Full Length Research Paper

Brown seaweeds administration generate psychotherapeutic response associated with brain norepinephrine modulation in rats

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Research in the area of herbal psychopharmacology has increased considerably over the past few decades in search of panacea for neuroprotection. Seaweeds are one of the herbal sources consumed in many Asian countries as medicine due to their remarkable bioprospecting properties and evident health benefits. Keeping in view the bioactive potential of seaweeds, the present study was designed to evaluate the psychotherapeutic potential of *Sargassum swartzii* and *Stoechospermum marginatum*, in association with the role of brain norepinephrine (NE) using a rat model. Adult male albino Wistar rats were divided into three groups (n=6) as control rats (CR), *S. swartzii* extract treated (SSET) and *S. marginatum* extract treated (SMET). Seaweeds were extracted using methanol and administered orally to rats for four weeks at a dose of 60 mg/kg. Behavioral changes for stimulant activities were assessed by activity cage and open field tests, while anxiety was observed in light-dark exploration test. Followed by scoring behavioral activities, rats were decapitated and brain samples taken out from the cranial cavity were immediately stored at -70°C until estimation of brain NE levels by high performance liquid chromatography-electrochemical detection (HPLC-ECD). Results exhibited an increase in ambulatory and anxiolytic activities by SSET and SMET rats with subsequent increase in brain NE as compared to CR. The increase in NE in SSET and SMET rats could be attributed to the lipolytic activity of seaweeds. However, the exact mechanism underlying the increase in NE needs further investigations. In conclusion, seaweed extracts showed significant psychostimulant and anxiolytic activity by ameliorating brain NE levels and could be studied further for isolation of active ingredients responsible for eliciting such a response.

Key words: Brown seaweeds, norepinephrine, psychostimulant activity, anxiety.

INTRODUCTION

Anxiety, depression and mood disturbance are widespread psychiatric disorders that are being treated with medicines of the natural closet from time immemorial (Kessler et al., 2005). A study comprising interviews of 2055 people suffering from psychological disorders, reported that more than 50% patients of depression and

anxiety were using medicines from natural sources (Kessler et al., 2001). Another study reported the use of herbal medicines by 44% of psychiatric patients interviewed during the last 12 months of their treatment (Elkins et al., 2005). Researches in the area of herbal psychopharmacology have gained the attention of scientific community especially after isolation of morphine from opium (Pengelly, 1997). Since then, plenty of herbal sources from the terrestrial environment were evaluated for bioprospecting and pharmacological potential (Spinella, 2001). It is important to note that oceans are considered as the largest reservoir of bioactive sources inhabiting half of the total global biodiversity and covers approximately 90% of the world's living biomass (Kim and Wijesekara, 2010). Among organisms of the ocean, seaweeds were the most studied bioactive source due to the presence of diverse biologically active ingredients different in structure and functions than other organisms (Paula et al., 2011; Li, 2009), their easy availability and possibility for cultivation (Dhargalkar et al., 2005). Seaweeds were reported to be consumed by humans (Liu et al., 2012) and animal (Hong et al., 2007), as food and medicine, for long. For nearly 2000 years, the traditional Chinese medicine has recommended the use of seaweeds for various kinds of pathological conditions (Liu et al., 2012). Even today, in Korea, brown seaweeds were reported to be utilized by mothers of newborn as folk medicine (Moon and Kim, 1999). The significance of seaweeds that may affect the central nervous system (CNS) has grown with preclinical *in vitro* and *in vivo* studies validating many phytotherapies for neuroprotection (Pangestuti and Kim, 2011). Seaweeds are considered as a useful bioactive natural source that could protect CNS against oxidative degradation (Jiao et al., 2011). Reports on lower incidence of psychiatric disorders in East Asian countries are also attributed to the use of seaweeds (Pangestuti and Kim, 2011). Another study reported bioprospecting potential of a seaweed specie via modulation of brain biogenic amines including norepinephrine (NE), which serves as the main chemical messenger of the noradrenergic system (Najam et al., 2010).

