Iranian Journal of Fisheries Sciences 22(1) 219-237 2023
DOI: 10.22092/ijfs.2023.128817

Research Article

Protective effects of greater lizardfish (*Saurida tumbil*) protein hydrolysates against depression and anxiety in male mice

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Received: March 2021 Accepted: July 2022

Abstract

Aquatic organisms that account for almost half of the world's biodiversity provide valuable sources of bioactive compounds exhibiting a number of physiological and health promoting functions, which make them promising ingredients for application in food and pharmaceutical. *Saurida tumbil* protein hydrolysate was prepared by papain enzyme hydrolysis and its anti-depressant and anti-anxiety effects were investigated in male mice. Fifty male mice were randomly assigned into control (C), chronic resistant stress induced depression and anxiety (CRS) and intervention (CRS mice receiving fish protein hydrolysate (FPH) at 100, 300 and 600 mg/kg) groups. Normal saline and FPH were given to the mice 30 min before inducing CRS by gavage for 21 days. Anxiety and depression were evaluated by elevated plus-maze and open field tests. Total antioxidant capacity (TAC) and malondialdehyde (MDA) level of mice serum were determined. The treatment of CRS mice with FPH significantly ameliorated the negative effects of CRS and significantly increased the number of entering into the open arms and also the time elapsed in the open arms of the elevated plus-maze (p<0.05). FPH administration also significantly increased the number of crossed squares and the number of grooming and rearing in open field tests (p<0.05). The administration of FPH at all studied doses to CRS mice increased TAC (p<0.05) and decreased MDA level (p>0.05). In conclusion, *S. tumbil* protein hydrolysate showed anti-depressant and anti-anxiety effects possibly through its antioxidant activity, which can be suggested for enrichment or designing human functional foods.

Keywords: Fish Protein hydrolysate, *Saurida tumbil*, Antioxidant, Anxiety, Depression

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Introduction

Depression and anxiety are two major mental diseases with high comorbidity (up to 70%), their incidences have increased significantly in recent decades (Azarfarin *et al*., 2018). According to the World Health Organization (WHO), depression and anxiety will be the second leading causes of morbidity by 2030, which imposes significant negative consequences and economic burdens on societies (Osborn *et al*., 2020). It is believed that depression and anxiety have common mechanisms and risk factors. Continued and prolonged exposure to oxidative stress and produced substances such as glucocorticoids can have harmful effects on cells, structure and function of some parts of the brain that are involved in emotion (amygdala and hippocampus), resulting in increased prevalence and severity of depression and anxiety (Murgatroyd *et al*., 2015; Botros *et al*., 2019).

Various chemical drugs are currently being introduced to treat depression and anxiety, however, these drugs are often associated with poor responsiveness and several side effects resulting in decreasing of patient's tendency toward these drugs. Therefore, research in order to find new effective natural compounds has become increasingly important (Khezri *et al*., 2016; Rabiei *et al*., 2017; 2019). Sea creatures accounting for almost half of the world's biodiversity, provide valuable sources of proteins and bioactive compounds including bioactive peptides, oligosaccharides, omega fatty acids, enzymes, minerals, pigments and biological polymers (Korhonen and Pihlanto, 2006; Khezri *et al*., 2016; Rabiei *et al*., 2017; 2019). The use of low value fish species and fisheries by-product for production and recovery of bioactive compounds such as fish protein hydrolysate will create value added and allow the optimized use of these resources. Bioactive peptides are protein particles (2-20 amino acids) that are inactive in their native protein and may be released during digestion (Agyei and Danquah, 2011; Nikoo and Benjakul, 2015). Bioactive peptides, in addition to being source of nitrogen and amino acids in the body, have several physiological and health functions. Moreover, resistance of low molecular weight bioactive peptides to gastrointestinal digestion and easily absorption made them a good option for health promotion and disease control (Shahidi and Zhong, 2008).

Greater lizardfish (*Saurida tumbil*), belongs to the family Synodontidae, is considered as a by-catch species in southern waters of Iran, which accounts for about 35% of by-catch in trawl catch of southern coasts of Iran (Niamaimandi *et al*., 2018). Protein hydrolysates extracted from different underutilized fish and fisheries by-products exhibit different health effects, such as antioxidative (Athmani *et al*., 2015; Rabiei *et al*., 2019), antimicrobial (Tang *et al*., 2015), antihypertensive (Erdmann *et al*., 2006; Murray and FitzGerald, 2007), immunomodulatory (Agyei and Danquah, 2012), anti-

