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Oral Supplementation of Sea Buckthorn *(Hippophae Rhamnoides L. Spp. Turkestanica)* Fruit Extract Modifies Haloperidol Induced Behavioral Deficits and Increases Brain Serotonin Metabolism

FARHAT BATOOL[1*], ASAD HUSSAIN SHAH[2], SYED DILNAWAZ AHMED[3] AND DARAKHSHAN JABEEN HALEEM[1]

[1.] *Neurochemistry and Biochemical Neuropharmacology Research Laboratory, Department of Biochemistry, University of Karachi, Karachi-75270. Pakistan*

[2.] *School of Life Sciences, John Maynard-Smith Building, University of Sussex, Falmer, Brighton BN1 9QG, UK*

[3.] *Department of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Azad Jammu and Kashmir, Rawalakot, Azad Kashmir Pakistan*

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ABSTRACT

Pulp and oils (fruit extract) from Sea Buckthorn (*Hippophae rhamnoides L.* spp. *Turkestanica*) (HRL) seeds and berries have been traditionally used in the treatment of different clinical and psychotic disorders and have significant implication in contemporary medicinal therapy. Schizophrenia is a chronic severe mental illness that affects approximately 1% of the population. Although the first generation of antipsychotic drugs such as chlorpromazine and haloperidol are widely prescribed for the treatment of schizophrenia, their beneficial effects are accompanied by extrapyramidal side effects (EPS). The present study was designed to investigate the effects of oral supplementation of HRL fruit extract (HRL-FE) on behavioral deficits and changes in brain serotonin (5-hydroxytryptamine; 5-HT) metabolism in rats administered with haloperidol repeatedly (two times a day at 09:00-09:30 A.M. and 05:00-05:30 P.M. at dose of 3.0 mg/kg body weight for two weeks. Results revealed that after two weeks of oral administration of HRL-FE (4.0 mg/kg body weight), rats exhibited significant ($p<0.01$) increases in locomotor activity in home cages and exploratory activity in open field arena. Repeated haloperidol treatment significantly ($p<0.01$) decreased brain tryptophan (TRP) and 5-HT and these decreases were reversed by 4.0 mg/kg body weight HRL-FE. These data suggest that HRL-FE plays a modifying role against the haloperidol induced behavioral deficits and could further extend as a nutritive therapy to conventional antipsychotic treatment in schizophrenia.

Key words: sea buckthorn (*Hippophae rhamnoides*), haloperidol, locomotor activity, tryptophan, brain serotonin

INTRODUCTION

Medicinal uses of a magical plant Sea buckthorn (*Hippophae rhamnoides L.* spp. *turkestanica*) (HRL) are well documented in Asia and Europe[(1-3)]. Modern medical science has made imposing progress in understanding the role of dietary supplementations in the maintenance of mental health and in the prevention of schizophrenia. Increasing scientific evidence is available to support the hypothesis that certain foods and food components have beneficial physiological and psychological effects over and above the provision of basic nutrients. Many major health problems (cancer, digestive problems, skin diseases, and cardiovascular symptoms etc.) are growing rapidly in the world especially in developing countries and their therapy with synthetic compounds are cost-effective and reported with side effects profile. Overwhelming evidence from clinical trial data indicates that a plant-based diet can reduce the risk of various diseases[(2)]. Many traditional food products including fruits have been found to contain components with potential health benefits[(3)]. Investigations on modern medicinal applications were initiated in Russia during the 1950s[(3)]. Recently, the research focus has shifted more to the biologically active components in foods that have the potential to