The NE neurons are located and distributed as small clusters in the brain stem, while their axons diffuse throughout the brain, for example, prefrontal cortex, hypothalamus, thalamus, hippocampus and amyglada (Goddard et al., 2010). Thereby, NE input received from the diverse targets influence wide range of brain functions, for example, memory retention, attention, mood, arousal and the response during stress (Sved et al., 2001). The involvements of NE in mediating psychostimulant functions were studied for many years and it was reported that irregularities associated with NE system may contribute to key symptoms of anxiety and mood disorders (Abercombie and Jacobs, 1987). A study observing elderly caregivers to Alzheimer's patients reported that subjects with high stress levels (caregivers) compared with non-care givers of much less stress level exhibit higher plasma norepinephrine levels and increase β-adrenergic receptor sensitivity (Mills et al., 1997). Conversely, facilitated NE transmission is attributed toward anxiolytic response. It is reported that NE system might have a modulatory role in both anxiogenic and anxiolytic effects that vary depending on the condition of stress (Ressler and Nemeroff, 1999). Therefore, NE system is postulated to play a primary role in pathophysiology and subsequent treatment of psychological disorders (Cameron et al., 2004).

Seaweeds are broadly classified as brown (Phaeophyta), red (Rhodophyta) and green (Chlorophyta) depending on color and pigmentation. Along Pakistan coast, brown seaweeds are considered as the most abundant algal group among three classes of algal flora (Hameed et al., 2000). *Stoechospermum marginatum* and *Sargassum swartzii* are the two abundant species among brown algae which are exclusively studied for diverse bioactivities but never been tested for pharmacological activities related to CNS *in vivo* (Shaikh et al., 1990; Dar et al., 2007; Sabina et al., 2005; Pujol et al., 2012). It should be noted that most of the studies on herbal medicines do not report isolation of single active compound since biological response relies on the synergistic interaction between chemical constituents in herbal sources (Williamson, 2001).It is more common for natural sources to have many potential psychoactive constituents which can be seen in studies using a combination of substances to get the desired results (Heinrich et al., 2004). Epigenetic assays of phytodol, a multi-compound herbal product used as an anti-inflammatory drug showed that gene expression profile of the whole herb and not an individual constituent is responsible for producing biological effects (Jordan et al., 2010). Based on findings mentioned earlier and remarkable nutritional and biological value of seaweeds, the current study is designed to investigate the psychoactive potential of *S. swartzii* and *S. marginatum*

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in connection with brain NE modulation.

MATERIALS AND METHODS

Algal

Healthy specimens of *S. swartzii* and *S. marginatum* were handpicked from the coast of Ormara, Baluchistan. Seaweeds were authenticated and archived at Center of Excellence in Marine Biology, University of Karachi for future correspondence. Collected seaweeds were rinsed with water to remove any debris or epiphytes and were shade dried in a dark room at 25±5°C temperature. Dried seaweeds were then ground with the help of mechanical grinder and soaked in methanol (5X). After each successive soaking, residues were filtered using Whatman no. 1 filter papers. Filtrates were pooled together, concentrated using rotary evaporator and further dried under vacuum to get the desired methanolic extracts.

Animals and dosing

Eighteen adult, 10 to 12 weeks old male Wistar rats weighing between 220 and 240 g were purchased from Agha Khan University Hospital, Karachi. All experiments were conducted in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals adopted by National Institutes of Health (Bayne, 1996). Rats were housed in individual cages at room temperature under 12 h dark/light cycle and were given standard diet and water *ad libitum*. Rats were acclimatized for 3 to 4 days before the start of the experiments. All experiments were conducted according to the protocol set by local animal care committee. At the start of experiment, rats were divided into three groups (n=6) as control rats (CR) (distilled water treated), *S. swartzii* extract treated rats (SSET) and *S. marginatum* extract treated rats (SMET), respectively. The treatment was given at a dose of 60 mg/kg daily for four weeks. Extracts and dist water were administered orally with the help of feeding tube attached to a 1 ml syringe. Careful consideration was taken, while administration of doses and it was made sure that the animals took in the entire dose. After behavioral trials,rats were decapitated randomly to avoid any order effect. Brain samples were removed from the cranial cavity and stored immediately at -70°C until estimation for NE was performed.

Open field test

The locomotor activity of rats in a novel environment was tested using open field apparatus (Kennett et al., 1985). The test is based on the principle in which ambulatory skills of rats were determined in an open novel space where escape was prevented by a surrounding wall. Test was performed in a quiet room under white light. The apparatus used in the present study consisted of a square area (76 × 76 cm) with opaque walls (42 cm high) having twenty five equal squares drawn on the floor of the apparatus. Rats were taken from their cages and placed in the center of the apparatus one at a time. The numbers of squares crossed by the rats with all paws were counted in a 10 min session. The tools were cleaned with water after each test to eradicate animal clues.

