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Evaluation of Antidepressant Like Effects of Ethanolic *Hypericum hookerianum* and its Glycosidic Flavonoid Enriched Extract in Reserpine Induced Swiss Albino Mice

¹S. Subakanmani, ¹S. Murugan and ²P. Uma Devi

¹Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimbatore, 641114, Tamil Nadu, India

²Department of Biology, School of Natural Science, Madawalabu University, Ethiopia

Corresponding Author: S. Murugan, Department of Biotechnology, School of Biotechnology and Health Sciences, Karunya University, Coimbatore, 641114, Tamil Nadu, India

ABSTRACT

The purpose of the study was to investigate the anti-depressant like effect of ethanolic extract of *H. hookerianum* (EEH_h 200 and 400 mg kg⁻¹ p.o.,) and its glycosidic flavonoid enriched extract of ethanolic extract of *H. hookerianum* (GFH_h 100 mg kg⁻¹ p.o.,) in reserpine induced (2 mg kg⁻¹ i.p.,) Swiss albino mice. The flavonoidal constituents in EEH_h and GFH_h was confirmed by HPLC. The behavioral analysis of forced swimming test, tail suspension test, locomotor activity in mice has been conducted. Brain superoxide dismutase, catalase, glutathione and lipid peroxidation were estimated biochemically. Brain monoamine oxidase (A and B) activity has been carried out by double beam spectrophotometer. All the values in this study indicated that the GFH_h posses well specified antidepressant activity than EEH_h due to higher concentration of flavonoids as confirmed by HPLC.

Key words: Forced swimming test, tail suspension test, HPLC, locomotor activity

INTRODUCTION

Depression is a general disorder, affecting over 120 million people worldwide and the epidemiological surveys conducted globally reported that the lifetime prevalence of depression is in the range of 10-15% (Lepine and Briley, 2011). Moreover, depression will become the second cause of illness-induced disability by the year 2020 (WHO., 2006). The major biochemical role of depression is the monoamine hypothesis, which describes that depression is caused by a functional shortage of monoamines (dopamine, norepinephrine, epinephrine and serotonin) at certain parts of the brain (Wouters, 1998). Monoamine oxidase is found in almost all tissues of the body and exists in two forms: MAO-A and MAO-B. Monoamine oxidase (MAO) is responsible for metabolizing brain monoamines like norepinephrine, dopamine and 5-HT i.e., serotonin. Serotonin is the main substrate for MAO-A and it is also the main target for the antidepressant Monoamine oxidase inhibitors. The MAO-B has a substrate preference for phenyl ethyl amine. Both enzymes effectively act on norepinephrine and dopamine. During depressive condition, the level of MAO (A and B) activities increased which leads to the higher usage of substrates; thereby it reduced the levels of monoamines (Knoll, 1997; Krishnan, 1998).

Even though the majority of the currently used antidepressant drugs [Tri Cyclic Antidepressants (TCA), Selective Serotonin Reuptake Inhibitors (SSRI) and Monoamine oxidase inhibitors (MAOIs)] ameliorate depressive symptoms, they also exert numerous undesirable

effects (Dhingra and Parle, 2012). Moreover, 30% of depressive patients do not react properly to the first-line treatment (Fava and Rush, 2006). Thus, the investigations of more efficacious and well-tolerated drugs are progressing in many developing countries. Among the probable approaches, the use of products obtained from natural resources has made a lot of unique and vital offerings to drug discovery (Newman *et al.*, 2003). In the recent past, much attention has paid on traditional herbal medicines for antidepressant drug evolution. The phytoconstituents from plants, particularly flavonoids have attracted progressively and they are considered as supplemental interventions to sustain health and treat diseases (Mancuso *et al.*, 2007; Stevenson *et al.*, 2007). The biological activity of flavonoids 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 and Rizvi, 2009; Darvesh *et al.*, 2010). Currently, many pharmaceutical companies have started new herbal formulation from plants which showed comparatively promising therapeutic activity.