inflammatory (Da Rocha *et al*., 2018; Kemp and Kwon, 2021), anti-thrombotic (Rajapakse *et al*., 2005; Nasri *et al*., 2012), anticancer (Picot *et al*., 2006), and neuroprotective (Lin *et al*., 2018; Hong *et al*., 2019) activities, which make them promising ingredients for application in food and pharmaceutical products. However, *S. tumbil* is a low value fish used for producing animal feed (Ugwuowo *et al*., 2019) and different works focused on introducing it as human food products, such as health mix containing fish powder (Rathnakumar and Pancharaja, 2018) and surimi products (Huda *et al*., 2001). Also, limited studies have been carried out on producing collagen and gelatin from skin, scale and bone (Taheri *et al*., 2009; Jaziri *et al*., 2022) as well as antioxidant protein hydrolysates from muscle (Bahram *et al*., 2022) of this fish species. In this study, the anti-depressant and anti-anxiety effects of *S. tumbil* protein hydrolysates are investigated on chronic resistant stress exposed mice.

Materials and methods

Fish preparation and enzymatic hydrolysis

Fresh S. tumbil specimens were purchased from local fish market in Bushehr, Iran in late December 2017, frozen and placed in Ziploc sealable plastic bags and transported in an ice box containing ice packs in 20 hours to the seafood science laboratory of Islamic Azad University of Ghaemshahr, Mazandaran, Iran. Fish were thawed, washed, filleted and minced twice using a meat grinder (MK-G28NR, Germany), then were kept in several plastic zip top bags at -20°C until use. Samples of minced fish (50 g) were placed in an Erlenmeyer flask and to which 100 mL sodium phosphate buffer solution (pH 6) was added (1:2 w/v) and the mixture was homogenized using a digital homogenizer (IKA, T25, Germany). The mixture was heated at 85°C for 20 min to inactivate endogenous enzymes using shaker water bath (Raad Teb Novin, SL910, Iran). Then, the mixture was cooled and to which the papain enzyme (Papain enzyme from papaya latex (1.5-10 units/mg; Solid, pH 6.0, 40°C) purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA)) at the ratio of enzyme to protein ratio of 1:25 was added and hydrolysis was carried out for 180 min at 55°C in shaker water bath. This condition was chosen based on our previous investigation on different enzyme ratios and different incubation times for hydrolysis of S. tumbil (Bahram *et al*., 2022). The pH of the hydrolysis mixture was kept at 6 which was optimum for papain enzyme. In the next step, to stop the enzymatic reaction the mixture was heated at 95°C for 15 min. Then, the resulting mixture was centrifuged at 8000g for 30 min at 4°C and the supernatant was collected and freeze-dried. The resulting protein hydrolysates was weighed and kept in plastic zip top bags at -20°C until use (Rabiei *et al.*, 2019; Bahram *et al*., 2022).

Determining the amount of protein and the protein hydrolysis yield

Total amount of minced fish protein was determined by Kjeldahl method (AOAC, 2005) and the amount of soluble protein in supernatant was determined using Biuret method (Layne, 1957). Protein hydrolysis yield was calculated using the following formula:

Protein hydrolysis yield (%) = 100 × Supernatant soluble protein content/total minced fish protein

The total yield of protein hydrolysates was calculated as a percentage of the weight of the substrate.

Evaluation of in vitro antioxidant activity DPPH radical scavenging activity Briefly, 1 mL of *DPPH solution* (0.1 mM DPPH prepared in 95% ethanol) *was added to* 1 mL of FPH at different concentrations and incubated for 15 min in *dark condition* at room temperature. Then absorbance of the mixture was recorded at 517 nm against blank *sample (*distilled water instead of FPH). DPPH radical scavenging activity was calculated using the following formula (Gülçin, 2006; Rabiei *et al*., 2019):

$$\text{DPPH radical scavenging activity (\%)} = [(A_{blank}-A_{sample}) /A_{blank}]\times 100$$

ABTS radical scavenging activity Freshly prepared ABTS solution (reacting 10 mL of 7.4 mM ABTS with 10 mL of 2.6 mM potassium persulfate for 12h in the darkness at room temperature) was diluted with methanol to obtain an absorbance of 1.1±0.02 at 734 nm. Then, 2850 µL of ABTS solution was added to 150 µL of FPH solution at different concentrations and after incubation at 23±2°C for 1 h, the absorbance was recorded. Blank sample was prepared using 150 µL of distilled water instead of the FPH. ABTS scavenging activity was determined using the following formula (Gülçin, 2006; Rabiei *et al*., 2019):

$$\text{ABTS scavenging activity (\%)} = [(A_{blank}-A_{sample}) /A_{blank}] \times 100$$

