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Original Article

ANXIOLYTIC ACTIVITY OF MARINE MACROALGAE *SARGASSUM ILICIFOLIUM* AND *PADINA TETRASTOMATICA* IN MICE

SUBHASH R. YENDE*[1], UDAY N. HARLE[2], ABHAY M. ITTADWAR[1]

[1]Department of Pharmacology, Gurunanak College of Pharmacy, Nagpur (India), [2]Clinical Research Consultant, Mahalaxmi Nagar, Nagpur (India)
Email: subhashyende@gmail.com

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ABSTRACT

Objective: The present study was designed to investigate an anxiolytic effect of chloroform and ethanol extract of *Sargassum ilicifolium* (SI) and *Padina tetrastomatica* (PT) in mice.

Methods: Acute toxicity study was done as per OECD 423 guidelines. Based on acute toxicity studies, doses of 200, 400 and 600 mg/kg of SI and PT extracts were selected, and the anxiolytic activity was assessed using Elevated plus maze (EPM) and light/dark exploration (L/DE) tests.

Results: Diazepam (2 mg/kg), the ethanol extract of SI (400 mg/kg) as well as ethanol extract of PT (600 mg/kg) significantly increased time spent and entries into the open arm in EPM test. In L/DE test Diazepam, chloroform extract (600 mg/kg) and ethanol extract (400 mg/kg) of SI as well as chloroform extract (600 mg/kg) and ethanol extract (600 mg/kg) of PT significantly increased time spent in light box and transition between the boxes.

Conclusion: in the present investigation, ethanol extract at 400 and chloroform extract at 600 mg/kg of *Sargassum ilicifolium* as well as ethanol extract at 400 and 600 mg/kg and chloroform extract at 600 mg/kg of *Padina tetrastomatica* exhibited an anxiolytic effect in the experimental model of anxiety. However, additional research will be necessary to investigate the mechanism underlying this anxiolytic activity.

Keywords: *Sargassum ilicifolium, Padina tetrastomatica,* Brown algae, Anxiolytic activity

INTRODUCTION

Anxiety, an emotional state is one of the most frequently occurring psychiatric disorder [1]. This is increasingly recognized as a highly prevalent and chronic disorder with incidences of 18.1 % and prevalence of 28.8% [2]. Despite a steady increase in the development of anxiolytic agents, the prevalence of the disorder remains stable that could be attributed to the unknown neurobiological understanding of pathophysiology or the inconsistent efficacy of current pharmacological treatment. Selective Serotonin Reuptake Inhibitors (SSRIs) are the commonly prescribed drugs for the treatment of depression and several anxiety disorders. Unfortunately, the onset of action of SSRIs is often delayed by 3–6 w. The existence of this delayed action combined with the fact that one-third of patients do not respond to treatment [3]. Benzodiazepine class agents have widespread therapeutic potentials and are generally prescribed medication for the treatment of several forms of anxiety, but these agents have prominent side effects, such as sedation, dependence, cognitive and psychomotor impairment [4, 5]. These are some of the factors that cause interest for many researchers to evaluate new compounds from a natural source with fewer unwanted side effects. Many traditional herbs and herbal medicines have been reported as anxiolytic agents. However, the marine flora has not explored up to that extent for their CNS activity. Hence, we undertook the study to evaluate CNS potential of some marine macroalgae.

Marine macroalgae or seaweeds are found in the coastal region between high tide to low tide and in the subtidal region up to a depth where 0.01 % photosynthetic light is available. Marine macroalgae have created a promising significance in the biomedical area, mainly because of their contents of bioactive substances. Polysaccharides, terpenoids, phlorotanins, fucoidans, sterols, and glycolipids show wide range of pharmacological properties which includes anticancer, anti-inflammatory, antimicrobial, antiviral, antioxidant, hypoglycemic, hepatoprotective and neuroprotective activities [6, 7]. Also, some marine organism and marine macroalgae showed the potential as a source of new drugs for the treatment of neurological disorders [8, 9]. Due to natural constituent and availability, marine flora are believed to provide less untoward effect profiles and provide greater effectiveness as compared to the synthetic drug.