Hypericum hookerianum is a small woody yellow flowered shrub found in India. It is mainly available in the high altitudes of hills and commonly known as the Golden Lotus of *H. perforatum*. These *Hypericum* species are mainly used for the treatment of neurodegenerative diseases. *Hypericum hookerianum* Wight and Arnott are a well known ornamental plant among the 20 different species of *Hypericum* found in India. Different extracts of *H. hookerianum* have already been reported to posses wound healing (Mukherjee and Suresh, 2000) antibacterial (Mukherjee *et al.*, 2001), antitumor (Dongre *et al.*, 2007), antiviral (Vijayan *et al.*, 2004) and anxiolytic (Subakanmani and Umadevi, 2012) activities. Due to the neuroprotective potential of *Hypericum* species, the present study aimed to explore the promising underlying mechanisms of antidepressant-like effect of ethanolic extract of *H. hookerianum* (EEHh) and its Glycosidic Flavonoid enriched extract (GFHh) in reserpine induced Swiss albino mice.

MATERIALS AND METHODS

Collection and validation of samples: The aerial parts of *H. hookerianum* were collected from the high altitudes of Nilgiris (Western Ghats), Tamil Nadu, South India in the month of October, 2011. The plant was authenticated by Dr. S. Rajan, Field Botanist, Survey of Medicinal Plants and Collection Unit (Central Council for Research in Homoeopathy), Department of AYUSH, Ooty, TamilNadu, South India. The collected *H. hookerianum* was subjected to shade drying for about 5 weeks. The dried *H. hookerianum* was crushed to powder mechanically by pulverizer, sieved and stored in airtight container for further analysis.

Chemicals: All the chemicals used in this study were of analytical grade (Sigma Life Sciences Mumbai, India). The plant extracts EEHh (200 and 400 mg kg⁻¹) and GFHh (100 mg kg⁻¹) were dissolved in water and administered orally. The depression inducer, reserpine (Methyl (3β,16β,17α,18β,20α)-11,17-dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy] yohimban-16-carboxylate) was dissolved in glacial acetic acid and diluted to a concentration of 2 mg kg⁻¹ using distilled water and standard drug imipramine (3-(10,11-dihydro-5H-dibenzo[b,f]azepin-5-yl)-N,N-dimethylpropan-1-amine) was dissolved in distilled water to obtain 10 mg kg⁻¹ and both the drugs were administered intraperitoneally.

Preparation of EEHh by soxhlation method: The shade dried aerial parts of *H. hookerianum* was extracted with petroleum ether, chloroform and ethanol successively by the soxhlation method at room temperature and concentrated over water bath and evaporated under reduced pressure and then lyophilized.

Separation of GFHh by acid hydrolysis method: Exactly 25 g of *H. hookerianum* (EEHh) was dissolved in 100 mL 2N HCl: MeOH (1: 1 v/v), sealed in a screw-cap tube and heated on a steam bath for 30 min. The mixture was extracted with an equal volume of ethyl acetate using separating funnel. The upper organic layer was separated and subsequently evaporated to dryness under reduced pressure (Hasler *et al.*, 1992). The residue was dissolved in ethanol and simultaneously the presence of flavonoids was analyzed by HPLC. The aqueous layer was analyzed for sugar using Fehling's solution, which was found to be positive and confirmed the presence of glycosidic derivatives.

HPLC analysis of EEHh and GFHh

Instrumentation and chromatographic conditions: Flavonoids analyses were carried out using an high pressure liquid chromatographic system (Shimadzu, USA) consisting of a solvent delivery pump (Model LC-10 ADvp), a variable wavelength UV/VIS detector (Model SPD 10 AVP), a manual injection valve (Rheodyne®, USA) with a 20 μ L loop and degasser (DGU 14A) and CLASS-VP™ System Software is used for data collection and analyses. The mobile phase of acetonitrile: water (HPLC graded water pH adjusted to 4.5 with ortho phosphoric acid) in various ratios 50:50, 55:45, 60:40, 70:30 were tested and the chromatograms were recorded at 256 nm with a flow rate of 1 mL min⁻¹. For this analysis, 1 mg of the standards (quercetin and ruini) and 20 mg of the plant extracts were used.

Experimental animals: Swiss albino mice of either sex, weighing 20-30 g were used in the present study and they were obtained from the Small Animal Breeding Station, Agricultural University (Mannuthy, Thrissur, Kerala, India). They were housed individually with *ad libitum* access to food and water under controlled laboratory conditions and were exposed to a 12 h cycle of light and dark. All the observations were made at room temperature in a noiseless diffusely illuminated room between 9.00-17.00 h. The experimental protocols were approved by the Institutional Animals Ethics Committee (IAEC) as per provisions of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India (KMCRET/PhD/11/2011). The entire procedure was scheduled from December 2011-April 2012.