Hydroxyl radical scavenging activity

Briefly, one mL of working solution (1.865 mM 1, 10-phenanthroline) was added to 2 mL of FPH solution. The mixture was homogenized and to which 1 mL of H_2O_2 (3% v/v) was added. The mixture was kept for 60 min at 37°C in a water bath, and then the absorbance was recorded at 536 nm. The same prepared sample without added H_2O_2 was regarded as blank and the same prepared sample without added FPH was regarded as negative control. Hydroxyl radical scavenging activity was measured using the following formula (Gülçin, 2006; Rabiei *et al.*, 2019):

Hydroxyl radical scavenging activity (%) = [(Asample-Anegative-control)/(Ablank-Anegative-control)] × 100

Animals and grouping

Male mice (25-30g) spending two weeks of adaptation (23±2°C, 12h light/12h dark, freely access to standard food and water) were randomly assigned into 5 groups (n=10): the control group only *received* normal saline by gavage for 21 days (C), chronic restraint stress group underwent 6 hours daily restraint stress and received normal saline for 21 consecutive days (CRS), interventional groups underwent chronic restraint stress and *received* FPH at doses of 100 (CRS-FPH100), 300 (CRS-FPH300), and 600 (CRS-FPH600) mg/kg, respectively, 30 min before CRS for 21 days by gavage. After 21 days of treatment, the elevated plus maze and open-field tests were used to measure depression and anxiety. After *behavioral examination (*measurement of depression and anxiety), blood serum and also brain samples were taken from the mice and stored at -80°C until biochemical measurements. All animal procedures were in accordance with the Guideline for the Care and Use of Laboratory Animals (Rabiei et al., 2019).

Elevated plus maze test

Elevated plus maze test was used to measure anxiety in mice. This test was performed in a relatively dark and silent chamber. Each mouse was placed gently in the center (elevated 50 cm above the floor) of the device facing the open arm (the apparatus has two opposite open arms and two opposite closed arms) and allowed to explore for 5 minutes. The number of entries and the time spent in each arm were recorded (Carola et al., 2002).

Open-field test

Open-field test is commonly used to study behaviors such as locomotor activity, depression and anxiety in laboratory animals. The device is a quadrant box (30× 30× 15cm) with the floor divided into squares of 10×10 cm. Each mouse was placed in the central square and the number of squares crossed with four paws as well as rearing and grooming behavior were measured for 6 min (Carola *et al.*, 2002).

Measurement of serum malondialdehyde (MDA) level

Samples of serum (200 µL) were mixed with 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% thiobarbituric acid and 200 µL of 8.1% sodium dodecyl sulfate. The mixture was mixed with 700 µL of distilled water and heated in a boiling water bath for 60 minutes. After cooling under tap water, distilled water (1 mL) and n-butanol/pyridine solution (5 mL) was added to the reaction mixtures and shaken vigorously. Then, the resulting solutions were centrifuged at 4000rpm for 10 min and optical absorbance of the supernatant at 532 nm was recorded (Rabiei *et al.*, 2014).

Measurement of serum total antioxidant capacity (TCA)

Ferric reducing antioxidant power (FRAP) assay was used to measure the total antioxidant capacity of serum and

tissue homogenate. 25 µL of serum was added to 1.5 mL of working FRAP solution (acetate buffer (10 mL, 0.25M, pH= 3.6), TPTZ (5 mL, 10 mM, prepared in 40 mM HCl) and $FeCl_3.6H_2O$ (2.5 mL, 20mM) and incubated at 37°C for 10 minutes. Then, optical absorbance at 593 nm was recorded (Rabiei *et al*., 2014).

Statistical analysis

All experiments were carried out in triplicate and the results were presented, as mean±standard deviation. Data were analyzed using SPSS20 software. Normal distribution of data was checked using Kolmogorov-Smirnov test. Analysis of Variance (ANOVA) followed by Duncan's test was used to identify statistical differences between means. P value less than 0.05 was considered statistically significant.

Results

S. tumbil muscle used for producing protein hydrolysate had 74.80±1.99, 19.71±1.68, 4.41±0.09 and 1.29±0.01 % (wet weight) for moister, protein, fat and ash, respectively. The yield of protein hydrolysis was 47.2%, which indicated high efficiency of the papain enzyme in hydrolysis of minced fish. The inhibitory activities of different concentrations of protein hydrolysates against DPPH, ABST and hydroxyl free radicals are shown in Figure 1. *S. tumbil* protein hydrolysates at a concentration of 2 mg/mL exhibited significant inhibitory activity against DPPH, ABST and hydroxyl free radicals (p<0.05).