Activity cage test

For evaluation of exploratory activity in familiar environment, transparent perspex cages of 29×26×2 cm size with sawdust covered floor were used (Bushra et al., 2012). Activity in the home cage was determined by counting the number of cage crossings for 10 min after the acclimatization period of 5 min as described earlier

(Bushra et al., 2012). Stimulant activity of test and control rat was monitored at the same time to avoid time effect. The experiment was conducted in a quiet room to avoid disturbance.

Light-dark exploration test

The test is based on the initial model as described by Crawley and Goodwin (1980). Rats were exposed to a plastic box with two compartments. The dimensions of both compartments were of the same size (26 × 26 × 26 cm), with a door (12 × 12 cm) between the separating walls of the compartments which allow rats to move freely between compartments. One compartment of the plastic box is left transparent and is exposed to light while the other one was made dark by painting the walls black. Under normal conditions rats tend to spent more time in the dark region due to the conflict between exploratory drive and risk avoidance in bright light. The test ran for 10 min after the rats were placed in the light compartment facing away from the partition. Time spent in light region and numbers of light/dark transitions were scored.

High performance liquid chromatography-electrochemical detection (HPLC-ECD)

NE was extracted from the known weight of brain tissues in extraction buffer (0.4 M perchlorate containing 0.1% sodium metabisulphite, 0.01% EDTA and 0.1% cysteine). Estimation of brain NE was carried out by the method using HPLC-ECD as described earlier (Abbas et al., 2013). The homogenate was centrifuged at 14000 rpm at 4°C. Supernatant was passed through 5 µ shim-pack ODS® (stationary phase) separating column (150 mm length; 4 mm). The mobile phase consisted of methanol (10%), octanesulfonic acid (0.023%) and EDTA (0.005%). The pH was maintained to 3.4 with the help of 0.1 M phosphate buffer. The detection was achieved at an operating potential of +0.8 V for brain NE.

Statistical analysis

The data obtained were expressed as mean ± standard deviation (SD). Differences between various means were calculated by one way analysis of variance (ANOVA). Post hoc analysis was carried out by using Newman-Keuls test. Values were considered significant at p<0.05.

RESULTS

Behavioral results

S. swartzii and S. marginatum extracts were administered to rats for four weeks. Rats were tested for ambulatory behavior in a novel environment using open field apparatus. SSET, SMET and CR were placed in a an open field and number of squares crossed in 10 min time interval showed an increase in locomotor activity by SSET and SMET rats as compared to CR (Figure 1; df=15.2, F=21.652, p<0.005). Similarly, stimulatory activity in a familiar environment was tested by activity cage test. The floor of the activity cage was covered with sawdust to give a home cage environment to rats. SSET and SMET rats showed an increase in stimulatory activity during a 10 min session in the activity cage as compared to CR animals (Figure 2; df=15.2, F=7.913, p<0.005).This
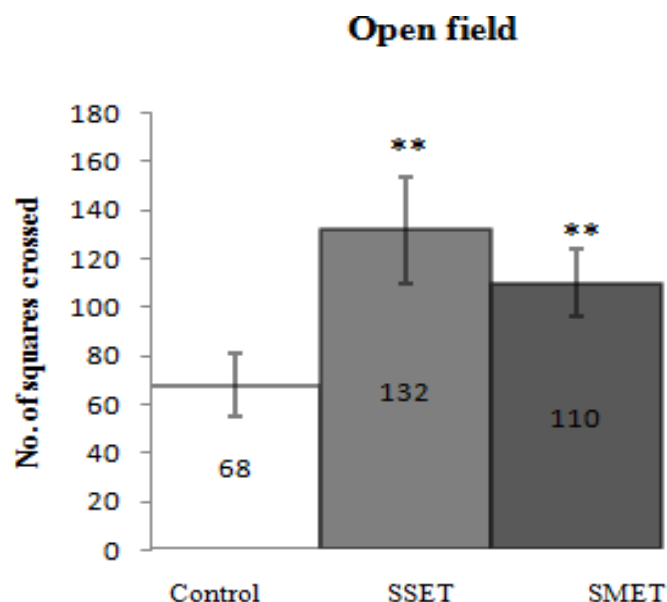
Open field

