The modulatory effect of Al-Assi river trout fish meal on OCD manifestations and molecular mechanisms in BALB/c Mice

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ABSTRACT

Background: Obsessive-Compulsive Disorder (OCD) is a type of anxiety disorder that is marked by intrusive and distressing thoughts, as well as repetitive behaviors. Trout fish (Oncorhynchus mykiss) is a functional food that might have potential therapeutic effects on many neurological disorders including OCD.

Objective: This study aims to explore the effects of Al-Assi River trout fish meal, a dietary source of tryptophan, on obsessive-compulsive disorder (OCD) symptoms and related molecular pathways in BALB/c mice.

Methods: OCD mice were divided into five groups: one control group without any treatment, one group treated with fluoxetine (a selective serotonin reuptake inhibitor), and three groups fed with different doses of trout fish meal (0, 7.5, and 15 g/kg body weight). The mice were subjected to various behavioral tests, such as the marble test, tail suspension test, sucrose preference test, and forced swim test, to evaluate OCD and depressive-like behaviors. Moreover, the expression and protein levels of genes involved in the serotonergic and GABAergic systems were measured.

Results: The results indicated that trout fish meal had dose-dependent effects on OCD-like behaviors, revealing exacerbation at lower doses and improvement at higher doses. For instance, in the marble test, OCD mice fed with 7.5 g of trout fish/kg body weight buried more marbles than those fed with 15 g/kg of trout fish (4.5 vs 3.33 out of 6, p>0.05). In the tail suspension test, the immobility time of OCD mice treated with fluoxetine was numerically lower than that of the untreated OCD mice (63.6 vs 87.3 seconds, p>0.05). Furthermore, normal mice had different baseline gene
expression profiles than OCD mice. Normal mice had the highest fold increase of Gabra gene expression (3.75) compared to the untreated OCD group, followed by groups treated with 7.5 and 15 g of trout fish/kg body weight (2.02 and 1.44, respectively).

Conclusions: This study suggests that dietary interventions rich in tryptophan, such as trout fish meal, may have modulatory effects on OCD symptoms and molecular mechanisms in mice. However, the optimal dosing and individual variability need to be considered. More research is required to clarify the underlying mechanisms and to evaluate the potential efficacy of trout fish meal in treating OCD in humans.

Keywords: BALB/c mice, OCD, qPCR, Western blotting, Gabra, Serotonin, Trout fish, Tryptophan.

INTRODUCTION

Obsessive-compulsive disorder is a neuropsychiatric condition that falls within the category of anxiety disorders, characterized by the presence of distressing and intrusive cognitive phenomena termed obsession and concomitant behavioral manifestations known as compulsions. These obsessions consist of recurrent and persistent thoughts, images, or urges that are experienced as intrusive and unwanted, leading to marked distress. The ensuing compulsions represent repetitive behavioral or mental acts aimed at neutralizing or alleviating the distress stemming from obsessions [1].

Scientifically, the etiology of OCD involves intricate interactions among genetic predisposition, neurobiological modifications, and environmental influences. Neuroimaging inquiries have unveiled conspicuous structural and functional deviations within cortical-subcortical circuits that govern affective processing, fear conditioning, and cognitive adaptability. Specifically, anomalous neural operation in regions such as the orbitofrontal cortex, anterior cingulate cortex, and basal ganglia have been implicated in the pathophysiology of OCD [2].
OCD is precipitated by disturbances in communication between deeper brain structures and the frontal cortex. These regions of the brain primarily utilize serotonin for communication, which elucidates the potential efficacy of augmenting serotonin levels in alleviating OCD symptoms [3].

Gamma-aminobutyric acid (GABA) is the neurotransmitter most strongly associated with serotonin. GABA relies on serotonin for its optimal function. GABA exerts its influence via a negative feedback mechanism that counteracts heightened excitatory neurotransmitters. This feedback mechanism, however, hinges on the neuromodulatory effects of serotonin [4].

Currently available evidence continues to endorse the primary use of selective serotonin reuptake inhibitors (SSRIs) as the first-line pharmacological intervention for OCD. Generally, 40% to 60% of OCD patients will witness a partial decrease in symptoms upon treatment with an SSRI [5]. SSRIs obstruct the reuptake of serotonin in the brain, augmenting its availability. The term "selective" pertains to their predominant impact on serotonin, as opposed to other neurotransmitters. Prominent SSRIs encompass fluoxetine (Prozac), fluvoxamine (Luvox), paroxetine (Paxil), citalopram (Calera), escitalopram (Lexapro), and sertraline (Zoloft) [5].