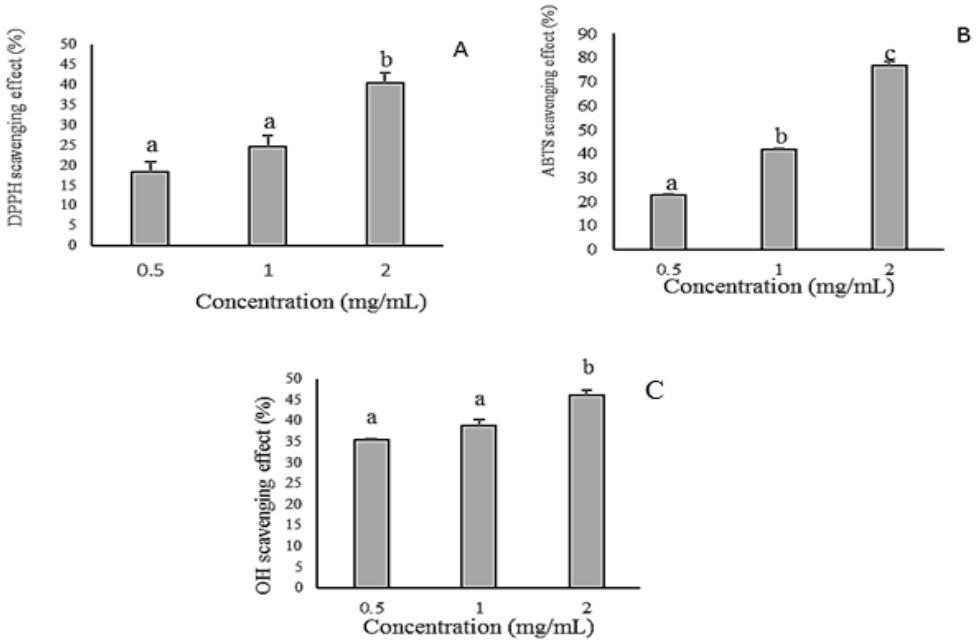

Figure 1: Inhibitory activity of different concentrations of Saurida tumbil protein hydrolysates against DPPH (A), ABST (B) and hydroxyl (OH) free radicals (C). Error bars represent standard deviation. Different letters indicate significant difference among concentrations of fish protein hydrolysate (p<0.05).

In this study, elevated plus maze and open-field tests were used to evaluate anxiety and depression in mice. The results of elevated plus maze test for number of entering into the open/ closed arms and also the time spent in open / closed arms are presented in Figures 2 and 3, respectively. Based on the results, exposure of mice with CRS significantly decreased number of entering into open arms and increased number of entering into closed arms (p<0.05). In a similar way, exposure of mice with CRS significantly reduced the time spent in open arms and significantly increased the time elapsed in closed arms (p<0.05). These results indicated the induced anxiety and depression, and also the decreased exploratory activity in CRS treated mice. Our results showed that treatment of mice exposed to CRS with fish protein hydrolysates at doses of 100, 300 and 600 mg/kg could significantly ameliorate negative effects observed for CRS groups. Administration of FPH significantly increased the number of entering into the open arms and also increased the time spent in open arms and it significantly decreased the number of entering into the closed arms and decreased the elapsed time in the closed arms (p<0.05). In our study, the best-improved effects were observed with increasing the dose of fish protein hydrolysates.

