

EVALUATION OF ANTIDEPRESSANT-LIKE EFFECTS OF AQUEOUS EXTRACT OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L. SSP. *TURKESTANICA*) FRUITS IN EXPERIMENTAL MODELS OF DEPRESSION

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Abstract

Herbs have been used as food and for medicinal purposes for centuries. Research interest has focused on various herbs that possess antidepressant properties and may be useful adjuncts in helping the management of depression in humans. The present study was therefore designed to investigate the antidepressant-like effects of aqueous fruit extract of Sea buckthorn (*Hippophae rhamnoides* L. ssp. *turkestanica*) in animal models of depression. In first phase of study test rats were treated with long term oral administration of Sea buckthorn (40 mg/kg P.O.) and controls received an equal volume of fresh water for two weeks. In the second and last phase of study, two groups of animals were exposed to repeated restraint stress (one group from water treated and other group from SBT-FE treated) for next one week. All groups of animals were separately submitted to forced swim test (FST), open field test (OFT) and elevated plus maze (EPM) tests for the bio-screening of fruit extract with antidepressant profile. Results revealed that the immobility time in the FST was significantly ($p < 0.05$) reduced and prolonged struggling (numbers of jumps) was observed particularly in rats orally administered with SBT-FE (40 mg/kg P.O.) following one week stress when compared with their respective controls. Open field ambulation was also significantly ($p < 0.05$) increased in a similar manner. Number of entries in open arms (OA) and % time spent in OA were also significantly increased ($p < 0.01$) and were more pronounced in SBT-FE treated rats following exposures to repeated restraint stress when compared with their controls. Thus, it is suggested that aqueous fruit extract of Sea buckthorn exhibited significant antidepressant-like effects in animal models of depression and may be served as a potential resource for natural psychotherapeutic agent, against depression.

Introduction

Sea buckthorn (*Hippophae rhamnoides* L. ssp. *turkestanica*) known as “Holy fruit of Himalayas” has been cherished by native Tibetans for centuries as a traditional medicine for its incredible nutritive qualities. Today, several studies on Sea buckthorn fruit and countless studies on the biological properties found in the plant have shown that its small berry promote good health and improve nervous system health. Sea buckthorn is native to Eurasia, and is mainly known in North America as an attractive ornamental shrub (Beveridge *et al.*, 2002; Titinen *et al.*, 2005). It has silvery deciduous leaves and colorful orange berries that persist most of the winter (Li & Schroeder, 1996; Zeb *et al.*, 2004). Sea Buckthorn has been used medicinally in China for at least twelve centuries, and Sea Buckthorn oil (from the pulp and seeds) is currently used clinically in hospitals in Russia and China. Fruit berries are rich in a variety of antioxidant chemicals (vitamins C and E; several carotenoids, including beta-carotene (pro-vitamin A); flavonoids; certain enzymes, and other substances) (Shah *et al.*, 2007; Batool *et al.*, 2009a; Shah *et al.*, 2010). China designated its Sea Buckthorn sports drinks “Shawikang” and “Jianibao” as the official beverages for its athletes at the Seoul Olympic games in 1988, and Russian cosmonauts were also supplied with Sea Buckthorn beverages, to enhance their health and resistance to stress (Yang & Kallio, 2002; Geetha *et al.*, 2003).

Depression is an important global public-health issue and is associated with substantial disability (Goodwin & Bunney, 1971; Gold *et al.*, 1988; Murray & Lopez, 1997). It is a chronic illness that affects mood, thoughts, physical health and behavior of any individual and has been estimated to affect upto 21% of the world's population (Paykel, 2006). Synthetic antidepressants taken in appropriate doses are often associated with their anticipated side effects like dry mouth, inability in

driving skills, constipation and sexual dysfunction (Sarko, 2000) and majority of patients are reluctant to take this treatment. Accordingly, natural medicinal plants may be important sources of novel antidepressant drugs and the usage of plant extracts may be proven better in the management of stress and depression. In oriental countries, many medicinal plants from natural resources, especially Chinese medicine, such as *Plantago asiatica*, *Scrophularia ningpoensis*, and *Hypericum perforatum* were successfully used to treat or prevent depression-like disorders (Schulz, 2006; Sanchez-Mateo *et al.*, 2007). In our previous studies, we have reported that the aqueous fruit extract of Sea buckthorn showed significant anxiolytic and antipsychotic activities (Batool *et al.*, 2009a; Batool *et al.*, 2009b; Batool *et al.*, 2010). On the basis of these findings, the present study was therefore, undertaken in an effort to develop a novel medicinal material with potential antidepressant profile. In continuation of our research on the plant, the aim of this investigation was to evaluate the probable mechanisms of antidepressant-like activity of Sea buckthorn (*Hippophae rhamnoides*) in behavioral models of depression in laboratory rats. The study would possibly help to establish that aqueous fruit extract of the Sea buckthorn plant which may have potential therapeutic value for the management of depressive disorders.