Among these SSRIs, Fluoxetine stands out as one of the most widely employed for OCD treatment. The efficacy of fluoxetine in addressing OCD was validated in randomized multicenter phase III clinical trials. Combined outcomes from these trials disclosed that 47% of patients treated with the highest dose reported being "much improved" or "very much improved" after 13 weeks of therapy, compared to only 11% in the placebo group [5]. The American Academy of Child and Adolescent Psychiatry recommends SSRIs, including fluoxetine, as the primary therapeutic approach for children, alongside cognitive behavioral therapy (CBT), to manage moderate to severe OCD [6].

However, an alternative approach involving dietary choices may hold promise. Tryptophan is important for the synthesis of serotonin, a neurotransmitter that regulates mood, sleep, appetite, and cognition in humans. Tryptophan deficiency can lead to depression, insomnia, anxiety, and impaired memory. One of the sources of tryptophan in the human diet is fish, especially rainbow trout (Oncorhynchus mykiss). Rainbow trout is a high-protein food that contains about 17.3 grams of protein per 100 grams of edible portion [7]. The protein quality of rainbow trout is high, as it provides all the essential amino acids in adequate amounts. The tryptophan content of rainbow trout is about 0.3 grams per 100 grams of protein, similar to other fish species such as salmon, tuna, and cod [8]. Compared to the other amino acids found in rainbow trout, tryptophan has a lower concentration but a higher requirement for human health [8].

The Al Assi River hosts a distinctive variety of fish that are particularly abundant in tryptophan content, which is being explored as a potential source for elevating serotonin levels in scientific investigations [9]. This study aims to evaluate the impact of dietary trout fish meal on alleviating OCD-like behaviors in BALB/c mice, along with the underlying molecular serotonergic and GABAergic system.

**METHODS**

**Experimental Groups:** BALB/c female mice (8 weeks old) were used as the animal model for this study. The mice were housed in a temperature-controlled room with a 12-h light/dark cycle. They had free access to water and a standard pellet diet containing 24% protein, including 0.3% tryptophan, 4.5% fat, and 4% fibers (Lab CHOW T8604 Harlan USA) [10-11]. The mice were randomly divided into five groups of 6 mice each. OCD-like behavior was induced in mice of 4 groups (A, B, C, D) through intraperitoneal injection of 15mg of RU24969/kg body weight. Group E, the control, was not administered.
chemicals nor treated with dietary fish meal. Groups A, B & C received 0, 7.5, 15 g of trout fish meal/kg body weight daily and for 21 days. The trout fish meal, containing 0.21% Tryptophan on an as-is basis [8], was provided separately in a clean aluminum dish inside the cage. Group D received 15mg of fluoxetine/kg body weight intraperitoneally once a day for 21 days.

The calculated daily Tryptophan intake per mouse for groups B and C is 10.6 and 11.0 mg, respectively, 4 and 8% higher than the remaining groups (A, D, and E).

**BEHAVIORAL TESTS**

**Marble test:** The marble test is a straightforward method for assessing OCD-like behavior in rodents by observing how many marbles they hide, a behavior indicative of anxiety or OCD. Rodents that are more anxious or have OCD hide more marbles. The test was done as follows: 1) The cage was filled with wood chip bedding about 5-10 cm deep and made flat, 2) six glass marbles were placed on the bedding in a regular pattern, with about 4 cm between each one; 3) one animal was placed in each cage and left for 60 min; 4) the number of marbles that were mostly buried (more than 2/3 of their size) was counted and recorded [12].

**Tail suspension test:** TST is a method to study how immobile rodents become when they are depressed, which is a common symptom of OCD in humans and rodents. It is a widely used, reliable, and sensitive method to test the effects of antidepressant drugs. It is based on the fact that if a rodent is in a situation where it has no control, then it will give up and stop moving. Each mouse was suspended by its tail from a tube about 10 cm from the ground for five minutes. During this time, the mouse would try to escape and reach the ground. The time that each mouse was immobile was measured. Each mouse underwent a single test session conducted in isolation from other mice [13].