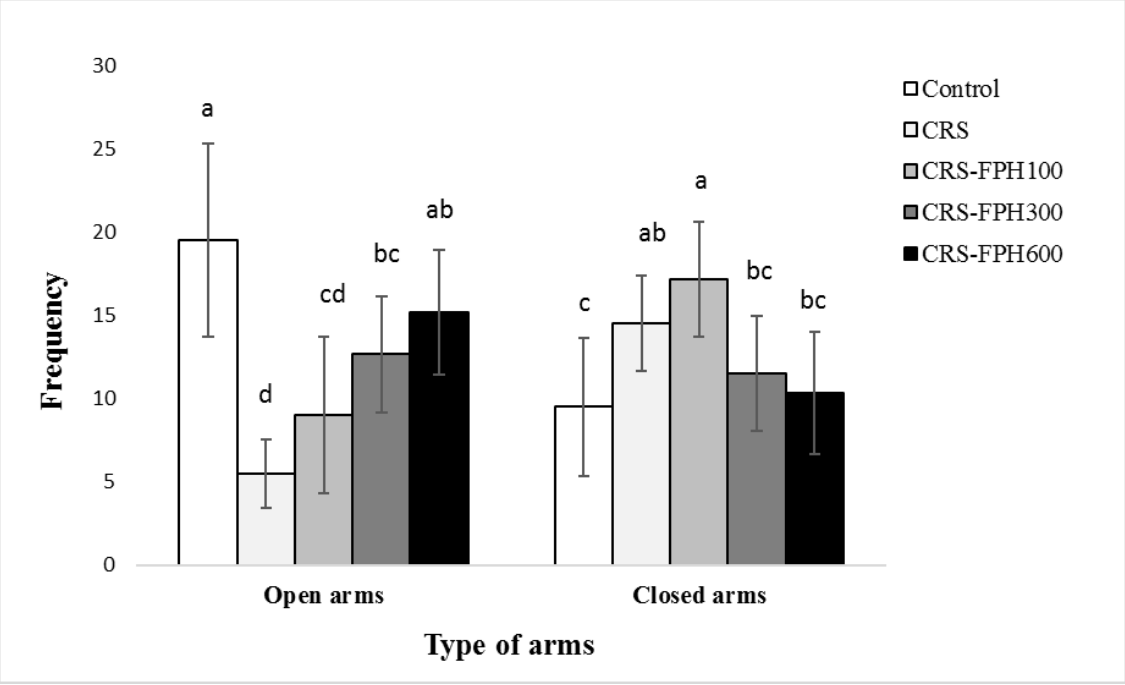

Figure 2: Number of entering into open arms and closed arms of Elevated Plus Maze. Control (mice received normal saline); CRS (mice underwent chronic resistant stress to induce depression and anxiety); CRS-FPH100, CRS-FPH300 and CRS-FPH600 (CRS mice received fish (S. tumbil) protein hydrolysate at 100, 300 and 600 mg/kg, respectively). Error bars represent standard deviation. Different letters indicate a significant difference (p<0.05) among different treatments.

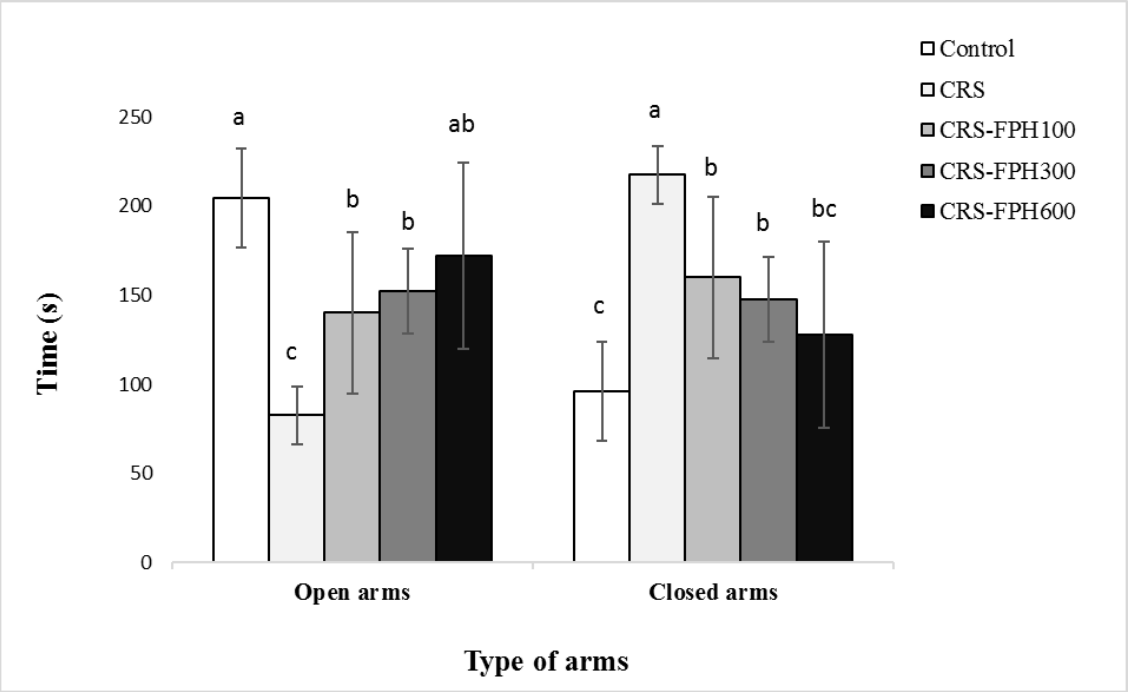

Figure 3: The time spent in open arms and closed arms of Elevated Plus Maze. Control (mice received normal saline); CRS (mice underwent chronic resistant stress to induce depression and anxiety); CRS-FPH100, CRS-FPH300 and CRS-FPH600 (CRS mice received fish (*S. tumbil*) protein hydrolysate at 100, 300 and 600 mg/kg, respectively). Error bars represent standard deviation. Different letters indicate a significant difference (p<0.05) among different treatments.