Materials and Methods

i. Plant material and the preparation of fruit extract: The mature and fresh fruits of the *Hippophae rhamnoides* L. were collected from District Rawalakot Azad Kashmir Pakistan in the month of October 2009 where the plant grows wildly under natural conditions and later on they were identified by a Taxonomist at the University of Azad Jammu and Kashmir AK Pakistan.

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The whole fruit (50 g) was cleaned and pounded to pieces with a squeezer. The extract was filtered and the filtrate was stored at -20 °C in a refrigerator. The fruit pulp was extracted manually by extracting seeds from 50 berries of Sea buckthorn. The calculation was done after weighing 100 berries and 100 sample seeds. The crude extract was then diluted in sterile double-distilled water to make aqueous fruit extract of Sea buckthorn (SBT-FE) at a dose of 40 mg/kg for the investigation. The fruit extract was administered orally via mouth (*Par os*; P.O.) to test animals while control animals received an equal amount of fresh tap water under controlled conditions. The extract was kept in dark at 4°C until further analyses.

ii. Animals and treatment schedule: Twelve male albino Wistar rats with an average weight of 200±10 g on arrival were purchased from International Centre for Chemical and Biological Sciences (ICCBS); Research Institute of Chemistry, University of Karachi, Pakistan. Animals were group-housed (two rats per cage) in an environmentally controlled room temperature (21±1 °C) and relative humidity (55±5 %) with a 12:12 h light/dark cycle (light on at 7:00 A.M.) A 5-day familiarization period was allowed before animals were used in the experimental procedures. All experiments were conducted in a balanced design and performed in strict accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, US National Research Council, 1996) and the Institutional Ethical Committee's guidelines for animal research. All solutions and fruit extract doses were freshly prepared on the days of treatment.

iii. Experimental protocol: Twelve animals were randomly divided into two groups (six animals in each group) and treated as: (1) Water treated and (2) SBT-FE (40 mg/kg P.O.) treated for two weeks. After completion of two weeks, animals were further sub-divided into four groups (three animals in each group) and treated as: (1) Water treated plus Unstressed (2) Water treated plus Stressed (3) SBT-FE plus Unstressed (4) SBT-FE plus Stressed for next one week. All behavioral procedures were carried out in animal models of depression (FST, EPM and OFT) for the evaluation of antidepressant-like effects of SBT-FE in groups of rats orally supplemented with aqueous SBT-FE (40 mg/kg P.O.) following exposure to repeated restraint stress for one week and their comparisons with water treated and unstressed vehicles. Each experiment was conducted in a separate behavioral room in a balanced design with fixed schedule under controlled conditions.

iv. Behavioral procedures: The rats were divided into various groups as described earlier. The behavioral profiles were evaluated both after repeated restraint stress in rats orally administered with water and aqueous fruit extract of Sea buckthorn at a dose of 40 mg/kg P.O and following 24 h withdrawal from the stress in rats. All animals were submitted to the following behavioral tasks: (a) forced swim test; FST (b) elevated plus maze test; EPM and (c) open field test; OFT.

Evaluation of antidepressant-like activity

i. Forced swim test (FST): Behavioral despair was proposed as a model to test for antidepressant activity by Porsolt *et al.*, (1978). Stressed and unstressed group of animals were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water (depth) at 25 ± 1°C; the total amount of time each animal remained immobile during a 5-min session was recorded (in seconds) as immobility time, as described previously (Brocardo *et al.*, 2008; Kaster *et al.*, 2005). Each rat was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those

movements necessary to keep its head above water. A decrease in the duration of immobility or struggling time is indicative of an antidepressant-like effect (Porsolt *et al.*, 1977).

ii. Open field test (OFT): To assess the effects of the fruit extract from Sea buckthorn on exploratory activity, experimental animals were evaluated in the open-field paradigm as previously described earlier (Batoool *et al.*, 2009a). Animals orally administered with SBT-FE (40 mg/kg P.O.) following repeated restraint stress were individually placed in an open field apparatus made up of Perspex plastic with dimensions (40×60×50 cm) and the floor was divided into 25 equal squares by lines. The latency to move from the centre square (in seconds) and numbers of squares crossed with all paws (crossing) were counted in a 5 min session. All groups of animals were monitored in a balanced design during experiments.