Figure 1. Effect of *S. swartzii* and *S. marginatum* extract treatment on locomotor activity in a novel environment was assessed using open field test. Rats were placed at the center of the field and numbers of squares crossed were scored. Data represent as mean ± SD (n=6).   ****p<0.05* is considered as significantly different from controls.
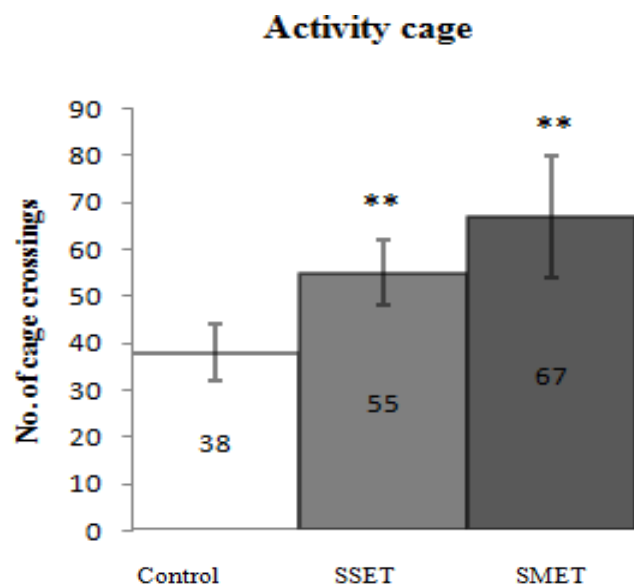
Activity cage

Figure 2. Effect of *S. swartzii* and *S. marginatum* extract treatment on stimulant activity in a familiar environment was assessed using activity cage test. Rats were placed in a transparent box with sawdust covered floor and numbers of cage crossings were noted. Data represent as mean ± SD (n=6).   ***p<0.05* is considered as significantly different from controls.

indicates that SSET and SMET treatment significantly increased stimulatory activity both in novel and familiar environments.
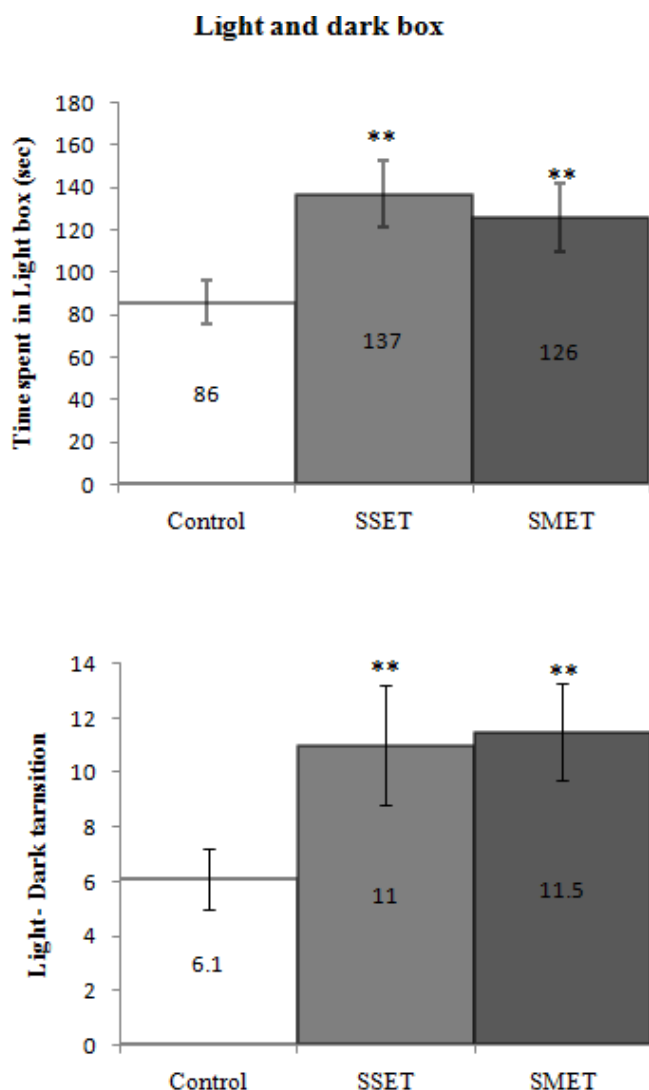
Following stimulatory activity, rats were tested for anxiety like effects in dark and light box. Current study showed that *S. swartzii* and *S. marginatum* extracts when given to rats for four weeks produce significant anxiolytic effects (Figure 3) exhibited by the increase in time spent in light region (df=15.2, F=18.945, $p<0.001$) and number of transitions between light/dark compartments by SSET and SMET rats as compared to controls (df=15.2, F=15.51, $p<0.001$).