Acute toxicity test: Three female nulliparous and non-pregnant albino mice were used and ethanolic extract of *H. hookerianum* at different doses 25, 200 and 2000 mg kg⁻¹, were administered orally. The animals were fasted before the oral administration of the extract and observed individually, after dosage, at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. The animals were observed for common behavior and any toxic symptoms produced by the extract.

Treatment and behavioral analysis: The plant extracts of EEHh and GFHh were administered to the respective group of mice for a period of fourteen days. The group I received the vehicle (1% Tween 80 solution) which served as control. Group II received reserpine (2 mg kg⁻¹ b.wt., i.p.) alone and served as the negative control without any treatment, group III reserpine induced and treated with EEHh (200 mg kg⁻¹), group IV reserpine induced and treated with EEHh (400 mg kg⁻¹), group V reserpine induced and treated with GFHh (100 mg kg⁻¹), group VI reserpine induced and treated with standard drug imipramine (10 mg kg⁻¹, i.p.) served as positive control. One hour after the last administration of doses, the animals were subjected to the behavioral testing and

which was performed in a dark room with minimal background noise. Hand-operated counters and stopwatches were used by a blind observer to score the behavioral parameters. Each experimental group consisted of 6 mice, which was chosen by means of complete randomized schedule.

Forced Swimming Test (FST): Forced swim test was proposed as one of the models to study antidepressant like activity. 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 animal was again forced to swim in a similar environment for a period of 6 min in a “Test session” and duration of immobility time for each mouse was recorded (Porsolt *et al.*, 1977b).

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.* (1985). 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 (Steru *et al.*, 1985).

Locomotor activity (LMA): The photocell activity cage was utilized to measure the degree of depression. The action of plant extracts and imipramine on spontaneous locomotor activity (photocell beam counts) were measured automatically by using Actophotometer (Medicraft photo actometer, model No: 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. The spontaneous locomotor activity of each mouse was recorded individually for 10 min using Actophotometer. Mice were re-tested for activity scores before and after the treatment (Reddy and Kulkarni, 1998).

Estimation of biochemical parameters

Estimation of superoxide dismutase: The activity of superoxide dismutase was assayed by the method of Kakkar *et al.* (1984). The obtained values were expressed as units $\text{min}^{-1} \text{mg}^{-1}$ of protein. One unit of the enzyme can be calculated by the amount of enzyme its inhibits the auto oxidation reaction by 50%.

Estimation of catalase: The catalase activity was assayed by the method of Sinha (1972) and the CAT activity was expressed as micro molar of H_2O_2 consumed/min/mg protein.

Estimation of reduced glutathione: The GSH in the mice brain was determined according to the method of Ellman (1959).

Estimation of MAO-A and MAO-B activity: The whole procedure of brain separation was completed within 5 min (Pan *et al.*, 2005). The mouse brain mitochondrial fraction was organized following the method of Schurr and Livonia (1976). The MAO activity was measured using a spectrophotometer (Yu *et al.*, 2002). The protein concentration was estimated by method of Lowry *et al.* (1951) using Bovine Serum Albumin (BSA) as standard. In MAO-A activity sodium phosphate buffer and 5-hydroxy tryptamine (i.e., serotonin-main substrate for MAO-A) were used as blanks and MAO-B activity sodium phosphate buffer and benzyl amine (main substrate for MAO-B) were used as blanks.

Statistical analysis: Data obtained from behavioral and *in vivo* experiments were expressed as mean and standard deviation (\pm SD, n = 6). Statistical differences between the treatments and the negative control (reserpine induced group) were evaluated by ANOVA (Bonferroni test for multiple comparisons). The p<0.05 was considered to be significant (*p<0.05, **p<0.01, ***p<0.001).

RESULTS

HPLC analysis: The presence of quercetin and rutin in EEHh and GFHh extracts was confirmed by HPLC analysis. Chromatograms of quercetin and rutin, EEHh and GFHh are depicted in Fig. 1a-c.

Acute toxicity study: The EEHh extract did not exhibit any sign of toxicity of mortality up to the dose level of 2000 mg kg⁻¹ b.wt., in mice and hence the extracts were considered as safe for further behavioral and neuropharmacological analysis.