iii. Elevated plus maze test (EPM): For the assessment of antidepressant-like activity of aqueous fruit extract of Sea buckthorn (40 mg/kg P.O.) in stressed and unstressed rats, the elevated plus maze (EPM) apparatus was used. EPM was consisted of two open arms (OA; 16 x 5 cm) and two closed arms (CA; 16 x 5 12 cm) and was elevated (60 cm) above from the floor (Pellow and File 1986). A 5-min session was used for each rat to determine (1) number of entries in an OA and (2) total time spent in an OA in %. Rats orally administered with SBT-FE plus repeated stress and controls were individually placed at the centre of the EPM with heads facing the OA i.e., fear-inducing environment. All behavioural procedures were done in a balanced design.

v. Statistical analysis: In this investigation, the results were expressed as mean ± standard deviation (n = 12). The data were analyzed statistically by one way analysis of variance (1-ANOVA) and different group means were compared by Newman-Keuls test, while $p < 0.05$ was considered statistically significant in all cases. The software package Statistica was used for analysis of data.

Results

Figure 1 shows the antidepressant-like effects of oral administration of aqueous SBT-FE (40 mg/kg P.O.) in forced swimming test (FST) in rats repeatedly restraint for one week. Repeated treatment with the aqueous SBT-FE at dose of 40 mg/kg significantly ($p < 0.01$) increased struggling (numbers of jumps) in stressed rats or tries to escape from the water-filled cylinder became more prolong when compared with their respective controls. This observed fact taken as an antidepressant-like effects of SBT-FE and can be explainable in terms of decreased immobility where helpless despair syndrome is minimum in stressed rats when exposed to FST. It is suggested that aqueous SBT-FE has the ability to inhibit immobility induced following repeated restraint stress procedure in animals.

In this investigation, the results presented in Fig. 2 shows that oral administration of aqueous SBT-FE (40mg/kg P.O.) in rats repeatedly treated with restraint stress for one week showed significant ($p < 0.01$) increases in exploratory activity in an open field arena over a 5-min period when compared with their respective water treated unstressed subjects. It is suggested these increases in locomotor activity in stressed animals in an open field arena indicate the antidepressant-like effects of the extract and can be correlated with possible increases in brain serotonin metabolism. Hence, it is interesting to speculate that stressed-induced or drug-induced decreases in locomotor activity can be reversed or normalized by the oral administration of aqueous SBT-FE as observed from the present findings.

The results are presented in Fig. 3, which shows the effects of oral administration of aqueous SBT-FE (40 mg/kg P.O.) in elevated plus maze (EPM) test. Significant decreases ($p<0.01$) were observed in aversive behavior of rats following exposure to EPM model. Results have shown that SBT-FE (40 mg/kg P.O.) significantly ($p<0.01$) increased exploration (in terms of numbers of entries in an open arms) and adaptation to aversive / or anxiogenic stimuli (in terms of % time spent in open arms) in group of rats treated with repeated restraint stress procedure for one week as compared to their water treated unstressed subjects. The results indicate that aqueous SBT-FE demonstrated antidepressant-like effects by increasing the exploration and % time spent particularly in stressed rats when exposed to open arms (OA: fear-inducing environment) of an elevated maze experimental model.

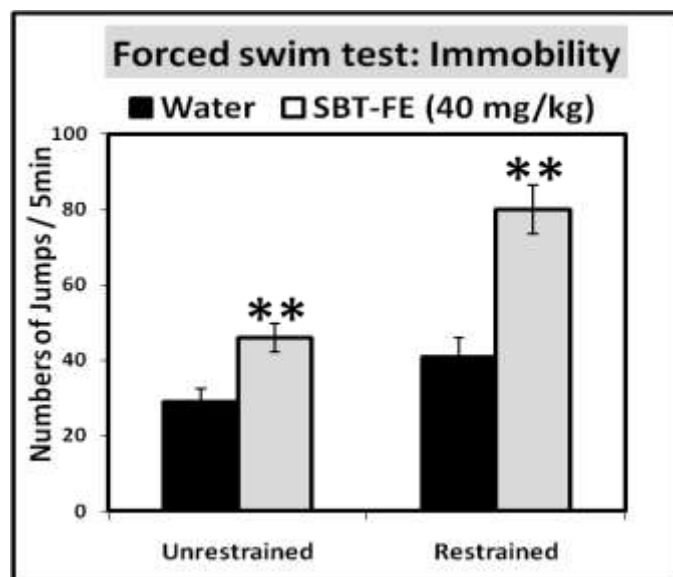


Fig. 1. Effects of oral administration of Sea Buckthorn (*Hippophae rhamnoides* L.) aqueous fruit extract (40 mg/kg) on struggling (immobility) in rats following exposure to repeated restraint stress. Values are means \pm S.D. ($n=12$). Significant differences by Newman-Keuls test: * $p<0.01$ from respective SBT-FE plus unstressed animals, + $p<0.01$ from water plus unstressed animals following two-way ANOVA.

