

Abstract
Depression is one of the most common psychiatric disorders with high mortality, morbidity and economic burden worldwide. Herbal medicines are an important part of the culture and traditions and are widely accepted due to their low incidence of adverse effects. Hippophae salicifolia D. Don (HS) is widely used in northern Himalayan region as food, fuel, medicine, veterinary care, cosmetics, agricultural tools and bio-fencing. Berries of this plant have rich micronutrients with potent antioxidant potential. The present study is designed to study potential use of berries in mental depression. HS extract 250 and 500mg/kg and imipramine 25 mg/kg was administered to rats for 7 days. HS extract decreased the immobility time in Tail Suspension Test and Forced Swim Test on par with the standard drug Imipramine, without any significant change in spontaneous motor activity. HS increased the brain dopamine (DA), serotonin (5-HT) and norepinephrine (NE) levels and inhibited the activity of monamine oxidase (MAO-A) concomitantly, it also increased the brain glutathione levels (GSH) and decreased brain lipid peroxides (MDA). The results of the present study indicate that HS shows antidepressant activity in part by increasing the synthesis of NE and by inhibiting the metabolism of NE and 5HT and also by inhibiting lipid peroxidation due to potent antioxidant potential.

Key words: Antidepressant activity, Hippophae salicifolia berries, monoamines, antioxidant activity

Introduction
Depression is one of the most common psychiatric disorders with high mortality, morbidity and economic burden worldwide (1, 2). Stressful events are the precipitating factors for the onset of depression (3). Dysfunction in the neurotransmitter levels result in the systemic effect with hyper activation of hypothalamic pituitary adrenal axis (HPA) besides psychological and behavioral consequences which result in hypercortisolemia causing a wide array of organ and immune changes (4). Depression is usually treated with the antidepressant drugs, which cascade serious side effects. So, globally there is greater interest in herbal remedies.

Herbal medicines are an important part of the culture and tradition. Today, most of the population is reliant on herbal medicines for their health care needs. Apart from their cultural significance, this is because herbal medicines are more accessible and affordable (5). Traditional knowledge helps scientists to target plants that may be medicinally useful (6).

In ancient Greece, leaves of sea buckthorn were used as horse fodder for...
improving weight and shiny hair, thus gaining the sea buckthorn genus a Graeco-latin name ‘Hippophae’ (Hippo-Horse, Phaos-to shine). It belongs to family Elaeagnaceae. *Hippophae salicifolia* (HS) plant is traditionally utilized by local people of Central Himalaya in multidimensional aspects as food, fuel, medicine, veterinary care, cosmetics, agricultural tools and bio-fencing. HS berries have high content of natural, potent antioxidants including: Ascorbic Acid (Vitamin C), Tocopherols (Vitamin E), Carotenoids, Flavonoids-isorhamnetin, quercetin, ω-3, ω-6 fatty acids, Kaempferol, Catechins, Proanthocyanidins and Chlorogenic Acids (7) and rich in mineral elements such as nitrogen, phosphorus, iron, manganese, boron, calcium, aluminium, silicon and others. Potassium plays an important role in the ionic balance and helps in maintaining the tissue excitability of the human body (8).

*Hippophae salicifolia* has attracted a great deal of attention from scientists and engineers all over world due to its concentrated ecological and socio-economical benefits. The present study aims at evaluating whether the edible fruit HS can also serve as an antidepressant.

**Material and Methods**

*Hippophae salicifolia* berries extract was commercially procured from the Chansha Organic Herb Inc., China. It was subjected to various chemical tests in order to detect the presence of different phytoconstituents (9).

**Animals:** Adult healthy Swiss Albino mice of either sex (20-30g) were used. The animals were acclimatized for the laboratory conditions for a period of ten days i.e. room temperature (27±3°C), relative humidity (65±10%), and 12h light/dark cycle. All animals were fed with rodent-pellet diet and water was allowed *ad libitum* under strict hygienic conditions. The experiment was conducted after prior approval from Institutional Animal Ethical Committee (No.1677/PO/a/12/CPCSEA/21).

**Acute toxicity study:** The acute toxicity study was conducted as per the OECD guidelines 423. Observations were made and recorded systemically 1, 2, 4 and 24 h after dose administration for skin changes, morbidity, aggressiveness, sensitivity of the sound and pain, as well as respiratory movements.