Neurochemical results

After scoring behavioral activities rats were decapitated and brain samples were taken out for estimation of NE. The present study showed that long term treatment with *S. swartzii* and *S. marginatum* significantly increase brain NE levels as compared to control rats. Results of the behavioral trials correlate with the increase in brain NE in SSET and SMET rats as compared to CR (Figure 4; df=15.2, F=10.989, $p<0.001$). However, the exact mechanism behind the increase in NE warrants further experimentation.
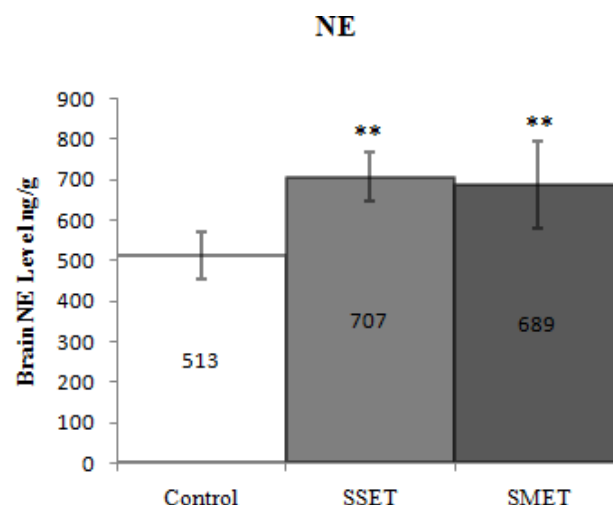
DISCUSSION

The present study reported an increase in brain NE levels in SSET and SMET rats as compared to CR. The role of NE in alteration of various behaviors have been reported from decades, while the importance of NE as a potential target for the treatment of various psychological conditions has gain interest recently (Morilak et al., 2005). Many behavioral states are controlled by brain NE modulation which include but not limited to vigilance, arousal, anxiety and inhibition of depressive symptoms. The NE neurons have acquired the important cellular position in the brainstem nuclei, while its receptors are projected in almost every part of the brain (Smythies, 2005). The increase in brain NE concentration is modulated either by NE synthesis or its degradation (Bonisch and Bruss, 2006). Other mechanisms involve in increasing brain NE are either inhibition of enzymes monoamine oxidase or catechol-o-methyl transferase (Huotari et al., 2002). The production of NE in brain also varies notably by means of its precursor L-tyrosine and significantly increases by tyrosine modulation (Brodnik et al., 2012). Free tyrosine levels are controlled by tissue flux through fat metabolism while its entry in the brain increases after competition with other amino acids for a common transport system (Fernstrom, 1990). It was

Light and dark box

NE

Figure 4. Light-dark exploration. Rats treated with 60 mg/kg extracts of *S. swartzii* and *S. marginatum* spent more time in brightly lit open area and made significant transition between the two compartments then the vehicle treated rats. Data represent as mean ± SD (n=6).   **p<0.05* is considered as significantly different from controls.

Figure 3. Effect of *S. swartzii* and *S. marginatum* extract treatment on brain NE was assessed using HPLC-ECD. The brain samples were drawn from the cranial cavity of the rats after decapitation following behavioral tests. Data represent the concentration (ng/g) as mean ± SD (n=6). **p<0.05* is considered as significantly different from controls.

reported in a study that obese mice getting treatment of lipolytic caffeine showed significant reduction in body fat along with increase in brain norepinephrine (Chen at al., 1994). It is also very much recognized that brown seaweeds are a potent inhibitor of lipid accumulation (Park et al., 2011). Thus it could be deduced that increase in brain NE levels of SSET and SMET (oral administration of extracts for four weeks) rats might be due to the lipolytic activity of seaweeds. The increase in lipid metabolism could have increased free tyrosine

concentration in plasma which in turns increased brain tyrosine levels by competing with other amino acids. This increase in the brain tyrosine, precursor of NE, is subsequently involved in increasing brain NE in SSET and SMET rats. However, the exact mechanism behind the increase in NE levels in rat brains warrants further investigations.

