test in reserpine –treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and reserpine induced group ; # p<0.001 when compared with reserpine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
In TST, all the groups showed normal mobility during training session. But on the 7th day, reserpine induced group showed increased immobility period (p<0.001) when compared with the control group. On the other hand, EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and IMP (10 mg/kg) treatment significantly decreased the immobility period when compared with reserpine induced group (p<0.001). Similar results were also observed on the 14th day of treatment (Fig 5.6). Among all other extracts, GFHh diminished the immobility period and their activity is similar to standard drug imipiramine.

5.5.1.3. Effect of EEHh and GFHh on Rota Rod test (RRT) in reserpine – treated mice

During training sessions, animals showed maximum grip strength in all rpm (5-25). In rota rod test, significant (p<0.001) decrease in motor co-ordination (i.e., very less grip strength) activity was observed among reserpine induced group when compared to control group. But the reduced grip strength was significantly reversed (p<0.001) in EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and IMP (10 mg/kg) treated groups when compared to reserpine induced group (Fig 5.7. A and B).
Fig 5.7. Effect of EEHh and GFHh on Rota rod test (A) - % of grip strength before treatment (B) % of grip strength after treatment at different rpm in reserpine- treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and reserpine induced group ; # p<0.001 when compared with reserpine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
5.5.1.4. Effect of EEHh and GFHh on Loco Motor Activity (LMA) in reserpine treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and reserpine induced group ; # p<0.001, ns- when compared with reserpine induced and treatment groups using one- way ANOVA followed by Bonferroni’s test as a post-ANOVA test.

In LMA, reserpine induced group showed less number of photocell beam counts when compared to control group (p<0.001). When the reserpine induced group were treated with EEHh (400 mg/kg), GFHh (100 mg/kg) and IMP (10mg/kg), significant increase in photocell beam counts (p<0.001) was observed (Fig 5.8). Among the extracts tested, GFHh (100 mg/kg) increased the photocell beam counts which was comparatively similar to standard drug imipiramine.

5.5.1.5. Effect of EEHh and GFHh on reserpine induced hypothermia in reserpine –treated mice

The reserpine induced group presented significant alterations in hypothermia (i.e., reduced normal body temperature) when compared with control group (p<0.001). Administration of EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and IMP (10 mg/kg) antagonized the hypothermia induced by reserpine (p<0.001) (Fig.5.9).
5.5.2. Evaluation of *in vivo* antioxidant potential of EEHh and GFHh in brain of reserpine induced Swiss Albino mice

5.5.2.1. Effect of EEHh and GFHh on enzymic antioxidants in brain of reserpine treated mice

The effect of EEHh, GFHh, and IMP on enzymic antioxidants (SOD, CAT, GPx and GST) activities is shown in Fig 5.10 (A, B, C and D). Biochemical analysis showed significant decrease in SOD, CAT, GPx and GST activities in reserpine induced group when compared with control group (p<0.001).

The animals treated with 200 mg/kg of EEHh exhibited increased activities of all enzymes SOD, CAT (p<0.005), GPX and GST (p<0.01) when compared with control group. The other treated groups, EEHh (400 mg/kg), GFHh (100 mg/kg) and IMP (10 mg/kg) showed significant increase in all the enzyme activities (p<0.001) when compared with reserpine induced group. Interestingly, GFHh (100 mg/kg) treated group showed improved SOD, CAT, GPx and GST activities than EEHh (400 mg/kg) treated group. The GFHh treatment remarkably improved antioxidant defense in reserpine induced mice which is similar to standard drug imipiramine.
Fig 5.10. Effect of EEHh and GFHh on enzymic antioxidants (A) Superoxide Dismutase (B) Catalase (C) Glutathione peroxidase and (D) Glutathione -S- Transferase in brain of reserpine-treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and reserpine induced group and # p<0.001, $ p<0.01 and @ p<0.05 when compared with reserpine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
5.5.2.2. Effect of EEHh and GFHh on non-enzymic antioxidants in brain of reserpine treated mice

The effect of EEHh and GFHh on non-enzymic antioxidants of reserpine induced mice is presented in Fig. 5.11. ((A), (B), (C) and (D)).

Data are expressed as mean ± SD. a p < 0.001 compared with control and reserpine induced group and # p<0.001 when compared with reserpine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
5.5.2.3. Effect of EEHh and GFHh on LPO in brain of reserpine-treated mice

Biochemical analysis of LPO indicated that the level of MDA was significantly increased (p < 0.001) in the reserpine induced group due to increased free radical generation, as compared to control group. But administration of EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and IMP (10 mg/kg) significantly (p < 0.001) brought down the level of MDA which indicated the decrease in LPO level, when compared with the reserpine induced group (Fig.5.11. (A)).