Effects of EEHh and GFHh on FST: The climbing and swimming activity of mice was recorded in FST. 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

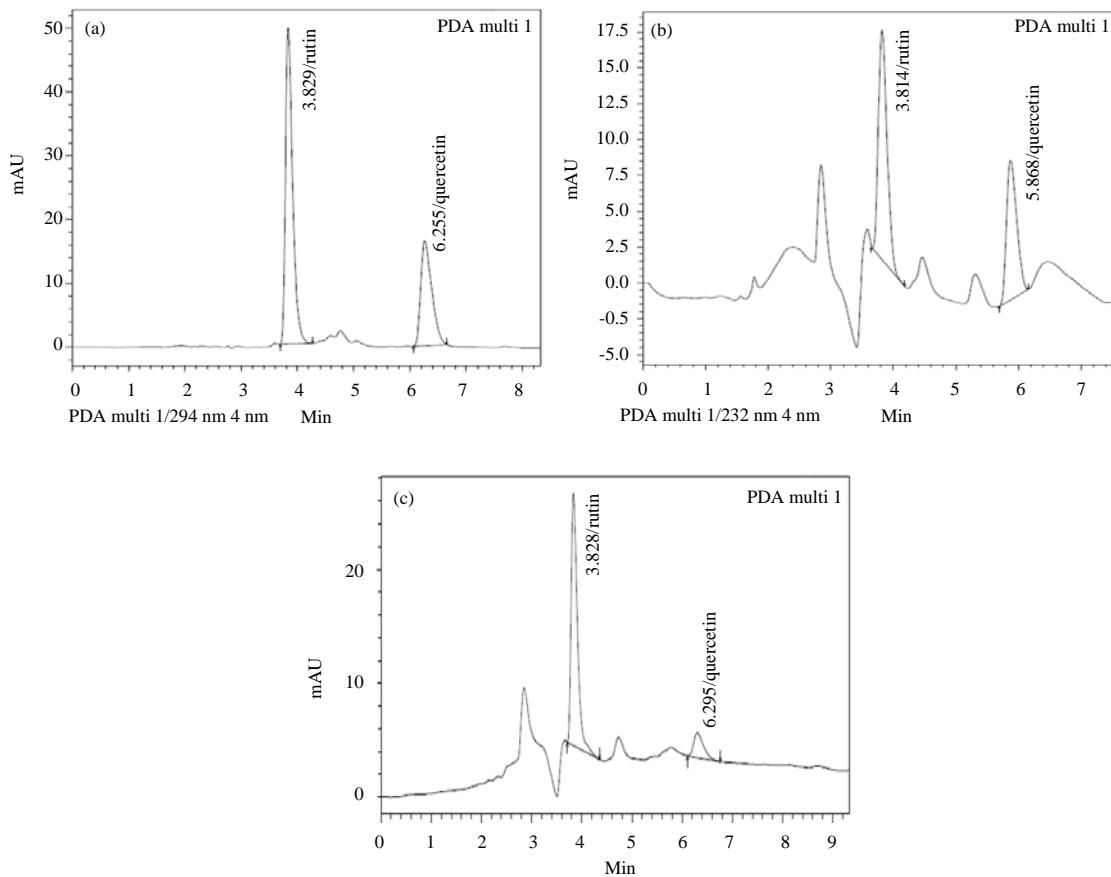


Fig. 1(a-c): Chromatogram-HPLC analysis of (a) Quercetin and rutin standards, (b) EEHh and (c) GFHh

increased immobility period when compared with the normal control group. The other groups (i.e., reserpine induced) that were treated with EEHh (200 and 400 mg kg^{-1}) and GFHh (100 mg kg^{-1}) showed increased climbing and decreased immobility period when compared with reserpine induced group ($p<0.001$). Similar results were also observed on the 12th day. Upon comparison with all other extracts, GFHh showed more climbing and swimming effects and the values are comparatively similar to standard imipiramine (Fig. 2).

Effects of EEHh and GFHh on TST: In TST, all the groups showed normal mobility period during training sessions. But on the 6th day, reserpine induced group showed an increased immobility period ($p<0.001$) when compared with normal control group. The other groups (i.e., reserpine induced) treated with EEHh (200 and 400 mg kg^{-1}), GFHh (100 mg kg^{-1}) and imipiramine (10 mg kg^{-1}) showed a decreased immobility period when compared with reserpine induced group ($p<0.001$). Similar results were also observed on the 12th day (Fig. 3). When comparing the all other extracts, GFHh showed less immobility period and values are comparatively similar to standard imipiramine.