Based on the results of open field test, exposure of mice with CRS significantly decreased number of crossed squares and number of rearing and grooming behaviors compared to the control group (p<0.05), indicating induced anxiety-like behaviors. Treatment of CRS exposed mice with 100, 300 and 600 mg/kg fish protein hydrolysates ameliorated the negative effects of CRS and significantly increased number of crossed squares and frequency of rearing and grooming compared to the CRS-mice (p<0.05). The best-improved effects were observed with increasing dose of fish protein hydrolysates (Fig. 4).

The results of serum total antioxidant capacity (TAC) and malondialdehyde (MDA) level in experimental groups are shown in Table 1. Serum antioxidant capacity in the CRS group was significantly lower than that of control group and serum malondialdehyde level showed significant increase (p<0.05), indicating presence of oxidative stress. In our study, treatment of CRS exposed mice with different doses of fish protein hydrolysates resulted in amelioration of negative effects caused by CRC exposure. FPH administration significantly increased serum antioxidant capacity (p<0.05) and decreased serum malondialdehyde levels (p>0.05), which are in line with the result of DPPH, ABST and hydroxyl free radicals scavenging activities.

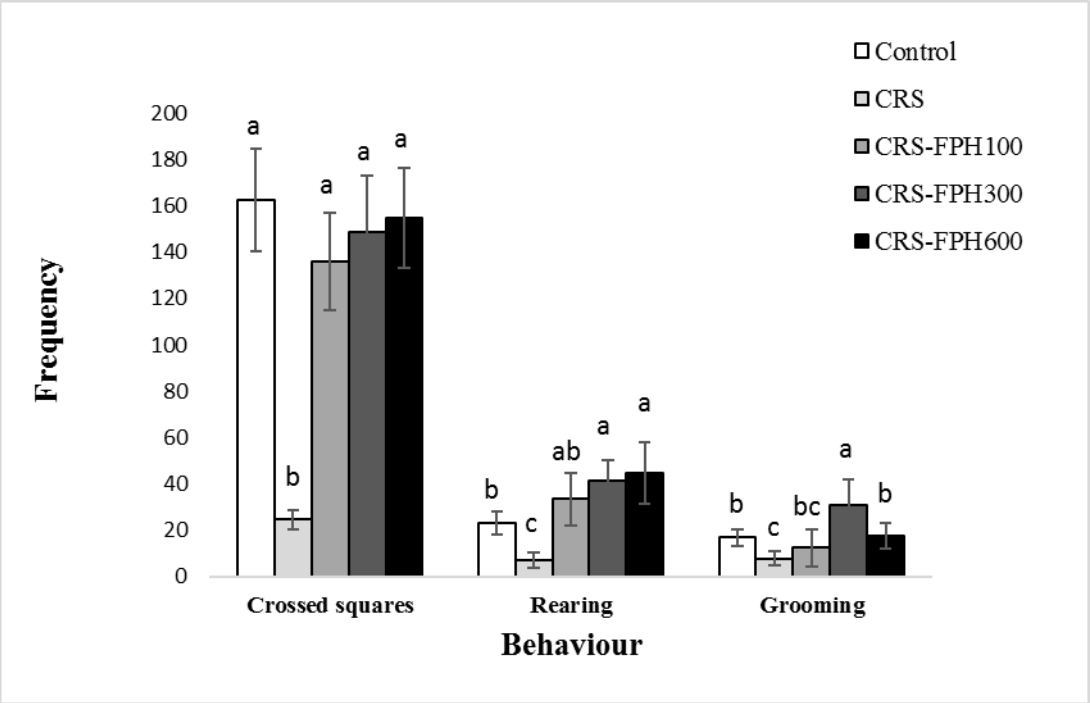

Figure 4: The number of crossed squares, rearing and grooming in the open filed test. Control (mice received normal saline); CRS (mice underwent chronic resistant stress to induce depression and anxiety); CRS-FPH100, CRS-FPH300 and CRS-FPH600 (CRS mice received fish (S. tumbil) protein hydrolysate at 100, 300 and 600 mg/kg, respectively). Error bars represent standard deviation. Different letters indicate a significant difference ($p< 0.05$) among different treatments.

Table 1: Total antioxidant capacity (TAC) and malondialdehyde (MDA) level in experimental groups (±standard deviation).