Sargassum ilicifolium (Turner) C. Agardh is tropical and sub-tropical brown algae, distributed in the open intertidal coast of Gujarat, Maharashtra, Goa, Karnataka and Lakshadweep. Ethyl acetate extract of *Sargassum ilicifolium* has been reported to possess immunomodulatory activities [10], alcoholic extract of this seaweed reported for antibacterial activity [11], analgesic and anti-inflammatory activity [12] and antioxidant and anticancer activity [13]. Furthermore, the antidepressant-like activity of *Sargassum ilicifolium* had been previously reported in mice models of depression [14].

Padina tetrastomatica (Hauck) is a marine brown alga found in the coastal areas of India. Methanolic extract of *Padina tetrastromatica* have reported for spasmogenic, anti-fertility, hypotensive properties [15] and *in vitro* antioxidant activity [16, 17]. Sulphated polysaccharide from *Padina tetrastromatica* showed significant anti-inflammatory activity against carrageenan induced paw edema [18]. Also, it reported to possess anti hepatitis B virus activity [19]. Furthermore, chloroform, ethanol, and water extract of *Padina tetrastromatica* showed antimicrobial activity against gram positive, gram negative bacterial and fungal test [20].

However, the anxiolytic effect of *Sargassum ilicifolium* and *Padina tetrastomatica* has not been investigated. Hence, we evaluated the anxiolytic activity of *Sargassum ilicifolium* and *Padina tetrastomatica* using experimental models as Elevated Plus Maze (EPM) test and Light/Dark Exploration (L/DE) Test.

MATERIALS AND METHODS

Animals

Swiss albino mice (25-30 g) were used for the experiment purpose. The animals were housed in solid-bottomed polypropylene cages and acclimatized to animal house conditions (temperature 25±2 °C, 12 h light: 12 h dark cycle and relative humidity 50±5%). The mice

were fed with commercial standard diet and water *ad libitum*. The experiments were designed and conducted in accordance with the guideline of CPCSEA and approved by Institutional Animal Ethical Committee (Approval No. GNCP/IAEC/2011-12/P'cology-01).

Drugs and chemicals

Diazepam (Calmpose Inj. Ranbaxy, India) was purchased from a local vendor and used as reference drug. Chloroform (Hi Media Laboratories, Mumbai), Ethanol (Research Lab Fine Chem, Mumbai) were used for extraction of SI and PT. The drug was dissolved in distilled water and all extracts were suspended in 5 % Tween 80.

Seaweed collection and extracts preparation

The brown seaweeds, *Sargassum ilicifolium* (SI) and *Padina tetrastomatica* (PT) were collected from the inter-tidal rocky shore of Bhatkarwada, Ratnagiri coast in Nov-Dec 2011. The seaweed species was identified by Professor B. B. Chaughule, Emeritus Professor, Department of Botany, University of Pune, Pune (India). The fresh sample was washed with sea water followed by fresh water to remove salts, epiphytes, microorganisms and other suspended materials, and dried at room temperature. The air-dried and coarsely powdered sample was extracted successively using Soxhlet apparatus by Petroleum Ether, Chloroform, and Ethanol.

Phytochemical analysis

The Chloroform and ethanol extracts of SI and PT were analyzed for the presence of phytochemicals by qualitative analysis [21, 22].

Acute toxicity studies

The Acute toxicity was determined on Swiss albino mice as per OECD-423 guidelines [23]. The overnight fasted animals were administered extracts orally at the dose level of 2000 mg/kg body weight and were continuously observed for 2 h to detect changes in the autonomic or behavioural responses and then, monitored for any mortality for the following 7 d.

Experimental setup

The animals were randomly assigned to experimental groups (6 mice per group) as follows (table 1):

Elevated plus maze (EPM) test

Elevated plus maze is the simplest apparatus to study the anxiolytic response of almost all types of anti-anxiety agents. Modified method as described earlier by Lister (1987) was used in this study [24]. The maze consisted of two opposite open (30 cm×7 cm×0.5 cm) and two opposite closed (30 cm×7 cm×15 cm) arms, extending from a central platform (7 cm ×7 cm) and elevated to a height of 45 cm above the floor. Mice were individually placed on the center of the maze facing an open arm, and the number of entries and the time spent in the closed and open arms were recorded during a 5 min observation period. Arm entries were defined as the placement of all four paws into an arm [25].