* Author for correspondence. Tel: +92-213-4511794;
Fax:+92-213-5377846;
E-mail: batool@uok.edu.pk; batool_fb@yahoo.com

optimize mental well being and reduce risk of psychosis and other diseases. The use of phytochemical or ingredients from Sea buckthorn or other medicinal plants is a safe way for combating against these diseases. Different parts of Sea buckthorn have been used as traditional therapies for diseases. The fruit, leaves, bark and seeds of Sea buckthorn contain over 190 nutrients. Sea buckthorn is exceptionally rich in vitamin E with vitamins A, C, D and K, etc[4]. Its active ingredients include carotene, flavonoids, phytosterols, serotonin, and eighteen amino acids covering all eight essential amino acids, i.e., isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan (TRP) and valine[5]. Later research has shown that over long periods of time a ninth amino acid, histidine, is also essential, as well as twenty-four mineral compounds including iron, zinc, calcium, magnesium, selenium, iodine, etc., that are important to good health[4-5]. Different preparations of Sea buckthorn in the form of oils and pulp (fruit extract) are available and recommended for various clinical, cosmetic and health purposes[6]. In spite of several usages of Sea buckthorn plant, the most important and popular part are berries, from which the juice is extracted. Research in the early 1960s has reported that 5-hydroxytryptamine (5-HT; serotonin) is isolated from bark and pulp (fruit extract) of HRL[7-8] while other studies have revealed that serotonin has a prominent role in the treatment of mental disorder like schizophrenia[9-10]. Furthermore, it has been reported that dietary manipulations of drug effects on the role of brain serotonin are accountable to treat enigmatic disorder schizophrenia. Many neurotransmitter substances are present in foods, and therefore, can directly influence brain chemistry.

Motor-related side effects are commonly encountered in the treatment of schizophreniform psychoses with so-called "classical" antipsychotic drugs such as haloperidol that are known to block central dopamine (DA) receptors with their DA-D_2 antagonistic potential[11-12]. The ability of antipsychotic drugs to modulate serotonergic as well as dopaminergic function has been suggested to be important for their efficacy and side-effect profile[13]. However, it is also known that there are interactions between dopaminergic and serotonergic neurons in the central nervous system (CNS) which may be of relevance to the extrapyramidal symptoms (EPS) in rats[13]. Serotonergic system is known to play a key role in the modulation of the activity of dopaminergic neurons. The nature of the modulation seems to be inhibitory. Thus lesions of the serotonergic cell bodies in the raphe nuclei augmented apomorphine-induced locomotor behavior[11,13]. There is also suggestive evidence from early laboratory studies for important DA/5-HT interaction in the mediation of EPS functions, as displayed in the catalepsy model in rats[14]. In view of possible role of serotonin, the aim of the present investigation was to monitor the effects of oral administration of HRL-FE on haloperidol induced behavioral deficits and changes in rat brain 5-HT metabolism. The results suggest the contribution of serotonin and its precursor amino acid TRP as adjuncts for the treatment of haloperidol induced EPS symptoms and will also help in the development of nutraceuticals as a nutrient therapy in schizophrenia.

MATERIALS AND METHODS

I. *Berries Collection and Fruit Extract Formulation*

Sea buckthorn (*Hippophae rhamnoides L. spp. Turkestanica*) (HRL) berries from Northern areas (Hussainabad, Sakardu) of Pakistan were collected in the first week of October when the plant grows wildly under natural conditions. These berries were kept in plastic pots and transported to University of Agriculture, Rawalakot, Azad Kashmir. The fresh fruit were then cleaned and pounded to pieces with a squeezer[15]. The extract was filtered and the filtrate was stored at -20°C in a refrigerator. The 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 40% HRL juice. To use 40% preparations of Sea buckthorn pulp, the calculations were done by considering the wet seed weight as well. It was estimated that 50 berries of Sea buckthorn would yield pulp weight of 4.0 g dissolved in 100 mL of water. This 4.0 g of pulp did not include seeds weight. The juice solution was administered orally *via* a feeding tube to groups of rats for two weeks continuously at a dose of 4.0 mg/kg body weight at an equivalent volume of 1.0 mL/kg body weight in each group of rats in a balanced design.