Reduced glutathione (GSH) and vitamins (C and E) are non-enzymic antioxidants which are also involved in defense mechanism against free radicals. The effect of EEHh, GFHh and IMP (10 mg/kg) on non-enzymic antioxidants is depicted in Fig 5.11 (B, C and D). Biochemical analysis indicated the decreased GSH content, vitamin C and E levels in reserpine induced group when compared with control group (p<0.001).

Here, the animals treated with EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and IMP (10 mg/kg) showed significant increase in all the non-enzymic antioxidants (p<0.001) when compared with reserpine induced group. Among the extracts, GFHh treatment remarkably improved the non-enzymic antioxidants in reserpine induced mice which is similar to standard drug imipiramine.
5.5.3. Effect of EEHh and GFHh on brain neurotransmitters in reserpine-treated mice

The levels of brain neurotransmitters (dopamine, serotonin, adrenaline, nor-adrenaline) of reserpine induced mice are shown in the Fig.5.12 (A), (B), (C) and (D).

Fig 5.12. Effect of EEHh and GFHh on brain neurotransmitters – (A) Dopamine  (B) Serotonin  (C) Epinephrine (Adrenaline) and (D) Nor-epinephrine (Nor-adrenaline) in reserpine-treated mice

Data are expressed as mean ± SD. a p < 0.001 compared with control and reserpine induced group and # p<0.001, $ p<0.01 and @ p<0.05 when compared with reserpine induced and treatment groups using one way ANOVA followed by Bonferroni’s test as a post- ANOVA test.
5.5.3.1. Effect of EEHh and GFHh on brain dopamine and serotonin in reserpine-treated mice

In spectrofluorimetric analysis, significant decrease in brain serotonin and dopamine levels were observed (p<0.001) in reserpine induced group when compared with control group. Upon treatment, increase in brain dopamine and serotonin levels were observed for 200 mg/kg dose of EEHh (p<0.01), while the 400 mg/kg of EEHh, 100 mg/kg of GFHh and 10 mg/kg of IMP administered groups also caused significant increase (p<0.001) in dopamine and serotonin when compared with reserpine induced group (Fig 5.12. (A) and (B)).

5.5.3.2. Effect of EEHh and GFHh on brain adrenaline and nor-adrenaline in reserpine-treated mice

In this study, the brain adrenaline and nor-adrenaline levels were significantly decreased in reserpine induced group when compared to control group (p<0.001). In addition to the increased brain adrenaline and nor-adrenaline levels observed for 200 mg/kg dose of EEHh (p<0.01), while the 400 mg/kg of EEHh, 100 mg/kg of GFHh and 10 mg/kg of IMP also caused significant increase (p<0.001) in brain adrenaline and nor-adrenaline levels when compared with reserpine induced group (Fig.5.12. (C) and (D)).

5.5.3.3. Effect of EEHh and GFHh on brain MAO A and MAO B activity in reserpine-treated mice

The effect of EEHh and GFHh on brain MAO A and MAO B activity in reserpine induced mice is depicted in Fig.5.13 (A) and (B)).

In this experiment, increased activities of brain MAO A and MAO B were observed in reserpine induced group when compared with control group (p<0.001). In addition to the decreased brain MAO A and MAO B activities observed for 200 mg/kg dose of EEHh (p<0.01), while the 400 mg/kg of EEHh, 100 mg/kg of GFHh and 10 mg/kg of IMP caused significant decrease in brain MAO A and B activity (p<0.001) when compared with reserpine induced group which indicated the MAO inhibitory effects.
Fig 5.13. Effect of EEHh and GFHh on brain MAO A and B activity (A) MAO A and (B) MAO B in reserpine-treated mice

Data are expressed as mean ± SD, a p < 0.001 compared with control and reserpine induced group and # p<0.001, @p<0.05 and when compared with reserpine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.

The brain Monoamine Oxidase (MAO A and B) inhibitory activity of EEHh (200 and 400 mg/kg), GFHh (100 mg/kg) and IMP (10 mg/kg) are listed in table 5.1.