Light/dark exploration (L/DE) test

This test is based on the innate aversion of rodents to brightly lit areas and on their spontaneous exploratory behavior in response to a novel environment and to light [26]. The apparatus consisted of two boxes (25×25×25 cm) joined together. One box was made dark by covering its top with plywood and a 40 W lamp illuminated the other box.

The light source was placed 25 cm above the open box. The mice were placed individually in the center of the light box and observed for the next 5 min for a number of crossing to light compartment and time spent in the light box [25].

Table 1: Grouping of animals and treatment

Group	Treatment	Dose
Vehicle	Distilled water	1 ml/100 g, p. o.
Diazepam	Diazepam	2 mg/kg, i. p
CSI 200	Chloroform extract of *Sargassum ilicifolium*	200 mg/kg, p. o.
CSI 400	Chloroform extract of *Sargassum ilicifolium*	400 mg/kg, p. o.
CSI 600	Chloroform extract of *Sargassum ilicifolium*	600 mg/kg, p. o.
ESI 200	Ethanol extract of *Sargassum ilicifolium*	200 mg/kg, p. o.
ESI 400	Ethanol extract of *Sargassum ilicifolium*	400 mg/kg, p. o.
ESI 600	Ethanol extract of *Sargassum ilicifolium*	600 mg/kg, p. o.
CPT 200	Chloroform extract of *Padina tetrastomatica*	200 mg/kg, p. o.
CPT 400	Chloroform extract of *Padina tetrastomatica*	400 mg/kg, p. o.
CPT 600	Chloroform extract of *Padina tetrastomatica*	600 mg/kg, p. o.
EPT 200	Ethanol extract of *Padina tetrastomatica*	200 mg/kg, p. o.
EPT 400	Ethanol extract of *Padina tetrastomatica*	400 mg/kg, p. o.
EPT 600	Ethanol extract of *Padina tetrastomatica*	600 mg/kg, p. o.

All extracts were dissolved in 5 % Tween-80 while; diazepam was dissolved in distilled water. The extracts were administered orally for seven days and anxiolytic activity was carried out on last day of treatment.

Locomotor activity

The locomotor activity (horizontal activity) was measured using digital actophotometer (INCO, Ambala, India). Each mouse was placed in the actophotometer for 5 min and locomotor score was obtained [27].

Statistical analysis

Results were expressed as mean±SEM. The data was analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test, and $P<0.05$ was considered as statistically significant.

RESULTS

Phytochemical analysis

The qualitative phytochemical analysis revealed the presence of sterols, terpenoids, flavonoids, alkaloids, glycosides and saponin in chloroform and ethanol extract of SI and PT (table 2).

Acute toxicity studies

Since no mortality was observed at 2000 mg/kg as well as at 5000 mg/kg. Therefore, doses of 200, 400 and 600 mg/kg of SI and PT extracts were selected for further study.

Elevated plus maze test

In EPM test, ethanol extract of SI (400 mg/kg) and diazepam exhibit significant ($p<0.01$) increased in time spent in the open arm, whereas there was significant ($p<0.05$) decreased in time spent and number of entries in the close arm, compared to the vehicle treated group. While, CSI (200, 400, 600 mg/kg) and ESI (200, 600 mg/kg) did not produce a significant effect on behaviour of mice in EPM (fig. 1). Also ethanol extract of PT (600 mg/kg) and diazepam significantly ($p<0.01$) increased time spent in open arm, EPT (400 and 600 mg/kg) significantly ($p<0.05$) increased number of entries in open arm and EPT (600 mg/kg) significantly ($p<0.01$) decreased time spent in the open arm compared with vehicle-treated group.

While CPT (200, 400, 600 mg/kg) and EPT (200, 400 mg/kg) showed no significant change on EPM test (fig. 2).

Light/dark exploration test

In Light/Dark exploration test, diazepam significantly (p<0.01) increased the time spent in the light box as well as the number of crossing between light and dark boxes. The treatment with CSI (600 mg/kg) and ESI (400 mg/kg) significantly (p<0.05) increased the time spent in the light box, while ESI (400 mg/kg) significantly (p<0.05) increased number of crossing between light and dark boxes. However, no significant difference was observed on this parameter at CSI (200, 400 mg/kg) and ESI (200, 600 mg/kg) as compared to the vehicle-treated group (fig. 3).