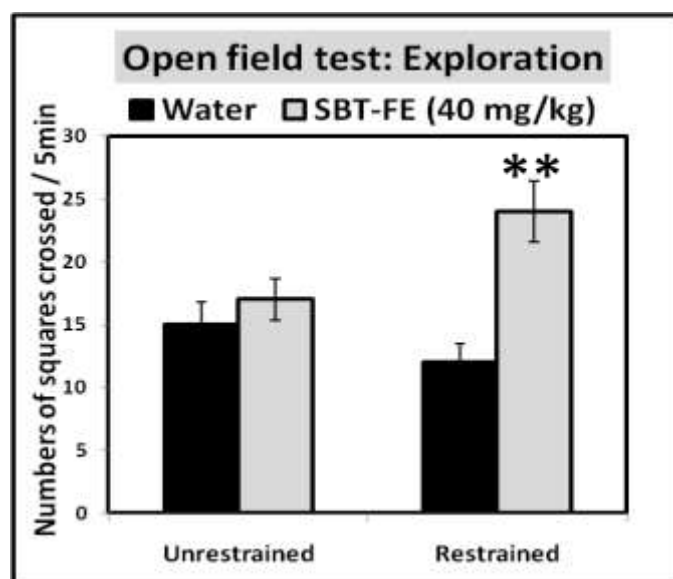


Fig. 2. Effects of oral administration of Sea Buckthorn (*Hippophae rhamnoides* L.) aqueous fruit extract (40 mg/kg) on exploratory activity in rats following exposure to repeated restraint stress. Values are means \pm S.D. ($n=12$). Significant differences by Newman-Keuls test: * $p<0.01$ from respective SBT-FE plus unstressed animals, + $p<0.01$ from water plus unstressed animals following two-way ANOVA.

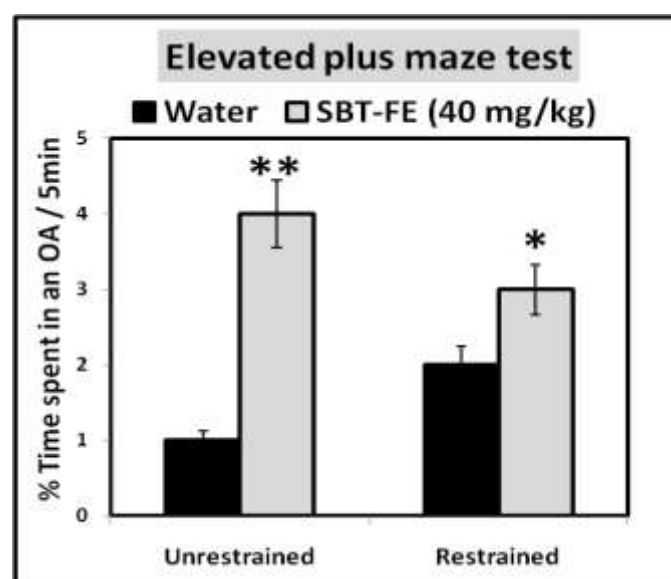
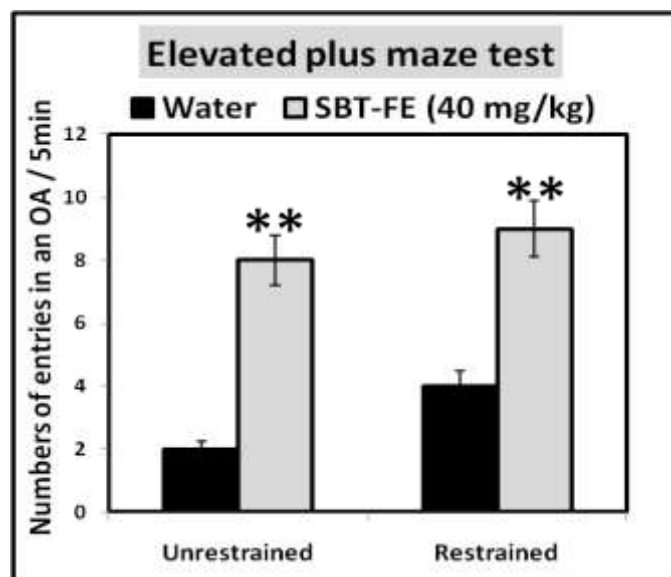


Fig. 3. Effects of oral administration of Sea Buckthorn (*Hippophae rhamnoides* L.) aqueous fruit extract (40 mg/kg) on (a) numbers of entries in an OA and (b) % time spent in an OA in rats following exposure to repeated restraint stress. Values are means \pm S.D. ($n=12$). Significant differences by Newman-Keuls test: * $p<0.01$ from respective SBT-FE plus stressed animals, + $p<0.01$ from water plus unstressed animals following two-way ANOVA.