Table 5.1: Brain Monoamine Oxidase (MAO A and B) inhibitory activity of EEHh and GFHh

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>% activity of brain MAO A</th>
<th>% activity of brain MAO B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control group</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Reserpine Induced (RI; 1mg/kg)</td>
<td>58.62% a</td>
<td>57.75% a</td>
</tr>
<tr>
<td>3</td>
<td>RI +EEHh (200 mg/kg)</td>
<td>47.8% b</td>
<td>44% b</td>
</tr>
<tr>
<td>4</td>
<td>RI +EEHh (400 mg/kg)</td>
<td>34.78% #</td>
<td>33% #</td>
</tr>
<tr>
<td>5</td>
<td>RI +GFHh (100 mg/kg)</td>
<td>29.41% #</td>
<td>14.6% #</td>
</tr>
<tr>
<td>6</td>
<td>RI +IMP (10 mg/kg)</td>
<td>7.69 % #</td>
<td>12.06% #</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, a p < 0.001 compared with control and reserpine induced group and # p<0.001, @p<0.05 and $ p<0.01 when compared with reserpine induced and treatment groups using one-way ANOVA followed by Bonferroni’s test as a post-ANOVA test.
5.6. Discussion

Depression, the foremost civic health problem, is aggravated by exposure to persistent oxidative stress (Sakakibara et al., 2008). The abnormalities of noradrenergic, dopaminergic and serotonergic transmission pathways are known biological causes of mental depression (Berton and Nestler, 2006). Imipramine is an effective tricyclic antidepressant class of drug which showed undesirable side effects like headache, dizziness, nausea, gastrointestinal problems etc., (Skidmore-Roth, 2010). Hence, the novel antidepressant agents from medicinal plants with less side effects with less cost need to be exploited. The present study investigated the antidepressant like effect of EEHh and GFHh on reserpine induced animal by behavioral and biochemical analysis of brain antioxidants and neurotransmitters. Also the antidepressant like effect exhibited by plant extracts was compared with the standard drug imipramine.

According to Xu et al (2008), behavioral studies play a vital role in the evaluation of antidepressant drugs in rodents. Behavioral studies are not only used to study the antidepressant like activity of substances but also their neurobiological mechanisms (Steru et al., 1985; Bourin et al., 2005). FST is the most validated behavior model to study the antidepressant like effects of plant extracts (Martinez-Vazquez et al., 2012). In FST, the despair state of hopelessness or desertion seen in depressed mice is similar to human depression. One of the roles of antidepressant drugs is to reduce this behavior of abandonment in experimental rodents (Castange et al., 2001). EEHh and GFHh extracts exhibited increased swimming and climbing activities in mice which was comparable to classical antidepressant drug imipramine. Similar results were also obtained in several plant extracts of *H.perforatum* (Butterweck et al., 2000; Nolder and Schotz, 2002), *Byrsonima crassifolia* (Herrera-Ruiz, et al., 2011), *Apocynum venetum* (Zheng et al., 2013) and *Trichilia catigua* (Chassot et al., 2011) these extracts have also been demonstrated to contain flavonoidal constituents.
TST is commonly used to identify and characterize the effectiveness of antidepressant drugs (Cryan et al., 2005; Bourin et al., 2005). TST also induces a state of despair in animals similar to that of FST (Steru et al., 1985; Renard et al., 2003). In the present study, reserpine administration resulted in high immobility period and this condition was alleviated by EEHy, GFHy and imipiramine treatment. TST is a well characterized model and it is sensitive to serotonergic, nor-adrenergic and dopaminergic modulated pharmacological classes of drug (Cryan et al., 2005; O’Leary et al., 2007). In TST, Zeni et al (2013) proved the antidepressant activity of Aloysia gratissima extract using monoaminergic mechanism which is consistent with the current results.

EEHy, GFHy and imipiramine antagonized the hypothermia induced by reserpine that can permanently inhibit the vesicular uptake of monoamines, including nor-adrenaline, dopamine and serotonin and its metabolites. Similar results were also obtained in a study conducted by Qiang-Song wang et al (2013) using ethanolic extract of Zuojin Pill. Majority of the studies have reported the involvement of dopaminergic system in the pathophysiology and biological cause of depression (Nemeroff, 2007). Therefore the antidepressant like effects observed in the present study is associated with the central monoaminergic neurotransmitter system.

Among reserpine-induced animals, decreased locomotor activity was (number of photocell beam crossed) observed in actophotometer. The hypomotility of reserpine may be due to its monoamine theory of depression that leads to depleted monoamine in brain (Lijian et al., 2011). EEHy, GFHy and imipiramine treated groups possibly antagonized the hypothermia and hypomotility effects of reserpine. Kumar et al (2010) also reported similar results using H.perforatum extract.

In rota rod test, animals should have a balanced walk on a rotating cylinder and it is commonly used to check and measure the coordination of the motor skills of experimental rodents (Duan et al., 1999). In the current study, reserpine induced
mice significantly reduced the grip strength but the administration of EEHh, GFHh and imipiramine showed marked improvement in behavioral performances. Mohanasundari et al (2006) reported that *H. perforatum* showed motor coordination skill in rota rod test against 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) induced motor dysfunction which is consistent with the current results.