Furthermore, the treatment with diazepam, CPT (600 mg/kg) and EPT (600 mg/kg) showed significant (p<0.05) increase in the time spent in the light box. While diazepam, CPT (600 mg/kg) showed significantly (p<0.01) increased number of crossing between light and dark boxes. Whereas, CPT (200, 400 mg/kg) and EPT (200, 400 mg/kg) did not produce significant change on the Light/Dark exploration test as compared with vehicle-treated group (fig. 4).

Table 2: Qualitative phytochemical analysis of extracts of *Sargassum ilicifolium* and *Padina tetrastomatica*

Plant constituents	CSI	ESI	CPT	EPT
Tests for Tannins	-	-	-	-
Test for Steroids	+	+	+	+
Test for terpenoid	+	+	+	-
Tests for Flavonoids	+	+	+	-
Tests for Alkaloids	+	+	+	+
Tests for Carbohydrates	+	+	+	+
Test for Glycosides	+	+	-	+
Tests for Saponin	-	+	-	-

+positive; -negative

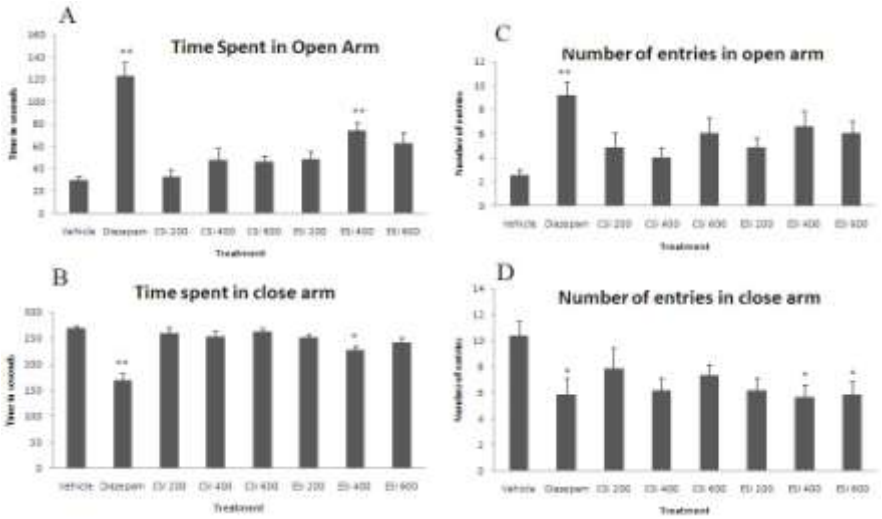

Fig. 1: Effect of *Sargassum ilicifolium* on (A) time spent(s) in open arm, (B) time spent(s) in closed arm, (C) entries in open arm and (D) entries in closed arm in elevated plus maze test
(n=6); * p<0.05, ** p<0.01, significantly differ from vehicle

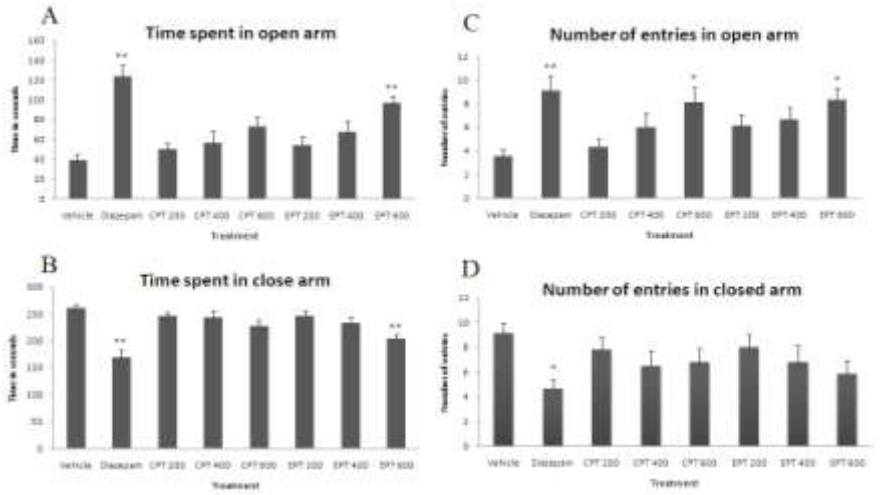

Fig. 2: Effect of *Padina tetrastomatica* on (A) time spent(s) in open arm, (B) time spent(s) in closed arm, (C) entries in open arm and (D) entries in closed arm in elevated plus maze test
(n=6); * p<0.05, ** p<0.01, significantly differ from vehicle