**Sucrose preference test (SPT):** SPT is a method to measure how much rodents like sucrose, which is a sign of enjoying rewards in OCD. It also shows how motivated and happy the animals are. Before the test, mice were used to having two bottles to drink from: one with 2% sucrose and one with water for four days in their cage. After this, mice could drink either the 2% sucrose or the water for three days. The amount of water and sucrose they drank was measured daily, and the bottles were switched daily to avoid any bias caused by a preference for a side. Sucrose preference was calculated as a percentage of how much sucrose they drank out of the total amount of liquid they drank and averaged over the three days of testing [14].

**Forced swim test:** The forced swim test measures how a rodent reacts to the fear of drowning, which is seen as a sign of how prone they are to negative emotions. It is often used to test how well antidepressants work, as well as the behavioral despair of giving up, which is another sign of depression and hopelessness in OCD. Mice were put in two trials where they had to swim in a water-filled cylinder made of acrylic glass that they could not escape. The first trial was for 15 minutes; after 24 hours, a second trial was done for 5 minutes. The mouse's time in the second trial was measured without moving more than needed to keep its head above water [15].

**Nestle shredding:** This test measures how much repetitive compulsive behavior mice have, a feature of OCD. It also resembles the human compulsion to collect or clean things and is a new, reliable, and realistic test. Each mouse was put in a different cage with a single nestle made of cotton fiber that was weighed before. The nestle was covered with a filter top. The mouse was left alone in the cage with the nestle for 30 min without water or food. The mouse was then removed and put back in its home cage after the test. The remaining nestle material not used by the mouse was taken out of the cage...
with forceps, dried, and weighed [12]. The difference in nestle weight before and after the trial was calculated for each test animal and recorded.

Molecular tests: Gabra & SCL6A4 gene expression and protein quantification in mice brains

Gene expression assessment using real-time PCR: At the end of the behavioral tests, mice were decapitated using a guillotine, and their brains were removed, cut into two halves, weighed, snap-frozen using liquid nitrogen, and stored at -80°C for further analysis. Several studies have recommended the use of the whole brain for the analysis of serotonin and Gaba as they are both highly expressed in various brain regions related to mood and anxiety [16].

Approximately 50 mg of brain tissue was minced and placed in a labeled conical tube. Total RNA was extracted from brain cells using the Zymo kit (Sigma, St. Louis, MO, USA) and quantitated using nanodrop spectrophotometry (ImplenNp80, 81829 Munchen Germany.). Total RNA was subjected to reverse transcription using the FIRE script Reverse Transcriptase (Sigma, St Louis, MO, USA). Briefly, 200 ng of extracted RNA were added to 1μL of 10x RT reaction premix with Oligo(dt) and random primers (SOLIS BODYNE, Tartu, Estonia), and the mixture was incubated at 65 °C for 5 min and cooled to room temperature. The cDNA was quantitated using iTaq Universal Syber-green PCR (Bio-Rad, CA, USA) and a specific set of primers targeting the Serotonin transporter and Gabra genes (Table 1). The following cycling conditions were adopted: initial denaturation of 95 °C for 30 sec, followed by 35 cycles of 95 °C for 15 sec, 60 °C for 1 min, melting curve 65 °C for 3 sec, 95 °C for 3 sec. The raw data was exported to Microsoft Excel for analysis [17]. The ΔΔct method was used to assess the fold increase of the investigated genes against β actin that was used as a housekeeping gene.

### Table 1. The set of primers used in this study: [17-18]

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession number</th>
<th>Forward primer</th>
<th>Amplicon length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabra1</td>
<td>XM_017314259</td>
<td>Forward: CCAAGTCTCCTTCTGGCTCAACA</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: GGGAGGCAATTTCTGGGCACGTGAT</td>
<td></td>
</tr>
<tr>
<td>SLC6A4</td>
<td>NM_010484.2</td>
<td>Forward: GTTGATGCTGCGGCTCAGATCT</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: GAAGCTCGTCATGCAGTTCACC</td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>NM_007393</td>
<td>Forward: AGGCCAACCGTGAAAGATG</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse: ACCAGAGGCATACAGGGACAA</td>
<td></td>
</tr>
</tbody>
</table>