II. *Animals*

Male Albino-Wistar rats with an average weight of 180 ± 20 g on arrival were purchased from Agha Khan University (AKU) and group-housed (two rats per cage) in an environmentally controlled room (ambient temperature 21 ± 1°C and relative humidity 55 ± 5%) on a 12:12 h light/dark cycle (lights on at 7:00 A.M.). A 5-day acclimatization period was allowed before animals were used in experiments. After this period and 24 h before the behavioral tests, the animals were individually housed in an environmentally controlled test room in transparent Perspex cages (dimensions 26×26×26 cm, W×L×H). Food (standard rat diet) and tap water were continuously available to animals during experiment. The rats used for the treatment were all experimentally naive animals. All experimental protocols were approved 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.

III. *Drugs and Injections*

Haloperidol (Serenace; manufactured under license from G.D. Searle and Co. U.S.A, by Searle Pakistan Ltd. Laboratories) was available in 5.0 mg/mL ampoules (injectable solution) diluted to desired dose (3.0 mg/kg body weights) in saline solution (0.9% NaCl). Injections were given twice daily between 09:00-09:30 A.M. and 05:00-05:30 P.M. for 2 weeks continuously and the route of administration used was intraperitoneal injections (i.p.). Control animals received an equal amount of vehicle on the same schedule. HRL-FE at a daily dose of 4.0 mg/kg body weight was administered *via* feeding tube with the same schedule of daily drug administration to experimental rats in a balanced design.

IV. *Experimental Protocol*

Twenty-four animals were randomly divided into two equal groups (twelve animals in each group): (1) saline injected and (2) haloperidol injected groups. These animals were injected with haloperidol at a dose of 3.0 mg/kg body weight (two times a day at 09:00-09:30 A.M. and 05:00-05:30 P.M.) for 2 continuous weeks. Control animals were injected with saline in volumes of 1.0 mL/kg body weight. On the 6th day of study, locomotor activity in home cages and open field arena were recorded 15 minutes postinjections of haloperidol and vehicle in experimental animals. After the completion of 1st week, animals of the two groups were further subdivided into four equal groups (six animals in each group): (1) saline plus water (2) saline plus HRL-FE (3) haloperidol plus water, and (4) haloperidol plus HRL-FE. On the 15th and last day of experiment 15 min postinjections activity parameters were recorded again. Food intake was monitored on the 6th and 15th day of experiment by providing rats with a weighed amount of food with proper measurement by subtracting the left over food in the hopper of the cages. Body weights of the rats were also monitored on the 6th and 15th day of study. Growth rate was calculated in terms of percentage of initial body weight. The animals were then decapitated on the 15th and last day of study to collect plasma and whole brain samples. Brain samples were stored at -70°C for the estimation of TRP, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by High Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC)[16].

V. *Behavioral Analysis*

(I) *Locomotor Activity In Home Cage*

The activity boxes used in the present investigation were specifically designed Perspex home cages (26×26×26 cm) with saw-dust covered floor. Experiment was conducted in a separate quiet room. Locomotor activity was observed in activity boxes for 10 minutes in terms of numbers of cage crossings in all groups in a balanced design[16].

(II) *Exploratory Activity in an Open Field*

The open field apparatus used in the present investigation consisted of a square area 76×76 cm with walls of 42 cm in height. The floor was divided into 25 equal squares. To determine the activity, a rat was placed in the centre square of the open field. Latency to leave the centre square and numbers of squares crossed with all four paws were scored for 5 minutes as described earlier[11,16]. The activity of saline injected rats and haloperidol injected and HRL-FE administered rats were monitored in a balanced design to avoid order effect on the 6th day between 10:00 and 2:00 h.

VI. *Neurochemical Analysis*

(I) *Brain Dissection*

Animals were decapitated and the brains were removed immediately from the cranial cavity after opening the skull from the frontal cortex rostrally and medulla oblongata caudally as described by Batool and Haleem[11]. The cerebellum was pinched out by forceps. The brain dipped in ice cold saline was placed with dorsal side up in the molded cavity of a brain slicer. Olfactory nucleus material was discarded and whole brains were stored at -70°C in order to assay biogenic amines by HPLC-EC. Plasma samples were also stored at -70°C for biochemical estimations.