Effects of EEHh and GFHh on LMA: Locomotor activity (LMA) of photocell beam was counted by digital Actophotometer. Reserpine induced group showed less number of photo cell beam counts and total activity, when compared to normal control group ($p<0.001$). The other groups (i.e., reserpine induced) treated with EEHh (200 and 400 mg kg^{-1}), GFHh (100 mg kg^{-1}) and imipiramine (10 mg kg^{-1}) showed increased photocell beam counts ($p<0.001$) and total activity ($p<0.001$) when compared with reserpine induced group (Fig. 4). The GFHh treated groups showed high cell beam counts are comparatively similar to standard drug imipiramine.

Effects of EEHh and GFHh on superoxide dismutase activity: There was a significant reduction in the SOD level of reserpine induced animals when compared with normal control group ($p<0.001$). Treatment with EEHh (200 and 400 mg kg^{-1}) increased the SOD levels significantly

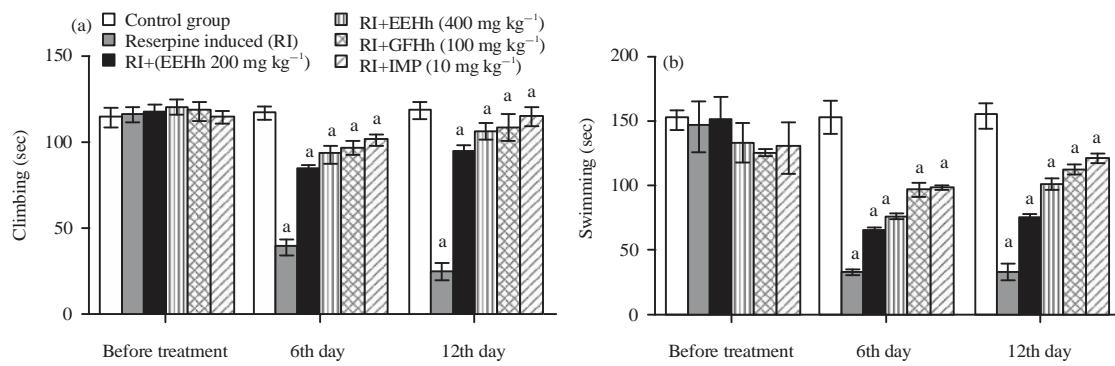


Fig. 2(a-b): Forced swimming test (a) Climbing and (b) Swimming. Effects of EEHh (200 and 400 mg kg^{-1}) and GFHh (100 mg kg^{-1}) or imipiramine (10 mg kg^{-1}) on FST with climbing and swimming behavior. Values were given as Mean \pm SD, p-values, $^a p<0.001$, when compared with control and reserpine induced groups (I and II), reserpine induced and other treated groups (II and III, IV, V, VI)

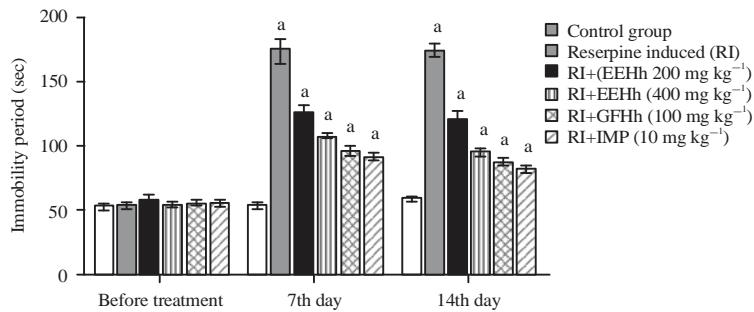


Fig. 3: Tail suspension test, effects of EEHh (200 and 400 mg kg^{-1}) and GFHh (100 mg kg^{-1}) or imipiramine (10 mg kg^{-1}) on TST with immobility period. Values were given as Mean \pm SD, p-values, ^a $p<0.001$, when compared with control and reserpine induced groups (I and II), reserpine induced and other treated groups (II and III, IV, V, VI)