**Experimental protocol for antidepressant activity:** Mice were randomly divided into 4 groups with six animals in each group. Group I received only vehicle (distilled water, daily) and served as control; Group II received standard antidepressant drug- Imipramine (25mg/kg p.o, daily); Group III received HS lower dose, 250mg/kg/p.o, daily. Group IV received HS higher dose, 500mg/kg/p.o daily. All the animals were received their respective treatment orally by gavage once daily for 7 days. At the end of experimental period (7 days of treatment) the animals were sacrificed by cervical dislocation to obtain brain samples for further studies.

**Test for locomotor activity:** The locomotor activity was measured using Actophotometer. It consists of cage which has 30 X 30 X 30 cm, and at the bottom six lights and photocells were placed in the outer periphery of the bottom in such a way that a single mouse blocks only one beam. Photocell is activated when the rays of light falls on photocells, the beam of light is interrupted as and when animal crosses the light beam, the number of interruptions were recorded for a period of 5 minutes (10).

**Tail suspension test:** The total duration of immobility by tail suspension was measured according to the method of Steru et al., (11). Mice both acoustically and visually isolated and suspended 50cm above the floor by adhesive tape placed approximately 1cm from the tip of the tail, immobility time was recorded during a 15 minutes test for animals of all groups.

**Forced swim test:** FST is the most widely used pharmacological *in-vivo* model for assessment of antidepressant activity. In this model, mice were forced to swim in condition from which they cannot escape and rapidly become immobile,
floating in an upright position and making only small movements to keep their heads above water. The development of immobility reflects the cessation of persistent escape directed behavior or learned helplessness, and a decrease in the duration of immobility, is interpreted as an antidepressant like effect. Mice were placed individually in a glass cylinders (height: 21 cm, diameter: 14.5 cm) containing 15 cm of water at 23 ± 1°C. First 2 min were allowed for acclimatization and the duration of immobility during 4 min was recorded (12).

**Estimation of neurotransmitters:** Mice were sacrificed after the treatment period (7 days) by decapitation and brains were rapidly removed. The brains were placed in 800µl of ice-cold 0.1M perchloric acid. Individual brain samples were homogenized and centrifuged at 20000 x g at 4°C and stored in a dark freezer at -70°C until further analysis. The pellets were dissolved in 10mM NaOH solution for protein determination using the Bradford protein assay (13). The homogenates were used for the estimation of monoamines like Noradrenaline (NA), Dopamine (DA) and Serotonin (5-HT) according to the method of Alburges et al., (14).

**Estimation of Monoamine oxidase A and B:**

Brain tissue was homogenized in ten volumes of cold sodium phosphate buffer (200mM, pHi-7.4) containing 320 mM sucrose, at 4°C for 30 seconds, using a Teflon glass homogenizer. The homogenate was centrifuged at 600g for 10min at 4°C to remove nuclei and further used for the estimations of MAO-A and MAO-B according to the method of Zheng and Liu (15) and Zhou et al. (16).

**Antioxidant studies:** Brain tissue samples were homogenized in 50 mM phosphate buffer (pH-7.0) containing 0.1 mM of EDTA to give 5% w/v homogenate. The homogenates were centrifuged at 10000 rpm for 10 min at 0°C in cold centrifuge, filtered and the resulting supernatant was used for further studies.

**Lipid peroxidation estimation:** MDA level was measured according to the method of Ohkawa et al., (17) at room temperature. 200 µl of supernatant was added to 50 µl of 8.1% sodium dodecylsulphate, vortexed and incubated for ten min at room temperature. 375 µl of thiobarbituric acid (0.6%) was added and placed in a boiling water bath for 60 min and then the sample was allowed to cool to room temperature. A mixture of 1.25ml of butanol:pyridine (1.5:1) was added, vortexed and centrifuged at 1000rpm for 5min. the colored layer (500µl) was measured at 532nm on a Spectrophotometer. The values were expressed in nmoles of MDA formed for mg protein/hr/min.

**Reduced glutathione assay:** Reduced glutathione was measured according to the method of Ellman, (18) at room temperature. 0.75 ml of supernatant was mixed with 0.75 ml of 4% sulfoalicylic acid then centrifuged at 1200 rpm for 5min at 4°C, from this 0.5 ml of supernatant was taken and added to 4.5 ml of 0.01 M DTNB and absorbance was measured at 412 nm using a UV-Visible Spectrophotometer (18).