(II) *HPLC-EC Determinations of Plasma TRP, Brain TRP and 5-HT Metabolites*

Whole brain samples were extracted and homogenized as described by Haleem[22]. Brain TRP, 5-HT and its metabolites were determined by HPLC-EC as described by Batool and colleagues[16]. A 5µm Shim-Pack ODS separation column of 4.0 mm internal diameter and 150 mm length was used. Separation was achieved by mobile phase containing 14% methanol, 0.023% octyl sodium sulfate and 0.0035% ethylenediaminetetraacetic acid (EDTA) in 0.1 M phosphate buffer of pH 2.9 at an operating pressure 2000-3000 psi on Shimadzu HPLC pump. Electrochemical detection was achieved on Shimadzu L-ECD-6A detector at an operating potential of 0.8 V (glassy carbon electrode vs an Ag/AgCl reference electrode). TRP was determined in a separate run at an operating potential of 1.0 V.

(VII) *Statistical Analysis*

In this investigation, the results were presented as a means ± S.D. Data on locomotor activity in home cages and open field were analyzed by 2-way ANOVA (repeated

measure design) followed by Newman-Keuls test. Neurochemical data on the effects of HRL-FE on the synthesis of brain TRP and 5-HT were analyzed by two-way ANOVA. *Post-hoc* comparisons, done with the Newman-Keuls test, when $p < 0.05$ were considered significantly different.

RESULTS

Figure 1 shows the effects of oral supplementation of HRL-FE (40% preparation; 4.0 mg/kg body weight) on growth rate (% change in body weight) and cumulative food intake/24h in rats treated with repeated saline and haloperidol. No significant effect of HRL-FE was found in growth rate ($F = 2.7$; $df = 1,20$; $p>0.05$). However, significant lower food intake ($F = 15.6$; $df = 1,20$; $p<0.01$) was found in rats treated with repeated haloperidol plus HRL-FE. *Post-hoc* analysis showed that oral supplementation of HRL-FE produced significant ($p<0.01$) decreases in food intake only in haloperidol treated rats, while significant increases ($p<0.01$) were observed in group of rats treated with repeated haloperidol plus water administration.

Figure 2 shows the effects of oral supplementation of HRL-FE (40% preparation; 4.0 mg/kg body weight) on locomotor activity in familiar environment (in terms of numbers of cage crossings; akinesia) and exploratory activity in novel environment (in terms of numbers of squares crossed in open field arena) in rats treated with repeated saline and haloperidol. A significant effect of HRL-FE ($F = 21.5$; $df = 1,20$; $p<0.01$) and haloperidol ($F = 15.6$; $df=1,20$; $p<0.01$) was observed. *Post-hoc* analysis showed significant decreases ($p<0.01$) were found in group of rats treated with repeated haloperidol. On the contrary, significant increases ($p<0.01$) were observed in rats repeatedly treated with haloperidol plus orally supplemented with HRL-FE.

Figure 3 shows the effect of oral supplementation of HRL-FE (40% preparation; 4.0 mg/kg body weight) on plasma TRP, brain TRP, 5-HT and 5-HIAA levels of