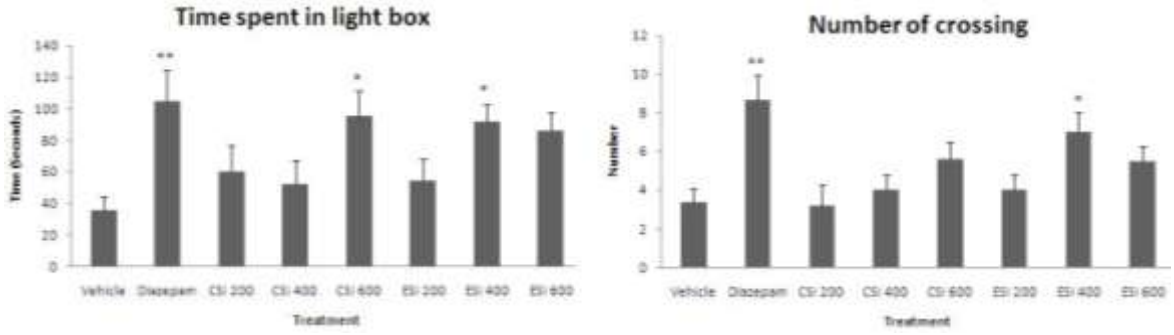

Fig. 3: Effect of *Sargassum ilicifolium* on time spent in light box and number of crossing in Light/Dark exploration test (n=6); * $p<0.05$, ** $p<0.01$, significantly differ from vehicle

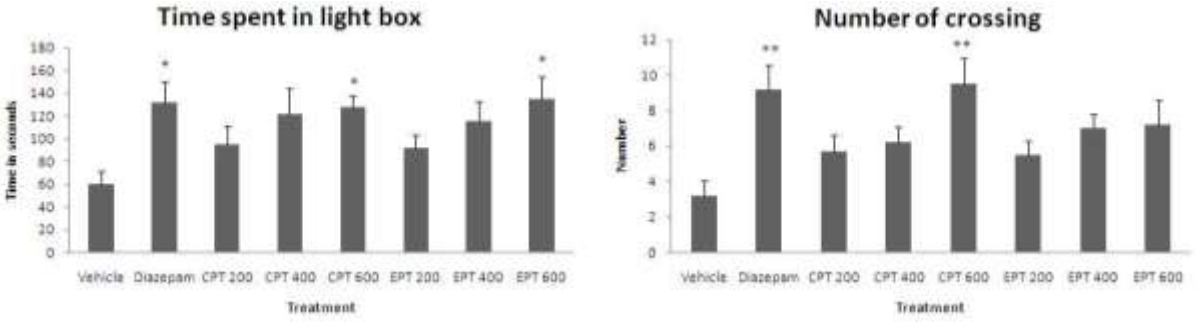

Fig. 4: Effect of *Padina tetrastomatica* on time spent in light box and number of crossing in Light/Dark exploration test (n=6); * $p<0.05$, ** $p<0.01$, significantly differ from vehicle

Locomotor activity

Diazepam ($p<0.01$) and EPT (600 mg/kg) ($p<0.05$) significantly reduced locomotor score. However, other doses of chloroform and ethanol extract of SI and PT did not produce a significant difference in the locomotor score of mice, when compared with vehicle-treated group (fig. 5 and fig. 6).