Quantitation of Gaba and serotonin proteins using Western blotting

An amount of 100 µg of proteins from brain homogenate was mixed with 16µl of Laemmlı buffer and 1 µl β-mercaptoethanol (Bio-Rad, USA). Each sample was then subjected to 95°C for 5 minutes and loaded onto 14-well polyacrylamide gels (Mini-PROTEIN TGX stain-free gels, Bio-Rad, USA). Gel electrophoresis was performed at 300V for 20 min, and then the banded peptides were transferred directly onto nitrocellulose membranes (NCM) (Bio-Rad, Germany). NCMs were blocked with 5% gelatin in Tris-Buffered Saline (TBS) for 2 hours and then washed two times for 5 min with Tween 20-TBS (TTBS). The NCMs were incubated with the primary antibodies specific for the protein of interest for one hour in 1% gelatin in TTBS. Finally, after washing with TTBS (2 times for 5 min each), NCMs were incubated with secondary antibodies conjugated to horseradish peroxidase in 1% gelatin in TTBS for an hour. The bands were detected using chemiluminescence reagents (Clarity Western ECL Substrate, Bio-Rad, CA, USA); the
banded proteins’ intensity was evaluated using Image Lab software (Bio-Rad, CA, USA). The primary antibodies included in this study were goat anti-mouse Gabra1 antibodies and goat anti-mouse serotonin antibodies [19].

**Statistical analysis:** The behavioral test parameters and the fold increase in the expression of the Gabra and serotonin transporter genes and protein levels were compared between various treatments using One-Way ANOVA followed by Tukey’s test for mean separation (SPSS.V.25, IBM, Chicago, Illinois, USA). Significant differences among means were presented at p<0.05.

**RESULTS**

**Behavioral tests:** The marble test revealed different numbers of buried marbles, with the highest score of 4.5 out of 6 belonging to Group C (OCD models given 7.5g of trout fish/kg body weight). However, the Nestle remaining weight test indicated that Group A (OCD model) had the lowest weight at 0.14 g, suggesting higher Nestle shredding activity than other groups.

Regarding the immobility time in the tail suspension test (TST), Group A (OCD model) had the longest immobility time at 87.3 seconds, followed by Group C at 75.3 seconds. On the other hand, Group B (OCD models injected with 15 mg of fluoxetine/kg body weight) had the shortest immobility time at 63.6 seconds. Similarly, in the forced swim test (FST), Group C had the longest immobility time at 86.8 seconds, followed by Group A at 77.8 seconds (Table 2).

Moreover, the sucrose test results showed a consistent and significant trend over three consecutive days. Group A had the highest daily sucrose intake on the 3rd day at 3.3 ml, followed by Group B at 1.5 ml. In contrast, Group D had the lowest intake at 1.3 ml on the 3rd day. These groups also had significantly lower water intake on days 1, 2, and 3 than the control group. For instance, on the 1st day, Group D consumed only 2.2 ml of water intake, while the control group consumed 3.9 ml (p<0.05) (Table 3).

**Table 2. Statistical identification of the behavioral test outcomes among various mice groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>OCD induction using RU24969</th>
<th>Treatment</th>
<th>Number of mice</th>
<th>Number of buried marbles (/6)</th>
<th>Immobility time in TST (sec)</th>
<th>Immobility time in FST (sec)</th>
<th>Nestle Remaining weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>yes</td>
<td>-</td>
<td>6</td>
<td>4.3</td>
<td>87.3</td>
<td>77.8</td>
<td>0.1</td>
</tr>
<tr>
<td>B</td>
<td>yes</td>
<td>15 mg fluoxetine /kg body weight</td>
<td>4</td>
<td>4</td>
<td>63.5</td>
<td>67.2</td>
<td>0.1</td>
</tr>
<tr>
<td>C</td>
<td>yes</td>
<td>7.5g trout fish /kg body weight</td>
<td>6</td>
<td>4.5</td>
<td>75.3</td>
<td>86.8</td>
<td>0.1</td>
</tr>
<tr>
<td>D</td>
<td>yes</td>
<td>15g trout fish /kg body weight</td>
<td>4</td>
<td>3.3</td>
<td>65.2</td>
<td>61.8</td>
<td>0.1</td>
</tr>
<tr>
<td>E</td>
<td>no</td>
<td></td>
<td>6</td>
<td>3.3</td>
<td>84.3</td>
<td>65.6</td>
<td>0.2</td>
</tr>
<tr>
<td>SEM'</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.26</td>
<td>3.4</td>
<td>3.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

'SEM = Standard Error of Mean

**Table 3. Sucrose and water intake of mice in various groups were included in the study.**
**Gabra and Serotonin gene expression:** The results showed that fluoxetine treatment (Group B) led to a 1.41-fold increase in Gabra expression. Additionally, trout fish consumption (Groups C and D) also increased Gabra expression by 2.02-fold and 1.44-fold, respectively, in OCD models. Interestingly, normal mice (Group E) had the highest Gabra expression, with a fold change of 3.75 compared to the control group (Table 4).