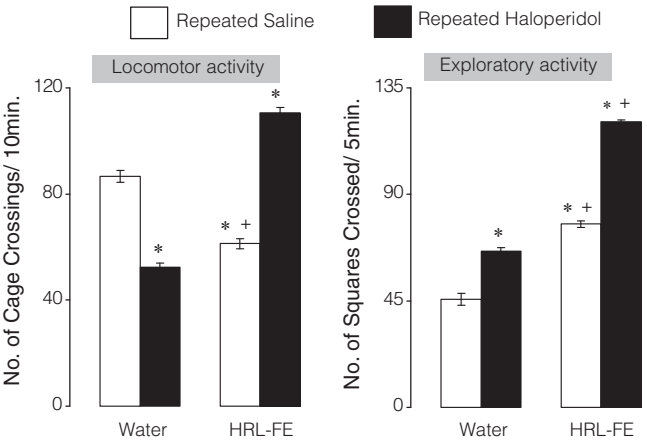

Figure 2. Effects of oral administration of *(Hippophae rhamnoides L.)* fruit extract (HRL-FE) on home cage locomotor activity and open field exploratory activity in rats treated with repeated saline and haloperidol. Values are means ± S.D. (n = 12). Significant differences by Newman-Keuls test: *p<0.01 from respective water treated animals, +p<0.01 from saline treated plus HRL-FE animals following two-way ANOVA.

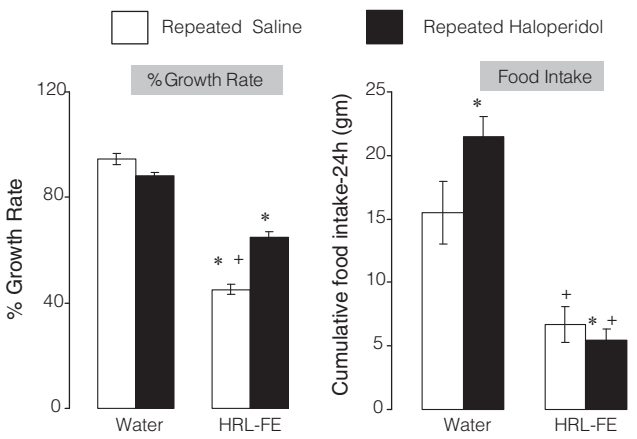

Figure 1. Effects of oral administration of *(Hippophae rhamnoides L.)* fruit extract (HRL-FE) on Food Intake and Growth Rate in rats treated with repeated saline and haloperidol. Values are means ± S.D. (n = 12). Significant differences by Newman-Keuls test: *p<0.01 from respective water treated animals, +p<0.01 from saline treated plus HRL-FE animals following two-way ANOVA.

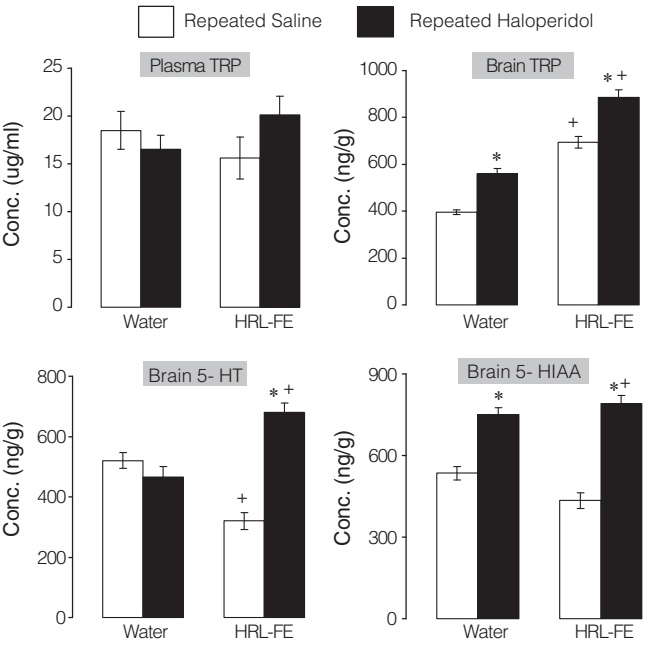

Figure 3. Effects of oral administration of (*Hippophae rhamnoides* L.) fruit extract (HRL-FE) on plasma TRP, brain TRP, 5-HT and 5-HIAA concentrations in rats treated with repeated saline and haloperidol. Values are means ± S.D. (n = 12). Significant differences by Newman-Keuls test: *p<0.01 from respective water treated animals, +p<0.01 from saline treated plus HRL-FE animals following two-way ANOVA.