**Statistical analysis:** All the data was expressed as mean ± SEM. Differences in mean values between groups were analyzed by one – way analysis of variance (ANOVA) followed by Dunnett's test.

**Results and Discussion**

The preliminary phytochemical analysis reveals that the hydroalcoholic extract of HS showed positive results towards tannins, phenolic compounds, Flavonoids and sugars. The present study is the first, to our knowledge, to show antidepressant-like activity of HS, as determined by the forced swimming test (FST), tail suspension test (TST). Tail suspension test and forced swim test are the widely used animal models of depression for the screening of antidepressant activity (11,12). The forced swimming and tail suspension-induced state of immobility in animals claimed to represent a condition similar to human depression and

**Antioxidant activity of Hippophae salicifolia berries**
Amenable to reversal by antidepressant drugs. It has been demonstrated that antidepressant drugs reduce this behaviour of abandonment in mice (19). In addition, several extracts from plants have been evaluated in this model with positive results (20-22). Animals are placed in an inescapable situation and the antidepressant-like activity is expressed by the decrease of immobility when compared with control groups.

In the present study we provided convincing evidence that the HS extracts administered by oral route produced specific antidepressant-like effects in TST and FST after 7 days treatment. The results presented here showed that extract at 250 and 500 mg/kg lead to a significant reduction in the immobility period after 7 days treatment in TST in a dose-dependent manner (Table 1, Table 2). In the present study we used mouse as animal and utilized FST and TST for the evaluation of antidepressant activity of HS. In our study, HS showed no change in locomotor activity at doses that produced antidepressant-like effect, indicating that the specific actions of this extract on the behavioral model are predictive of antidepressant activity (Table 3).

It is well accepted that increasing brain monoamine neurotransmitters is an effective way to treat depression (23, 24). The dysregulation of the neurotransmitters noradrenaline, serotonin and dopamine has been suggested to play a role in the pathogenesis of depression (25, 26). Generally, the most widely accepted hypothesis of the biological basis of depression implicate serotonin and noradrenergic system dysfunction.

In the present study, to probe the mechanism of action of HS, the effect of HS extract was studied on the brain NE, DA, serotonin levels (Fig 1A, 1B, 1C) and on the activity of MAO-A and B and on the indicators of oxidative stress (GSH, MDA). HS at higher dose

![Graphs showing effect of HS on dopamine, serotonin, and norepinephrine levels.](image-url)

**Fig. 1.** Effect of HS on (A) dopamine levels (B) Serotonin levels (C) Norepinephrine levels. Values are expressed as Mean ± SEM [n=6]; *(P<0.05), ***(P<0.01), ***(P<0.001) vs control group

Santh Rani et al
of 500 mg/kg significantly increased the NE, serotonin and DA. HS significantly increased the level of DA and NE with a significant decrease in the activity of MAO-A indicating increased NE is due to increased synthesis as DA levels are increased simultaneously with decreased metabolism as MAO-A activity is decreased. In addition there was no inhibition of MAO-B activity, indicating raise in DA is not due to inhibition of its metabolism (Fig 2A, 2B).

The dopaminergic system is also an important target implicated in the regulation of depression (27). DA involved in brain functions of behaviour, memory etc (28). A number of studies consistently reported a low DA and/or DA metabolite levels in patients with depressive illness (29, 30). In addition, it was demonstrated that chronic treatments with antidepressants such as amineptine improved the dopaminergic neurotransmission, which contribute to therapeutic effect of these drugs (31). HS increased the DA levels on par with standard drug imipramine, indicating the involvement of dopaminergic system in part in the antidepressant activity of these extracts on one hand on the other may be these increased DA levels responsible for the increase in NE levels as DA is precursor for the NE synthesis.

Recently, oxidative stress is closely correlated with depression. Increased lipid peroxidation (32, 33) and decreased antioxidant enzyme levels is reported in depressed patients (34) and preclinical studies have suggested that antioxidants have antidepressant properties (35). The reactive oxygen species (ROS) like hydroxyl radicals, superoxide anion, hydrogen peroxide and nitric oxide, produced during normal cellular metabolic functions, produce oxidative damage in brain (36, 37). The brain is more vulnerable to oxidative stress because of its elevated consumption of oxygen and the consequent generation of large amounts of ROS.

Lipid peroxidation (LPO), an index of oxidative stress was significantly increased in depressed patients (38). HS significantly decreased lipid peroxidation in a dose-dependent manner as compared to control (Fig 3A). Reduced glutathione levels was significantly decreased in depressed patients and was significantly increased in HS treated groups (Fig 3B).