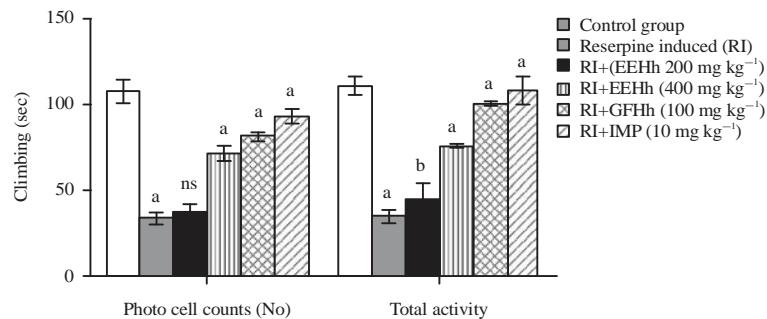


Fig. 4: Locomotor activity, effects of EEHh (200 and 400 mg kg^{-1}) and GFHh (100 mg kg^{-1}) or imipiramine (10 mg kg^{-1}) on LMA with photo cell beam counts. Values were given as Mean \pm SD. p values, ^a $p<0.001$, ^b $p<0.01$ when compared with control and reserpine induced groups (I and II), reserpine induced and other treated groups (II and III, IV, V, VI)

when compared to reserpine induced group ($p>0.05$ and $p<0.001$). The GFHh (100 mg kg^{-1}) and imipiramine (10 mg kg^{-1}) treated animal groups significantly restored the SOD levels of the brain ($p<0.001$). The GFHh (100 mg kg^{-1}) showed higher values of SOD activity when compared with all other extracts and the values are comparatively similar to standard drug imipiramine (Fig. 5a).

Effects of EEHh and GFHh on catalase activity: A significant reduction in the level of catalase activity was observed in reserpine induced animals ($p<0.001$) when compared to control group. Treated groups showed significantly increased catalase value when compared to reserpine induced group ($p<0.001$) except EEHh (200 mg kg^{-1}) treated group ($p>0.05$, non-significant), whereas, GFHh (100 mg kg^{-1}) showed higher catalase activity when compared with the other extracts (Fig. 5b).

Effects of EEHh and GFHh on reduced glutathione (GSH) activity: The GSH level was found to be significant fall in the reserpine induced animals when compared to normal control animals ($p<0.001$). There was no significant increase in GSH in EEHh (200 mg kg^{-1}) treated groups