repeated saline and haloperidol injected rats. Data on plasma TRP showed no significant effect of HRL-FE ($F = 1.7$; $df = 1,20$; $p>0.05$), insignificant effect of drug ($F = 2.6$; $df = 1,20$; $p<0.05$) and no significant interaction ($F = 1.3$; $df = 1,20$; $p>0.05$) between the HRL-FE and drug. Data on brain TRP showed significant effect of HRL-FE ($F = 24.8$; $df = 1,20$; $p<0.01$), significant effect of drug ($F = 24.5$; $df = 1,20$; $p<0.01$) and significant interaction ($F = 16.5$; $df = 1,20$; $p<0.01$) between the HRL-FE and drug. *Post-hoc* analysis showed that oral supplementation of HRL-FE did not alter the plasma TRP levels but significant increases ($p<0.01$) were observed in the levels of brain TRP.

Data on 5-HT levels showed significant effect of HRL-FE ($F = 24.7$; $df = 1,20$; $p<0.01$) and significant effect of drug ($F = 12.4$; $df = 1,20$; $p<0.01$) and significant interaction ($F = 12.5$; $df = 1,20$; $p<0.01$) was observed between the HRL-FE and drug. *Post-hoc* analysis showed that HRL-FE significantly increased ($p<0.01$) 5-HT levels in repeated haloperidol injected rats when compared with repeated saline plus HRL-FE administered rats. Data on 5-HIAA levels showed a significant effect of HRL-FE ($F = 12.8$; $df = 1,20$; $p<0.01$) and significant effect of drug ($F = 27.3$; $df = 1,20$; $p<0.01$) and significant interaction ($F = 10.1$; $df = 1,20$; $p<0.01$) was observed between the HRL-FE and drug. *Post-hoc* analysis showed that HRL-FE significantly increased ($p<0.01$) 5-HT levels in repeated haloperidol injected rats when compared with repeated saline plus HRL-FE administered rats. The increases in saline plus HRL-FE and repeatedly treated haloperidol plus HRL-FE were comparable.

DISCUSSION

To our knowledge, potential role of medicinal plant Sea buckthorn (*Hippophae rhamnoides L.)* (HRL) as a dietary nutrient in the modulation of haloperidol-induced behavioral deficits and increased brain 5-HT metabolism by HRL fruit extract supplementation to rats reported for the first time. Reported literature have shown that tryptophan and serotonin are the bioactive ingredients of the Sea buckthorn[3,5], and they have been known as promising adjuncts in the modulation of antipsychotic therapy to schizophrenic patients[17,18]. Dietary manipulation may serve as a safer and less invasive means than pharmacologic challenge to provoke serotonergic responsiveness in schizophrenia studies. Studies have also shown that repeated neuroleptic therapy in schizophrenic patients suppresses responsiveness of serotonergic system[19]. Observed food intake decreases (Figure 1) in the present study were explainable by the fact that carbohydrate rich diet can influence the availability of tryptophan to the brain and ultimately synthesis of brain 5-HT[20,21]. Thus the synthesis of 5-HT following a tryptophan load increased in the whole brain[22] and many brain regions except the striatum[13,23]. It is also reported that TRP load increases 5-HT synthesis in the brain and therefore may stimulate 5-HT release and functions[19,22]. The mechanism by which diet (or TRP) is thought to influence 5HT synthesis involves the following sequence: the composition of food consumed changes plasma levels of the large neutral amino acids (LNAA), which affect the rate of TRP transport into the brain, brain TRP levels and hence the rate of 5HT synthesis[23]. It has been suggested that, by this mechanism, dietary interventions might influence a range of behaviors and brain functions linked to serotonergic neurotransmission[22,23].