Fig 2. Effect of HS on (A) MAO-A levels (B) MAO-B levels. Values are expressed as Mean ± SEM [n=6]; *P<0.05 vs control group

Fig 3. Effect of HS on (A) MDA levels (B) reduced glutathione levels. Values are expressed as Mean ± SEM [n=6];*** (P<0.001) vs Control group

Antidepressant activity of *Hippophae salicifolia* berries
oxidative stress, damages the cell membrane (membrane fluidity, receptors, and ion channels) (38), which may result in calcium influx and cause cell death. In the depressed animal models (FST) as well, decrease in antioxidants and increase in lipid peroxidation was observed. In the present study also, depressed rats showed increased lipid peroxidation (Fig. 3A) and decreased reduced glutathione (GSH) (Fig 3B). The increased level of LPO observed in depressed (control) rats, indicates an excessive formation of free radicals and activation of LPO system.

Interestingly, our results evidenced a parallel increase in GSH, the most important antioxidant in response to treatment with extracts in depressed animals. The increase in activity

Santh Rani  et al

### Table 1. Effect of HS on Immobility time in TST.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th Day</th>
<th>1st Day</th>
<th>3rd Day</th>
<th>7th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>453.4±9.9</td>
<td>486.2±4.6</td>
<td>512.8±8.1</td>
<td>625.2±6.7</td>
</tr>
<tr>
<td>Imipramine</td>
<td>444.7±7.2</td>
<td>373.9±8.7”</td>
<td>223.5±3.8”</td>
<td>87.4±5.7”</td>
</tr>
<tr>
<td>HS lower</td>
<td>458.1±6.3</td>
<td>410.5±7.3”</td>
<td>362.2±5.9”</td>
<td>274.5±4.2”</td>
</tr>
<tr>
<td>HS higher</td>
<td>461.3±8.5</td>
<td>336.0±6.7”</td>
<td>250.2±5.4”</td>
<td>123.6±4.9”</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM [n=6]  ***P<0.001 vs control group

### Table 2. Effect of HS on Immobility time in FST.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th Day</th>
<th>1st Day</th>
<th>3rd Day</th>
<th>7th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.8±7.3</td>
<td>59.2±5.2</td>
<td>82.6±7.2</td>
<td>101.4±5.8</td>
</tr>
<tr>
<td>Imipramine</td>
<td>61.7±5.9</td>
<td>48.4±7.1”</td>
<td>39.5±4.7”</td>
<td>25.6±6.1”</td>
</tr>
<tr>
<td>HS lower</td>
<td>63.2±8.5</td>
<td>57.2±6.9”</td>
<td>50.5±6.9”</td>
<td>37.8±5.5”</td>
</tr>
<tr>
<td>HS higher</td>
<td>60.5±7.4</td>
<td>50.9±4.8”</td>
<td>43.1±8.4”</td>
<td>29.8±7.2”</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM [n=6]  ***P<0.001 vs control group

### Table 3. Effect of HS on Spontaneous Locomotor activity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th Day</th>
<th>1st Day</th>
<th>3rd Day</th>
<th>7th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>129.2±4.2</td>
<td>117.6±9.0</td>
<td>99.2±6.6</td>
<td>78.7±7.3</td>
</tr>
<tr>
<td>Imipramine</td>
<td>134.3±3.9</td>
<td>119.9±3.2</td>
<td>126.7±6.9</td>
<td>132.4±3.6</td>
</tr>
<tr>
<td>HS lower</td>
<td>121.9±5.6</td>
<td>120.2±3.8</td>
<td>122.3±11.4</td>
<td>124.8±7.9</td>
</tr>
<tr>
<td>HS higher</td>
<td>126.2±5.4</td>
<td>124.3±3.2</td>
<td>123.4±2.4</td>
<td>128.2±4.5</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM [n=6]
may provide an effective defense from the damaging effects of not only superoxide anion and hydrogen peroxide but also from damaging and highly reactive hydroxyl radical generated by Fentons reaction (39).

Conclusion

From our results, it can be concluded that HS showed antidepressant activity. The antidepressant like activity of *H. salicifolia* extract might be in part due to increasing the levels of noradrenaline and serotonin by increasing their synthesis and by inhibition of their metabolism by MAO-A and also due to its potent antioxidant potential.

References