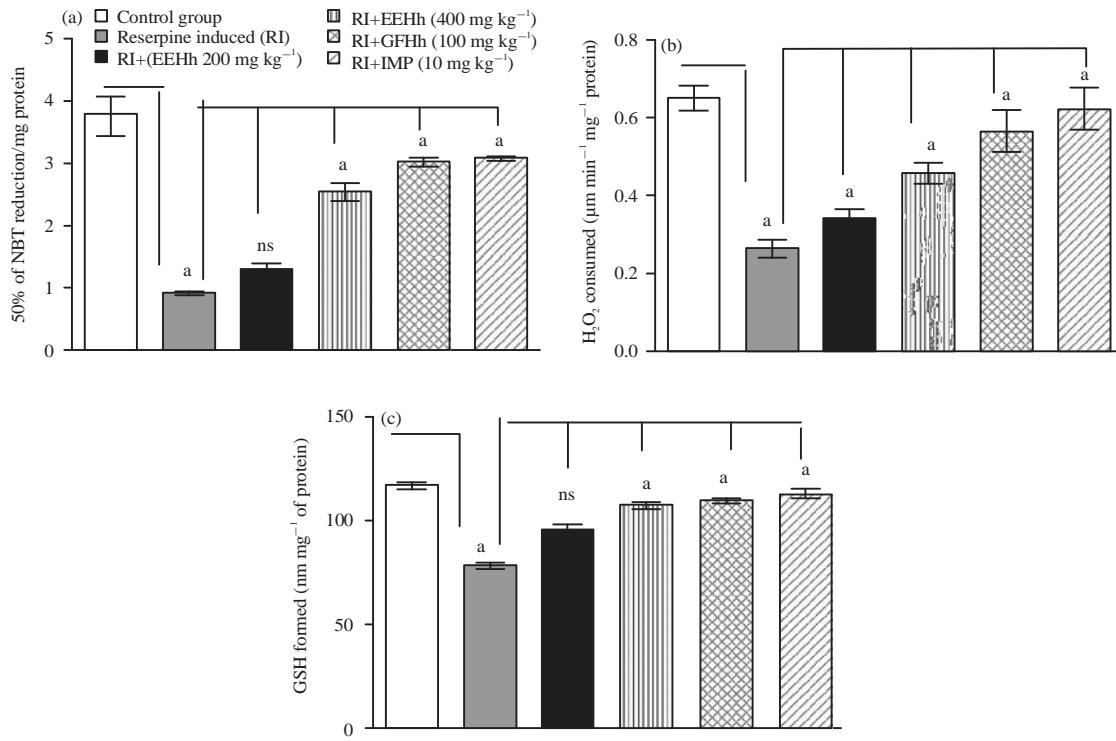


Fig. 5(a-c): Brain antioxidants, (a) Superoxide dismutase, (b) Catalase and (c) Reduced glutathione. Effects of EEHh (200 and 400 mg kg⁻¹) and GFHh (100 mg kg⁻¹) or imipiramine (10 mg kg⁻¹) on SOD, CAT, GSH activity in mouse brain respectively. Values were given as Mean±SD, p-values, ^ap<0.001 and ns: Non significant when compared with control and reserpine induced groups (I and II), reserpine induced and other treated groups (II and III, IV, V, VI)

Table 1: MAO-A and MAO-B activity

Treatments	Activity of MAO-A (%)	Activity of MAO-B (%)
Control	0.00	0.00
Reserpine induced (2 mg kg ⁻¹)	58.62	57.75
RI+EEHh (200 mg kg ⁻¹)	47.80	44.00
RI+EEHh (400 mg kg ⁻¹)	34.78	33.00
RI+GFHh (100 mg kg ⁻¹)	29.41	14.60
RI+IMP (10 mg kg ⁻¹)	7.69	12.06

RI: Reserpine induced

when compared with reserpine induced group. But animals treated with EEHh (400 mg kg⁻¹) and GFHh (100 mg kg⁻¹) significantly increased glutathione values were obtained (Fig. 5c).

Effects of EEHh and GFHh on activity of MAO-A and MAO-B: Table 1 describes the MAO-A and MAO-B activity of mice brain in all the groups. An increased activity of MAO-A and MAO-B were observed in the reserpine induced group when compared with control group (p<0.001). Treated groups (EEHh 200 and 400 mg kg⁻¹, GFHh 100 mg kg⁻¹, imipiramine 10 mg kg⁻¹) showed a decreased activity of MAO-A and MAO-B which is significant when compared with reserpine induced group and also showed significant percentage of inhibition (p<0.001).

DISCUSSION

The present study investigated the possible antidepressant like activity of EEHh and GFHh on reserpine induced animal by behavioral (FST, TST and LMA) and neurobiochemical (SOD, CAT, GSH, MAO-A and MAO-B) analysis and the presence of flavonoids was confirmed (quercetin and rutin) by HPLC.

Reserpine is a sympathetic drug that leads to hypothermia (decreases in body temperature) which depletes catecholamine in peripheral nervous tissues and in the 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 (Yu *et al.*, 2011). These irreversible changes can be antagonized by major classes of antidepressants. Different types of antidepressant drugs, such as tricyclic antidepressants and Selective Serotonin-Reuptake Inhibitors (SSRIs), as well as antidepressant herbal medicines like St. John's wort, are used to treat depression. However, most of the synthetic drugs exhibit side effects (Chambers *et al.*, 2006). In addition, disturbances of the drug-metabolizing enzyme systems were discovered with St. John's wort (Bolandghamat *et al.*, 2011) and thus, the search for novel antidepressants with fewer side effects is the prime aim of this study.

According to Xu *et al.* (2008), behavioral study plays a vital role in the evaluation of antidepressant drugs in rodents. In a sequence of publications, Porsolt *et al.* (1977b, 1978, 2001) and Cryan *et al.* (2002) have reported a new behavioral test in rodents, which was developed as a primary screening procedure to identify the effectiveness of antidepressants. The efficacy of FST with mice was reviewed by Petit-Demouliere *et al.* (2005) and also suggested that FST has good reliability and analytical validity. These tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including MAO inhibitors (Porsolt *et al.*, 1977a; Steru *et al.*, 1985). In the present study, oral administration of plant extracts (EEHh and GFHh) showed antidepressant like effect in FST i.e., increased swimming and climbing behavior, interestingly GFHh was more effective than EEHh.