Experimental groups	Serum TAC (/mL)	Serum MDA (µmol/mL)
Control	1401.58 ± 146.09^{a}	18.01 ± 5.90^{b}
CRS	588.55 ± 106.75^{d}	51.98 ± 4.43^{a}
CRS -FPH100	910.99 ± 25.19^{c}	22.72 ± 7.18^{b}
CRS-FPH300	971.78 ± 129.27^{bc}	19.16 ± 2.84^{b}
CRS-FPH600	1069.19 ± 72.10^{b}	20.64 ± 9.94^{b}

Control (mice received normal saline); CRS (mice underwent chronic resistant stress to induce depression and anxiety); CRS-FPH100, CRS-FPH300 and CRS-FPH600 (CRS mice received fish (*S. tumbil*) protein hydrolysate at 100, 300 and 600 mg/kg, respectively). Different letters in each column indicate significant difference among different treatments ($p<0.05$).

Discussion

Inhibitory activity of *S. tumbil* protein hydrolysate against DPPH, ABST and hydroxyl free radicals indicate its anti-oxidant and free radical scavenging activity, which is important as oxidative stress and free radical damage are among causative agents of depression and anxiety disorders. Our results on anti-oxidant activity of *S. tumbil* protein hydrolysates were comparable and even higher than those reported for gelatin hydrolysates prepared from skin and scale of sole fish (Viji *et al*., 2019), protein hydrolysate prepared from Argentine croaker (Da Rocha *et al*.,

2018) and protein hydrolysates and peptide fractions derived from *Colossoma macropomum* (De Quadros *et al*., 2019). *S. tumbil* protein hydrolysates can stabilize free radicals through donating electron by their peptides and amino acids, such as proline, valine, alanine, and leucine, which subsequently can terminate the chain radical formation. However, peptide size, sequence, amino acid composition, enzyme used, and degree of hydrolysis play important role in determining antioxidant activity (Viji *et al*., 2019; Rabiei *et al*., 2022).

In our study, exposure of mice with CRS significantly decreased number of crossed squares and number of grooming and rearing behaviors (open field test) as well as significantly increased number of entering into closed arms and time elapsed in closed arms (elevated plus-maze test) (p<0.05), indicating induced anxiety and depression-like behaviors. Treatment of mice with fish protein hydrolysates showed modification in CRS induced changes to return to normal levels, indicating anti-depressant and anti-anxiety effects of fish protein hydrolysates. So far, limited studies are conducted on neuroprotective and protective effects of fish protein hydrolysates and bioactive peptides against psychological disorders. In the study conducted by Bernet *et al*. (2000) it is observed that fish protein hydrolysates with commercial name of Gabolysat PC60 showed anti-anxiety effects in rats. The researchers related this effects to influencing of fish protein hydrolysates on HPA axis parameters. In another study, anxiolytic- and antidepressant-like effects of fish oil-enriched diet were reported in mice model (Zemdegs *et al*., 2018). Our results are in line with the above mentioned studies.

One of the main characteristics of exposure to stressful conditions is increase in the activity of HPA axis in response to release of corticotropin-releasing hormone (CRH), which increases chronic levels of glucocorticoids (Swaab *et al*., 2005; Marais *et al*., 2008). Glucocorticoids (including cortisol in human body and corticosterone in rodent's body) interact with their own receptors and provide numerous changes at level of neurons which provide the context for developing mental disorders, such as depression and anxiety (Azarfarin *et al*., 2018). Several mechanisms are involved in increasing level of glucocorticoids in brain and mental disorders. Based on the results of empirical studies, long-term interaction of neuronal cells with glucocorticoids reduces number of dendrites which subsequently changes neuronal plasticity. It seems that oxidative stress and neuronal inflammation play important role in these conditions (Lucca *et al*., 2009; Cohen *et al*., 2012). Repeated cellular contact with glucocorticoids increases production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and it inhibits detoxification capacity of endogenous antioxidants enzymes

(superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic antioxidants (vitamin C and glutathione) in brain, thus, neural cells are in danger of destructive effects of ROS and RNS (Bhattacharya *et al*., 2001).

Nerve cells are very sensitive to free radical damage due to their high levels of unsaturated fatty acids, high oxygen consumption, and a relatively weak antioxidant defense system. Free radicals produced during oxidative stress react with biochemical molecules, including membrane lipids, DNA and proteins, causing cell damage and death (Lucca *et al*., 2009). Therefore, oxidative damage of nerve cells is associated with significant memory and learning damage and emerging mood disorders, such as anxiety and depression (Eren *et al*., 2007).