Regarding serotonin gene expression, the study found that fluoxetine treatment (Group B) significantly increased expression by 2.03-fold compared to the control group A. In contrast, trout fish consumption (Groups C and D) decreased serotonin expression, with fold changes of -1.1 and -1.55, respectively. Notably, normal mice (Group E) exhibited a lower serotonin expression level, with a fold change of -2.77 compared to the control group A (Table 4).

**Table 4. Results of RT-qPCR analyses of Gabra and Serotonin gene expression in various mice groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>OCD induction using RU24969</th>
<th>Treatment</th>
<th>Number of Mice</th>
<th>Gene expression fold (increase+)/decrease (-)</th>
<th>Gabra</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>yes</td>
<td>-</td>
<td>6</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>yes</td>
<td>15 mg fluoxetine/kg body weight</td>
<td>4</td>
<td>+1.41</td>
<td>+2.03</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>yes</td>
<td>7.5g trout fish/kg body weight</td>
<td>6</td>
<td>+2.02</td>
<td>-1.1</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>yes</td>
<td>15g trout fish/kg body weight</td>
<td>6</td>
<td>+1.44</td>
<td>-1.55</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>no</td>
<td>-</td>
<td>6</td>
<td></td>
<td>+3.75</td>
<td>-2.77</td>
</tr>
<tr>
<td>SEM*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>3.175</td>
<td>2.041</td>
</tr>
</tbody>
</table>

1,2: Means in a column with different numerical superscripts are significantly different (p<0.05)
*SEM = Standard Error of Means.

**Gabra and Serotonin transporter second protein quantification:** When examining the GABRA protein expression in the various treatment groups compared to the control group A, which serves as the reference point with a value of 1, notable differences emerged. In Group B, where OCD models were injected with 15 mg of fluoxetine/kg body weight, there was a slight increase with a fold change of +1.10, indicating a marginal rise in
GABRA levels. Conversely, Groups C and D, which received different doses of trout fish, displayed decreases in GABRA protein expression, with fold changes of -2.03 and -1.36, respectively. On the other hand, Group E, representing normal mice, exhibited a modest increase in GABRA protein expression with a fold change of +1.24, indicating a slight elevation compared to the control group.

Regarding serotonin protein expression, the treatment groups showed distinct patterns compared to the reference control group A, which had a value of 1. In Group B, where fluoxetine was administered to OCD models, there was a decrease in serotonin expression with a fold reduction of 0.80. Group C, receiving 7.5g of trout fish/kg body weight, exhibited a notable increase in serotonin protein expression with a fold change of +1.0, suggesting a positive impact. However, Group D, receiving a higher dose of trout fish (15g/kg body weight), showed a decrease in serotonin expression with a fold change of -1. Remarkably, in Group E, normal mice displayed consistent serotonin protein expression with a fold change of 1.1 compared to the control group (Table 5).

Table 5. Variability of Gabra and Serotonin concentration as a function of treatment doses in various mice groups

<table>
<thead>
<tr>
<th>Group</th>
<th>OCD induction using RU24969</th>
<th>Treatments</th>
<th>Fold increase (+)/decrease (-) of proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GABRA</td>
</tr>
<tr>
<td>A</td>
<td>yes</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>yes</td>
<td>15 mg fluoxetine /kg body weight</td>
<td>+1.10²,³</td>
</tr>
<tr>
<td>C</td>
<td>yes</td>
<td>7.5 g trout fish/kg body weight</td>
<td>-2.03¹</td>
</tr>
<tr>
<td>D</td>
<td>yes</td>
<td>15g trout fish/kg body weight</td>
<td>-1.36¹,²</td>
</tr>
<tr>
<td>E</td>
<td>no</td>
<td>Normal mice</td>
<td>1.24¹</td>
</tr>
<tr>
<td>SEM²</td>
<td>-</td>
<td>-</td>
<td>1.758</td>
</tr>
</tbody>
</table>

1-3 Means in a column with different numerical superscripts are significantly different (p<0.05).
*SEM = Standard Error of Means.