The effects of long term SSET and SSET on psychostimulant and anxiolytic activity in a rat model were also observed. Present study showed that exploratory behavior tested in a novel environment using open field test and in a familiar environment using home cage paradigm is significantly increased in SSET and SSET rats as compared to CR. It was reported earlier that NE is α_1 receptor agonist in the brain which are widely distributed in CNS and are known to regulate motor activity, attention and vigilance (Aston-Jones et al., 1994). It was shown in a study that administration of NE injections has significantly increased stimulant activity via ameliorating functions of α1 receptors (Plazik et al., 1985). Furthermore the inactivation of NE decreases spontaneous locomotor activity in a novel and familiar environment (Mitchell et al., 2006). Thus, it could be inferred from the evidences above that the CNS stimulant activity is associated with the increase in brain NE levels (Rothman et al., 2001), while destruction of NE terminals subsequently exhibit marked reduction in stimulant activity (Nishi et al., 1991).

Therefore, the hypermotility in test rats as compared to

CR lead to the postulation that SSET and SMET have influenced the NE levels which is known to be involved in the mediation of locomotor activity and stimulant activity.

Anxiolytic behavior in SSET, SMET and CR rats was evaluated using light/dark test. Rats when placed in the light/dark box spent more time in the lit compartment as an index of anxiolytic behavior (Crawley and Goodwin, 1980). Studies also validated that administration of anxiolytics significantly increased locomotion and time spent in light region in light/dark box test (Imaizumi et al., 1994). Typically, anxiety is associated with the increase in brain NE level (Mathew et al., 2008). It was confirmed that NE neurotransmitter system activated by stress has a specific role in ameliorating anxiety (Tanaka et al., 2000). Conversely, it was reported that NE microinjections in brain dorsal periaqueductal gray area produces anxiolytic effects (Pelosi et al., 2009). It was further endorsed in a study that increase in NE activity is not only attributed to anxiety like behavior of stress, but also activation of NE contributes to the anxiolytic effect. The dual nature of this increase in NE is due to the difference in neurotransmission regulation (Ordway et al., 2007). Evidences suggest that NE system undergoes modification according to the response associated with condition of stress (Rodrigues et al., 2009). The pathological anxiety is attributed with time dependent phasic modulation within the NE system (Schulz et al., 2002). Hence, the treatment response in consequence of pharmacological modulation of NE is critical. In a report, it was revealed that pharmacological interventions targeting to increase NE system resulted in anxiolytic rather than anxiogenic response (Goddard et al., 2010). This was further authenticated by testing clonidine, a α2 adrenergic agonist which significantly stimulates the release of growth hormone (GH) in healthy individuals (Devesa et al., 1991). A more detailed study account was reported in a study comprising healthy individuals and anxiety patients. It was reported that patients with anxiety when administered with clonidine exhibit low levels of GH. Contemporaneously healthy subjects tested for response with clonidine exhibit an increase in GH levels, thus concluded that anxiety is associated with hyporeactivity of NE receptors (Cameron et al., 2004). Further from another point of view, it should be noted that NE neurons co expressed the neuropeptide galanin besides NE. Though, the release of galanin occurs from the same neuronal system its release does not affect brain NE levels, thus over expressed galanin springs into action by over expressed NE (Ordway et al., 2007). It was stated that the administration of galanin in rats induced anxiolytic like behavior (Bing et al., 1993).

Therefore, it could be deduced that SSET and SMET might have stimulated galanin form NE neurons besides NE and have produced an anxiolytic response. However, this needs to be evaluated further.

In the current study, brown seaweeds *S. swartzii* and *S. marginatum* were screened for psychotherapeutic profile. It has been suggested that seaweeds possess significant psychostimulant and anxiolytic activity by ameliorating brain norepinephrine levels. The present study emphasized the potential application of seaweeds as future pharmaceutical candidates for treatment of psychiatric disorders. However, the work can be further extended to evaluate the underlying mechanism behind the active principles in seaweeds for eliciting such a response and the exact causes of NE increase in brain after seaweeds administration.

Conflict of interests

The authors have not declared any conflict of interests.

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