In our study, CRS treated mice showed significant decrease in serum antioxidant capacity and increase in serum malondialdehyde level (p<0.05), indicating presence of oxidative stress. In previous studies on animal models of chronic stress, also significant increase in peroxidation of brain and serum lipids (Fontella *et al*., 2005), significant increase in protein peroxidation and oxidative damage of DNA in neuronal cells (Liu *et al*., 1996), and significant decrease of activity of antioxidant enzymes (catalase and superoxide dismutase) of brain and serum (Lucca *et al*., 2009) are reported. In our study, the treatment of mice with fish protein hydrolysates showed modification in CRS induced changes to return to normal levels, indicating anti-oxidant effects of fish protein hydrolysates which are in line with results of DPPH, ABST and hydroxyl free radicals scavenging activities. Therefore, it seems that anti-anxiety and anti-depressant effects observed for fish protein hydrolysates are associated with presence of low molecular weight peptides with high antioxidant activity in the protein hydrolysated sample, which can easily be absorbed by digestive system and, after reaching the tissues and cells, can play its own protective effects. In this regards, antioxidant effects of fish protein hydrolysates are evaluated in animal models. In the study of Khaled *et al*. (2012), treatment of lipid-rich diet induced diabetic rats with a *Sardinella aurita* protein hydrolysates significantly reduced lipid peroxidation and increased activity of the antioxidant enzymes (SOD, CAT and GPx) in the liver, but treatment with fish meat alone did not show such effects. Ktari *et al*. (2014) also reported that treatment of aloxane induced diabetic rats with *Salaria basilisca* protein hydrolysates at 400 mg/kg significantly reduced the serum and liver lipid peroxidation and significantly increased activity of antioxidant enzymes (SOD, CAT and GPx) in Kidney and liver. In another study, treatment of mice receiving cholesterol-rich diets with Zebra fish protein hydrolysates significantly reduced lipid peroxidation and increased activity of antioxidant enzymes (SOD and GPx) (Ktari *et al*., 2015). Similar results also reported

protective effect of fish protein hydrolysates against CCl_4 induces oxidative stress and its negative effects on kidney and liver of mice. These protective effects are linked to antioxidant activity of fish protein hydrolysates (Rabiei *et al*., 2019). Moreover, in another study antimicrobial effects of marine derived peptides are reported (Mosquera *et al*., 2016). Some functional properties, including emulsifying activity, emulsion stability, foaming activity, solubility, water holding capacity and oil absorption capacity are reported for fish protein hydrolysates prepared from cuttle fish, *Sepia pharaonis* (Raftani Amiri *et al*., 2016) and orangefin ponyfish, *Photopectoralis bindus* (Ramezani *et al*., 2020). *Caulerpa racemosa* (sea grape), an algae abundant in Indonesia which is not widely used by people, is used for producing protein hydrolysates suggested for using as sea food flavoring agent (Amin *et al*., 2021). Such studies suggest use of fish protein hydrolysates as ingredients in formulated diets, food and beverages. Moreover, protein hydrolysates can be used in fish nutrition and in this regard use of canola protein hydrolysate showed positive effects on growth performance, blood biochemistry, immunity and gastrointestinal microbiota of beluga (*Huso huso*) juveniles (Ebrahimnezhadarabi *et al*., 2021). So, further researche to elucidate all bioactive properties and mechanism of action of fish protein hydrolysates are suggested.

In summary, the results of the present study indicated anti-depressant and anti-anxiety effects of *S. tumbil* protein hydrolysates in chronic stress exposed mice which in part is seemed to be related to antioxidant and free radical scavenging activities of fish protein hydrolysates. Treatment of chronic stress exposed mice with *S. tumbil* protein hydrolysates significantly ameliorated negative effects of chronic stress in mice and significantly increased number of entering into the open arms and also the time elapsed in open arms of the elevated plus-maze ($p<0.05$). Fish protein hydrolysate administration also significantly increased number of crossed squares and number of grooming and rearing in open field tests ($p<0.05$), indicating anti-depressant and anti-anxiety effects of the studied protein hydrolysates. Administration of *S. tumbil* protein hydrolysate at all studied doses to chronic resistant stress exposed mice increased total antioxidant capacity of mice serum ($p<0.05$) and decreased serum malondialdehyde level ($p>0.05$). *S. tumbil* protein hydrolysate showed anti-depressant and anti-anxiety effects possibly through its antioxidant activity, which can be suggested for enrichment or designing human functional foods. Further clinical investigation on effects of fish protein hydrolysates in people with depression and anxiety is suggested.

Acknowledgements

The authors appreciate Qaemshahr Branch, Islamic Azad University for

providing laboratory facilities to conduct the experiments.

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