**DISCUSSION**

The aim of this study was to investigate the effects of trout fish meal, which is rich in tryptophan, on the behavioral and biochemical parameters of OCD models in mice. The results revealed that trout fish had different impacts on the OCD models depending on the dose administered. The OCD models administered 7.5 g of trout fish/kg body weight. They exhibited more compulsive behaviors, such as marble burying and Nestle shredding, than the OCD models given 15 g of trout fish/kg body weight or the control group. Moreover, the OCD models given 7.5 g of trout fish/kg body weight also showed more depressive-like behaviors, such as increased immobility time in the TST and FST, than the other groups. On the contrary, the OCD models given 15 g of trout fish/kg body weight showed similar or lower levels of compulsive and depressive-like behaviors than the control group. Additionally, the sucrose test results indicated that the OCD models had a higher preference for sucrose than the normal mice and that the trout fish treatment did not affect this preference.
These findings suggest that trout fish may have a dose-dependent effect on the serotonergic system of OCD models in mice. Tryptophan is an essential amino acid that is a precursor of serotonin, a neurotransmitter that is involved in the regulation of mood, anxiety, and compulsive behaviors. Previous studies have shown that tryptophan depletion can induce or exacerbate OCD symptoms in humans and animals, while tryptophan supplementation can improve OCD symptoms. However, the optimal dose of tryptophan for OCD treatment may vary depending on several factors, such as the baseline serotonin levels, the availability of other amino acids, and the presence of inflammation.

In this study, it is possible that the OCD models given 7.5 g of trout fish/kg body weight received insufficient tryptophan to increase their serotonin levels or had a lower absorption rate of tryptophan due to competition with other amino acids. This may have resulted in an intensification of their OCD and depressive-like behaviors due to a further reduction in serotonin availability. On the other hand, the OCD models given 15 g of trout fish/kg body weight may have received a sufficient or excessive amount of tryptophan to boost their serotonin levels, or they may have had a higher absorption rate of tryptophan due to lower competition with other amino acids. This increase in serotonin availability may have resulted in an improvement or normalization of their OCD and depressive-like behaviors.

The sucrose test results also support the hypothesis that trout fish may have a dose-dependent effect on the serotonergic system of OCD models in mice [20]. Previous studies have shown that sucrose preference positively correlates with serotonin levels in rodents and that tryptophan depletion can decrease sucrose preference, while tryptophan supplementation can increase sucrose preference [21]. In this study, it is possible that the OCD models had a higher baseline sucrose preference than the normal mice due to their lower serotonin levels, as suggested by their increased immobility time in the TST and FST. Moreover, it is possible that the trout fish treatment did not affect their sucrose preference because it did not alter their serotonin levels significantly enough to change their hedonic state.

The results of RT-qPCR analyses showed that fluoxetine and trout fish had different effects on the expression of Gabra and Serotonin genes in OCD models in mice. Gabra is a gene that encodes for the alpha subunit of the GABA-A receptor, a major inhibitory neurotransmitter receptor in the brain. Serotonin is a gene that encodes for the serotonin transporter, responsible for serotonin reuptake from the synaptic cleft. Both genes are implicated in the pathophysiology and treatment of OCD, as well as other neuropsychiatric disorders [22].

In the fluoxetine treatment (Group B), results showed a significant increase in both Gabra and Serotonin expression, suggesting a positive impact on the GABAergic and serotonergic systems. Fluoxetine is a selective serotonin reuptake inhibitor (SSRI), which blocks serotonin reuptake and increases its availability in the synaptic cleft [23]. Previous studies have shown that fluoxetine can also enhance the expression and function of GABA-A receptors in rodents, which may contribute to its anti-anxiety and anti-compulsive effects. Moreover, fluoxetine has been shown to be effective in reducing OCD symptoms in humans and animals [24].