Increased free radical generation in neurodegenerative diseases leads to a rapid consumption and depletion of endogenous scavenging antioxidants. Therefore researchers have introduced exogenous antioxidants like flavonoids and other phytoconstituents as well as scavengers to counter the oxidative damage in these diseases (Sanchez-Reus et al., 2007). Oxidative stress is described as increased production of ROS, LPO and concurrent oxidant related reactants which causes pathogenesis in depression. From clinical studies, it is understood that oxidative stress injures the cells that leads to destruction thereby decreasing the volume of hippocampus among depression case (Sapolsky, 2000). Response to the stress is a physiologically adaptable course of organisms under burden and leads to changes in the physiological function and metabolism (Rosch, 1997). The severity of depression is associated with imbalance in antioxidants (Yanik et al., 2004). Antidepressants could considerably improve the activity of antioxidant enzymes by increasing the GSH level and decreasing LPO level in depressive animal models on the assumption that *in vivo* antioxidant status necessitate antidepressant action (Zafir and Banu et al., 2009).

The cellular defensive role against reactive oxygen species (ROS) and free radicals include two important systems: the enzymatic (CAT, SOD and GPx) and non-enzymatic (GSH) which are chiefly involved in antioxidant defense mechanism (Mayo et al., 2002). In *in vivo* studies, a significant decrease in SOD and CAT levels were observed in reserpine induced group. The EEHh, GFHh and imipiramine restored the activities of antioxidant enzymes (SOD and CAT). Decreased activity of SOD leads to the poor removal of superoxide ion that can be
injurious to the organs. Moreover, enhanced SOD activity may be due to the involvement of EEHh and GFHh in scavenging O$_2$ generated from reserpine. There is a general agreement that quercetin and rutin act as the scavengers of reactive oxygen species (Haenen et al., 1999).

The increase of neural excitability is direct reflection of damage of cell due to oxidative stress. In this process, increase in catecholamine leads to automatic oxidation and caused O$_2$' generation. Consequently, H$_2$O$_2$ is formed by catalysed reaction of O$_2$'- that further generates more active hydroxyl ions (OH·). Poly unsaturated fatty acids in cell membrane easily react with OH· to produce lipid peroxides which causes damage to cell (Yao et al., 2005) and subsequently decompose to cytotoxic substances such as malonaldehyde (MDA). Therefore, MDA content can directly correlate and reflect the degree of lipid peroxidation caused by oxidative damage (Richter et al., 1987).

Long term treatment with neuroleptics like reserpine always increased the production of free radicals leading to oxidative stress and cellular damage (Balijepalli et al., 2001). The role of increased reactive oxygen species (ROS) and oxidative stress in the etiopathology of reserpine with involuntary movements of muscles has been proposed by Kulkarni et al (2004). Elkashef and Wyatt (1999) reported that rodents had significantly higher thiobarbituric acid reactive substances (TBARS) in the striatum of brain tissue, thereby suggesting the increase in peroxidation content (LPO), free radical production and oxidative stress in these animals.

The activity of glutathione dependent enzymes GPX and GST was decreased in reserpine induced group when compared with control group due to the neurotoxic effect caused by reserpine. But the treated groups, EEHh, GFHh and imipiramine increased the activity of GPx and GST when compared with reserpine induced group. The content of GSH was decreased in reserpine induced group when compared with the control group due to the antioxidant imbalance caused by neurotoxic effect. The GSH content in treated groups (EEHh, GFHh and
imipramine) was increased when compared with reserpine induced group suggesting their antioxidant capacity.

Reserpine induction led to depletion of monoamines which also weakened the antioxidant defense that was evidenced by depletion of reduced glutathione and catalase activity. This suggests that the reserpine induction caused significant oxidative damage, possibly by unbalancing oxidative and antioxidant defense mechanism. Quercetin has a protective role against reserpine induced oxidative damage and disorders (Naidu et al., 2004).

Vitamins E and C depletions are also involved in pathogenic pathways of clinical depression (Tagliari et al., 2010). Moreover, reduced vitamins E and C were also found to be associated with cognitive impairment that is observed in depression. In this experiment, reserpine induced groups showed reduced vitamin C and E levels and their levels were restored in EEHh, GFHh and imipramine treated groups. The mechanisms of antioxidant and antidepressant activities are complementary to each other which are evidenced by the presence of flavonoidal constituents in several plant extracts of *H. perforatum* (Bruno et al., 2005), *Glycyrrhiza uralensis* (Cheng et al., 2008), *Asparagus racemosus* (Singh et al., 2009) and *Artemisia absinthium* (Mahmoudi et al., 2009).