It is well established that haloperidol (a dopamine D_2 receptor antagonist) prevents hyperactivity induced by amphetamine decreases spontaneous locomotion and exploration and elicits a state known as catalepsy[18,24]. The dopamine system traditionally has been considered crucial to the control of motor activity[19]. With respect to the anatomical site of action a view has developed that striatum is involved in the control of motor behavior. Striatum, a region of brain involved in the control of motor activity, is rich in DA nerve terminals[19]. Factors that modulate brain serotonin metabolism have been shown to produce little effect in this region of the brain[21]. Early studies have shown that the administration of haloperidol for three weeks resulted in an increase in DA receptor binding. Subsequent studies showed a selective increase in dopamine D_2 receptor binding in rat brain following prolonged neuroleptic treatment[25,26]. The results of the binding experiments are consistent with behavioral studies on experimental animals. Thus chronic haloperidol treatment induced DA receptor hypersensitivity in rats as measured by enhanced hyperactivity and stereotypy to apomorphine after withdrawal of the neuroleptic[27]. In the present study, 2 weeks of treatment with haloperidol induced a time dependent increase in locomotor and exploratory activities in groups of rats orally supplemented with HRL-FE. Co-administration of HRL-FE at a dose of 4.0 mg/kg body weight completely reversed the behavior in a time dependent manner (Figure 2). Observed effects of repeated administration of haloperidol on behavioral deficits are explainable in terms of either an increase in the responsiveness of postsynaptic 5-HT_{1A} receptors or dopamine D_2 receptors or both. The results are therefore consistent with the notion that a decrease in the serotonergic influence on the activity of dopaminergic neurons is involved in the induction of EPS while a normalization of serotonergic influence in rats co-administered with HRL-FE could reverse the behavioral deficits induced by haloperidol treatment.

The important findings of the present study were that HRL-FE supplementation not only modified the locomotor activity but also increased brain TRP and 5-HT levels in the haloperidol injected rats. The levels of free TRP in plasma have been used as an index in the determination of brain TRP levels under different physiological and pharmacological conditions[18,22]. The increased precursor availability to the brain has a consequence on the

observed increases in brain 5-HT metabolism[21,22]. Our study suggested that these observed increases in brain 5-HT were associated with the increased availability of its precursor, TRP. This data can be interpreted as HRL-FE could have a potential to influence brain serotonin metabolism by altering the activity of rate-limiting enzyme tryptophan hydroxylase to the substrate. We have also observed increased level of 5-HIAA in the present study and number of studies have shown that increases in brain 5-HIAA levels are often taken as a measure of increased release or utilization of 5-HT in the synapse[16]. It is reported that oral administration of TRP-free amino acid mixture significantly decreased basal 5-HT and 5-HIAA levels 100 min after ingestion (65 and 81% of basal value respectively) and remained at this level for another 140 minutes suggesting that the rate of 5-HT formation varied directly with the availability of circulating precursor TRP, the source of which is dietary[28]. Findings of the present study support the idea that oral supplementation of Sea buckthorn as a nutraceutical in combination with repeated haloperidol treatment augmented the uptake of TRP in the brain and consequently increased brain 5-HT synthesis particularly to the groups of haloperidol treated rats supplemented with HRL-FE (Figure 3). Overall increases in the locomotor/ exploratory activity and brain serotonin metabolism suggest the possible role of medicinal plant Sea buckthorn and its fruit in extending therapeutic management of schizophrenia.

CONCLUSIONS

The present study suggests that oral supplementation of HRL-FE is effective in modifying haloperidol-induced behavioral deficits. In addition, HRL-FE reversed brain TRP and 5-HT decreases induced by repeated haloperidol treatment. It could be suggested that increased precursor availability due to dietary source of HRL-FE in rats lead to the increased brain 5-HT synthesis. Decreased food intake and increased brain TRP exhibits an imperative role of nutritional sources of Sea buckthorn rich in dietary TRP and 5-HT in its fruit extract. In relation to its medicinal, biochemical and nutritional aspects and emphasizing role of serotonin in schizophrenia, Sea buckthorn may be used as a nutritive therapy in psychotic patients. More issues will be addressed in future studies.

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