In trout fish treated groups (C and D), increased Gabra expression but decreased Serotonin expression was observed in OCD models. This suggests that trout fish may have a differential effect on the GABAergic and serotonergic systems. As mentioned earlier, trout fish are rich in tryptophan, a serotonin precursor. However, tryptophan can also be converted into other metabolites, such as kynurenine, quinolinic acid, and melatonin, which can modulate the activity of GABA-A receptors [25-26]. Therefore, it is possible that trout fish may increase the production of these metabolites, which may enhance the
expression and function of GABA-A receptors and may reduce the expression and function of serotonin transporters [25].

The control group of mice (Group E) had the highest Gabra expression, but the lowest serotonin expression compared to the control group A. This indicates that normal mice may have a higher baseline activity of the GABAergic system but a lower baseline activity of the serotonergic system than OCD models. This may explain why normal mice exhibited less compulsive and depressive-like behaviors than OCD models in the behavioral tests.

Western blot analyses showed that fluoxetine and trout fish had different effects on the protein expression of GABRA and Serotonin in OCD models in mice. Regarding fluoxetine treatment (Group B), a slight increase in GABRA protein expression but a decrease in Serotonin protein expression was observed compared to control group A. This suggests that fluoxetine may have a mixed effect on the GABAergic and serotonergic systems.

In addition, trout fish consumption in Groups C and D showed a decrease in GABRA protein expression but had different effects on Serotonin protein expression in OCD models. Group C, receiving 7.5g of trout fish/kg body weight, exhibited an increase in Serotonin protein expression, while Group D, receiving 15g of trout fish/kg body weight, showed a decrease in Serotonin protein expression. This suggests that trout fish may have a dose-dependent effect on the GABAergic and serotonergic systems. Group E had a modest increase in GABRA protein expression, but a consistent serotonin protein expression compared to control group A. This indicates that normal mice may have a higher baseline activity or expression of the GABAergic system but a stable activity or expression of the serotonergic system than OCD models. This corroborates the previous findings whereby normal mice showed less depressive-like behaviors than the OCD model.

**CONCLUSION**

In conclusion, this study underscores the importance of dosing and individual variability in response to tryptophan-rich dietary interventions such as fish containing various bioactive compounds [27]. When OCD mice were treated with 15 g of trout fish per kg of body weight, their OCD-like behavior was significantly reduced. However, when they were treated with 7.5 g of trout fish per kg of body weight, there was no noticeable difference. The molecular analysis revealed that trout fish consumption increased Gabra gene expression in the brains of OCD mice models. Nevertheless, OCD mice receiving 7.5 g of dietary trout fish per kg of body weight showed increased serotonin protein expression. In comparison, those receiving 15 g of trout fish per kg of body weight showed decreased expression of the same protein. This suggests that trout fish, probably through its tryptophan content, can influence the serotonergic and GABAergic systems in a dose-dependent manner, impacting OCD-like behaviors in mice. Further research is needed to elucidate the specific mechanisms underlying these effects and to explore their potential application in OCD treatment in humans.

**List of Abbreviations:**
- OCD - Obsessive-Compulsive Disorder, SSRI - Selective Serotonin Reuptake Inhibitor
- Gabra - Gamma-Aminobutyric Acid Receptor Alpha, qPCR - Quantitative Polymerase Chain Reaction, GABA - Gamma-Aminobutyric Acid, CBT - Cognitive Behavioral Therapy, RU24969 - A chemical compound used to induce OCD-like behavior in mice, TST - Tail Suspension Test, SPT - Sucrose Preference Test, FST - Forced Swim Test, Gabra - Gamma-Aminobutyric Acid Receptor Alpha, RT-qPCR - Reverse Transcription Quantitative Polymerase Chain Reaction, SEM - Standard Error of Mean, GABA - Gamma-
Aminobutyric Acid, β-Actin - Beta-Actin (a housekeeping gene), NCM - Nitrocellulose Membrane, TBS - Tris-Buffered Saline, TTBS - Tween 20-Tris-Buffered Saline, ANOVA - Analysis of Variance

**Competing interests:** The authors declare no financial or non-financial competing interests associated with interpreting the data or presenting information in this manuscript.

**Authors’ contribution:** FS conceived of the study and drafted the manuscript. FS and RS carried out the in-vivo trial and Laboratory analyses. MF and HS participated in the design of the study and performed statistical analysis. AJ provided the in-vivo trial facility. MF, HS, AJ, and MK helped draft the manuscript. All authors read and approved the final manuscript.

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