The correlation between depression and dopaminergic system was confirmed by the fact that antidepressant are targeted to act on dopaminergic system (Kiemk et al., 2002). Antidepressants that increase the availability of neurotransmitters (dopamine, serotonin, adrenaline and nor-adrenaline) at the nerve synapse may modulate the function of monoaminergic system endorsing the neurogenesis (Elhwuegi et al., 2004; Dailly et al., 2004). With regard to this notion, drugs like reserpine that cause depletion of brain neurotransmitters may induce depression (Matsuzaki et al., 2006). In the present study, EEHh and GFHh administered groups contained higher levels of brain monoamines (dopamine, serotonin, adrenaline and nor-adrenaline) meanwhile these extracts also exhibited antidepressant activity. The neuroprotective effect exhibited by glycosidic flavonoids and other compounds
present in EEHh and GFHh elevated the levels of brain neurotransmitters. The current findings are in consistent with the antidepressant effect demonstrated by ethanolic extract of *Apocynum venetum* in TST which is due to its flavonoidal constituents (Zheng et al., 2013). Even the extracts of *H.perforatum* increased the availability of serotonin, dopamine and/or nor-adrenaline which indicated their mechanism of action is similar to antidepressant drugs (Noldner and Schotz, 2002; Zhang, 2004; Wurglics and Schubert-Zsilavecz, 2006). Previous experimental results have endorsed the role played by herbal extracts and their phytoconstituents in antidepressant effect and their mechanism of action is due to their involvement in monoaminergic system (Rodrigues et al., 2002; Machado et al., 2009).

Dimpfel (2009) have also reported that quercetin, rutin flavonoids influenced the electropharmacogram of adult rats mimicking the act of moclobemide, a reversible inhibitor of MAO-A and also quercetin showed a certain similarity of electrical effects with classical antidepressant drug imipramine. In this experiment, EEHh and GFHh treated groups exhibited high MAO enzyme inhibitory activity similar to classical antidepressant imipiramine. However, quercetin is a glycosidic flavonoid that has the capacity to inhibit the activity of the enzyme MAO A, which is one of the main class of antidepressant agent (Racagni and Popoli, 2010). The major active principle flavonoids quercetin, rutin and hyperoside are present in *H.perforatum*. Among the flavonoids, hyperoside and rutin present in *H.perforatum* dominate the glycosidic compounds which are involved in the inhibitory effect of MAO A and B enzymes (Thiede and Walper, 1994).

Previous studies have suggested that the role played by quercetin and its glycosides in the antidepressant effects is a complex one. However, it is well-known fact, that in phytotherapeutics the crude plant extracts or semipurified mixture of enriched extracts usually showed more powerful effects than pure compounds. This may be due to the multiple target actions of complex mixtures but could also arise from synergistic interactions among their components (Fernandez et al., 2005).
In conclusion, EEHh and GFHh obtained from aerial parts of *H. hookerianum* presented antidepressant like effect in behavioral and biochemical analysis via the antioxidant and monoaminergic system. Interestingly, GFHh exhibited greater promising effect than EEHh. GFHh was found to possess high concentration of flavonoids and we proposed this finding to contribute in both behavioral and biochemical functions in reserpine induced depression. Thus, the GFHh is a promising candidate to treat drug induced depressive disorders.
Graphical summary - Antidepressant like activity of EEHh and GFHh in reserpine induced Swiss Albino mice

Swiss Albino mice

Reserpine induction

Treatment with EEHh and GFHh

Oxidative stress

Neuro protection by EEHh and GFHh

FST
- Decreased swimming
- Decreased climbing

TST
- Increased immobility period

RIHT
- Decreased body temperature

LMA
- Decreased photo cell beam counts

RRT
- Decreased grip strength

Decreased antioxidants
- SOD, CAT, GPx, GST
- Vitamin C, E and GSH
- Increased LPO
- Decreased brain serotonin, dopamine, adrenaline and nor-adrenaline
- Increased brain MAO A and B

Increased antioxidants
- SOD, CAT, GPx, GST
- Vitamin C, E and GSH
- Decreased LPO
- Increased brain serotonin, dopamine
- Decreased brain MAO A and B

FST
- Increased swimming
- Increased climbing

TST
- Decreased immobility period

RIHT
- Antagonize the reduced body temperature

LMA
- Increased photo cell beam counts

RRT
- Increased grip strength