Discussion

Natural products exhibiting antidepressant properties are one of the great interests for a number of reasons. The current therapeutic goal in the treatment of major depression is to improve quality of life by normalizing mood and reversal of functional and social disabilities associated with depression. In the present investigation, aqueous SBT fruit extract (40 mg/kg) exhibited significant antidepressant-like effects in FST. This model of depression is widely used for the screening of novel antidepressant drugs (Posrsolet *et al.*, 1977). The test is quite sensitive and relatively specific to all major classes of antidepressant drugs like tricyclics, selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs) (Posrsolet *et al.*, 1977; Detke *et al.*, 1995). In the present study, FST was used to evaluate the antidepressant-like effects of the extract SBT-FE in stressed and unstressed rats. In FST, immobility (despair in struggling) reflects a state of despair which can be reduced by numbers of synthetic agents, which are

therapeutically effective in depression (Dhingra and Sharma, 2006). In this test, rats are forced to swim in restricted space from which the escape is not possible and their struggling time of 5-min session ends at a point when the helpless despair syndrome produced. It has been shown that stress induces a state of helpless despair condition in animals, which is equivalent and claimed to a condition similar to human depression (Dhingra and Valecha, 2007). This attribution of animals' response to the development of depression process can be managed by the treatment with antidepressant medicines. In this study, a significant reduction in the immobility (the decrease in immobility is accompanied with an increase in swimming time) was observed following the long-term oral administration of aqueous SBT-FE at doses of 40 mg/kg (Fig. 1).

Open field behavioral model was used to study exploratory and locomotor activity in this investigation. Reported studies have shown that stress factors account for the decreases in mobility and functional responses against novel environment (Batoool *et al.*, 2009a). The purpose of including this test was to assess the general activity of the animals after performing FST. The results observed in the open field test showed that oral administration of aqueous SBT-FE (40 mg/kg P.O.) did not significantly increase the locomotor activity in unstressed groups of rats as compared with their control groups. However, aqueous SBT-FE administered rats following the exposure to repeated restraint stress showed significant ($p < 0.01$) increases in locomotor / exploratory activity on an open field arena (Fig. 2). It is therefore, suggested that the extract has the ability to reverse or normalize the locomotor suppressant behavior in laboratory animals and hence may help to cope with immobility factor associated with depression in humans. We have reported earlier that administration of aqueous extract of SBT at the dose of 40 mg/kg significantly altered the behavioral deficits induced by injections of atypical neuroleptic, haloperidol and increased brain serotonin metabolism in rats (Batoool *et al.*, 2001; Batoool *et al.*, 2009b). The present results are in general agreement with our previous studies in continuation to this plant and indicating its antidepressant-like activity in behavioral models of depression.

Stress-related anxiety is also one of the components of major depression in humans. EPM is rat model of anxiety that used for the screening of compounds with anxiolytic potential and used as a general research tool in neurobiology of anxiety and depression research (Batoool & Haleem, 1997; Zhang, 2004). In the present study, increases in the proportion of numbers of entries and % time spent in OA are taken as best index of reduced anxiety levels or reduction in stress-induced immobility (Fig. 3). It has been shown that depressive disorders have been associated with disturbances in brain serotonin and there is a great relationship between serotonin and depression as serotonergic system play a major role in the action of antidepressants (Millan, 2004). These results provided pharmacological support for the antidepressant-like activity of the plant and modifying the central nervous system involved in the control of locomotion and emotions.

In conclusion, this investigation provides the first evidence indicating that aqueous fruit extract from Sea buckthorn plant exhibit antidepressant-like effects in behavioral models predictive of antidepressant properties. These results possibly suggest that Sea buckthorn (*Hippophae rhamnoides* L.) fruit extract is effective as an antidepressant medicinal plant material. It is also suggested that the antidepressant-like effects of the fruit extract seem most likely to be mediated through an interaction with dopaminergic and serotonergic systems.

Furthermore, the antidepressant-like effects of the Sea buckthorn might well be considered and extend in future studies and possibly may helpful as potential therapeutic agent in the management of depressive disorders.

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