The TST is commonly used to identify and characterize the effectiveness of antidepressant drugs (Cryan *et al.*, 2005). In spite of the high use of this test to evaluate the antidepressant activity of new drugs, it is also a main tool to study the neurobiological mechanisms involved in antidepressant responses (Bourin *et al.*, 2005). The TST also induces a state of despair in animals similar to that of FST. This immobility, referred to as behavioral effect, is claimed to reproduce a condition similar to human depression (Steru *et al.*, 1985). In the present study, GFHh extract exhibited less immobility period when compared with EEHh.

Among reserpine-induced animals, decreased locomotor activity was (number of photocell beam crossed) observed in actophotometer when compared to control group. The results of the present study are similar to Yan *et al.* (2004). The hypo motility of reserpine may be due to its monoamine theory of depression that leads to depleted monoamine in brain, as a consequence, hypothermia and hypo motility were observed (Yu *et al.*, 2011). In treatment groups of EEHh and GFHh, GFHh showed more cell beam counts, which are attributed to more flavonoid content.

The cellular defensive role against ROS and free radicals includes two important systems: the enzymatic, which includes chiefly CAT, SOD and GPx enzymes and the antioxidant system. Undoubtedly, GSH is a tri peptide which provides a vital line of antioxidant defense (Mayo *et al.*, 2002). In the current study, a significant decrease in the concentration of SOD and CAT levels was observed in reserpine-induced group. But the EEHh and GFHh treated groups significantly

restored the activity of antioxidant enzyme levels (SOD, CAT) induced by reserpine. Decreased activity of SOD leads to the less removal of superoxide ion, which can be injurious to the organs. Moreover, the enhanced SOD activity in EEHh and GFHh groups might be involved in the scavenging of reactive oxygen species generated from reserpine. There is a general agreement that quercetin and rutin act as the scavengers of reactive oxygen species (Haenen and Bast, 1999). Reserpine induction leads to depletion of monoamines which also weakened the antioxidant defense as 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 cognitive dysfunction. Consequently, quercetin is considered as a potential therapeutic agent for the treatment of reserpine induced disorders (Naidu, 2004). The GFHh showed significant restored activities of all the contents when compared with EEHh owing to their higher concentration of flavonoids.

MAO (A and B) inhibition is a pharmacological approach to improve the synaptic effects of serotonin and nor-epinephrine in depressive disorders. It also promotes the effects of dopamine since MAO-A and MAO-B inhibition (Dunlop and Nemeroff, 2007) also metabolized this neurotransmitter. The effectiveness of MAO inhibitors in the treatment of major depression is not inferior to other groups of drugs such as tricyclic antidepressants and selective serotonin reuptake inhibitors but superior in the treatment of atypical depression (McGrath *et al.*, 1987).

In the current study, EEHh and GFHh extracts reduced the activity of MAO-A and MAO-B, in addition GFHh showed high mode of inhibitory activity against both the enzymes. Similar to classical antidepressants, many herbal antidepressant agents also inhibit MAO activity and revised monoaminergic neurotransmission (Zhang *et al.*, 2004). Previous studies have reported that quercetin and other flavonoids have shown MAO-A inhibitory capabilities (Chimenti *et al.*, 2006; Saaby *et al.*, 2009). MAO-A preferentially deaminates serotonin and nor-epinephrine. Thus, inhibition of MAO-A may lighten the symptoms of depression (Yamada and Yasuhara, 2004).

Dimpfel (2009) has also reported that the quercetin, rutin-glycosidic flavonoids influenced the electropharmacogram of adult rats in the same manner as moclobemide, a reversible inhibitor of MAO-A and also quercetin showed a certain similarity of electrical effects with classical antidepressant imipramine. In this study, antidepressant like effects of EEHh and GFHh owing to flavonoids which inhibited uptake of monoamines or MAO-A and MAO-B activity in the mice brain. Hence, *H. hookerianum* extracts may have potential therapeutic agents for the management of reserpine induced depressive disorders.

CONCLUSION

These findings confirmed the antidepressant potential of *H. hookerianum* in reserpine induced Swiss albino mice and this study is the first report on *H. hookerianum* antidepressant activity of its glycosidic flavonoids for reserpine induced models. Upon comparison with all the other extracts GFHh showed greater antidepressant like effect and the values are comparatively similar to that of standard drug imipramine. Further investigations on the isolation and identification of flavonoid compounds in the plant extract may lead to chemical entities for clinical use.

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