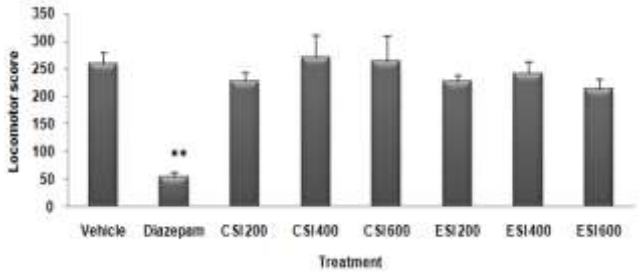

Fig. 5: Effect of *sargassum ilicifolium* on locomotor score in mice (n=6); ** $p<0.01$, significantly differ from vehicle

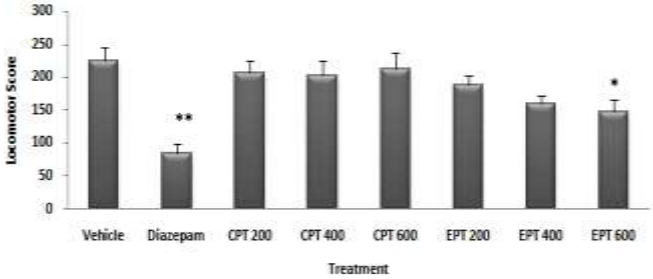

Fig. 6: Effect of *Padina tetrastomatica* on locomotor score in mice (n=6); * $p<0.05$, ** $p<0.01$, significantly differ from vehicle

DISCUSSION

The EPM test is a most popular and valid animal model for measuring anxiety by investigating aspects of physiological and pharmacological behaviour [28, 29]. This test has been widely used to screen anxiolytic or anxiogenic effects because of its use of natural conditions and stimuli to induce anxiety, such as a fear of a new, bright, and open space and the fear of balancing on a relatively narrow raised surface [30, 31]. In this study, the ethanol extract of SI (400 mg/kg) as well as ethanol extract of PT (600 mg/kg) significantly increased time spent and entries into the open arm of EPM, similar to those of diazepam.

The anxiolytic-like activity was also observed in light/dark exploration test. L/DE test is widely used with rodents as a model for screening anxiolytic and anxiogenic drugs, based on the innate aversion of rodents to bright areas and on the spontaneous exploratory behaviours of rodents in response to novel environments and light [26, 32]. In the present study, it was observed that chloroform extract (600 mg/kg) and ethanol extract (400 mg/kg) of SI as well as chloroform extract (600 mg/kg) and ethanol extract (600 mg/kg) of PT significantly increased time spent in light box and transition between the boxes of L/DE test. Diazepam also causes an increase in time spent and latency time to leave the light box in L/DE test.

The locomotor effect of extracts of SI, PT and diazepam, were tested in the present study. However, none of the doses except EPT (600 mg/kg) and diazepam affected spontaneous locomotor activity. Which indicated that ESI (400 mg/kg), CSI (600 mg/kg) and EPT (600 mg/kg) induced anxiolytic effect with an absence of sedative action.

Furthermore, considerable research has been shown that the anxiolytic-like effects of diazepam are mediated through an activation of brain neurotransmitter γ-amino-butyric acid (GABA) at the GABA receptor complex [33]. In another study, we have reported antidepressant-like activity of *Sargassum ilicifolium* in Forced Swim test and Tail Suspension test in mice [14]. The antidepressant-like activity of *Sargassum ilicifolium* was believed to be mediated

through noradrenergic or serotonergic mechanism. Also, Zhang *et al.* (2004) reported that monoaminergic system [serotonin (5HT) and norepinephrine (NA)] in a brain has been postulated to plays an important role in the pathophysiology of anxiety disorders [34]. Which supported by several preclinical and clinical reports indicating dysfunction of the monoaminergic system may be implicated as a promising mechanism in the pathophysiology of anxiety disorders [35-37]. In this regard, anxiolytic-like effects of SI and PT in the present study might be dependent on modulation of GABA and/or monoaminergic (NA and 5HT) system. Further investigations are required to clarify the detailed anxiolytic mechanism.

CONCLUSION

From the results, it can be concluded that chloroform and ethanolic extract of *Sargassum ilicifolium* and *Padina tetrastomatica* at a dose of 400 and 600 mg/kg respectively possess anxiolytic activity in EPM and LDE test, possibly due to the presence of different phytochemicals like alkaloids, steroids, terpenoids, flavonoids present therein. However, further research will be necessary to investigate the mechanism underlying this anxiolytic activity.

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CONFLICT OF INTERESTS

Declared none

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