POTENTIAL ANTIDEPRESSANT EFFECT OF OLIVE OIL IN ADULT MALE RATS EXPOSED TO RESTRAINT STRESS: ROLE OF BRAIN STEM SEROTONIN

By

Noha A. Nassef

Medical Physiology Department, Faculty of Medicine, Ain Shams University. Cairo, Egypt.

ABSTRACT

Background: Olive oil (OO) has neuroprotective effects and is inversely associated with depression risk. However, the role of neurotransmitter systems in the pathophysiology of depression is still unclear.

Objectives: In the present study, the antidepressant effect of olive oil and the underlying neurochemical mechanism were investigated.

Materials and methods: Twenty eight male albino rats were allocated into four equal groups: Control group, OO-supplemented group (OO was given in a daily dose of 300 μl/kg by gavage 5 days a week for 2 consecutive weeks, restraint stress (RS) group, and OO+RS group. Forced swim test was performed and immobility time was measured. Serotonin, malondialdehyde (MDA) and glutathione peroxidase (GPx) in the brain stem homogenate, and cortisol level in the serum were measured.

Results: Restraint stress group showed significant decrease in immobility time during forced swimming test, along with significant increase in serum cortisol, serotonin and MDA, and significant decrease in GPx. In the OO+RS group, immobility time was significantly reduced compared to restraint stress group and compared to control group. Cortisol, serotonin and MDA decreased and GPx increased.

Conclusion: Olive oil has antidepressant potential which could be mediated via its antioxidant not its neurochemical effects.

Keywords: Olive oil, antidepressant, glutathione peroxidase, serotonin.

INTRODUCTION

Restraining is one form of animal models that was used in studying the stress-induced depression and behavioral dysfunctions (S?ntha et al., 2016). The role of neurotransmitter systems in the pathophysiology of depression was previously investigated. Some neurotransmitters and hormones are involved in the pathogenesis of depression such as CCK, gastrin-releasing peptide, NPY, BDNF, ghrelin and Leptin (Lang et al., 2015). Among these neurotransmitters, serotonin is claimed in pathogenesis of depression (Cowen and Browning, 2015). Serotonergic innervations are widely spread throughout the brain with cell bodies of origin lying in the raphe nucleus. After synthesis, serotonin (5-HT) is transported by the vesicular monoamine transporter and stored in vesicles at the neuronal presynaptic endings. When neurons fire, these vesicles fuse with the synaptic membrane and release 5-HT into the synaptic cleft. Released 5-HT can bind to many different receptors of which several subtypes are involved in the actions of antidepressants and antipsychotics.
(Richardson-Jones et al., 2010). However, not all depressed patients show serotonin abnormalities, and not all patients benefit from drugs enhancing serotonergic neurotransmission. Also, some antidepressant drugs have no effect on serotonin neurotransmitter (Jans et al., 2007). Furthermore, pharmaceutical drugs are not very effective and they also have side effects. Mediterranean diet, containing high amounts of olive oil, has been inversely associated with depression risk (Lang et al., 2015). Furthermore, olive oil was described to have antidepressant and anxiolytic effects and was recommended as a treatment for depression and anxiety (Perveen et al., 2013). Olive oil is also known for its antioxidant effects, while oxidative stress and inflammation were reported to be involved in the pathophysiology of depression (Lang et al., 2015). However, meta-analysis study reported that omega-3 FA did not produce significant effects in the treatment of depression (Bloch and Hannestad, 2012). Therefore, the aim of the present study was to investigate the antidepressant effect of olive oil and the underlying neurochemical mechanism.

MATERIALS AND METHODS

Chemicals: Extra virgin olive oil (OO) was obtained from Siwa, Egypt.

Animals: Twenty eight male albino rats of local strain were purchased from an experimental animal farm in Helwan, and were kept in the Physiology Department Animal House under standard conditions of boarding and feeding with free access to water. Animals were kept in their cages, (30cm width x 50cm length x 20 cm height, 3-4 rats/cage), well ventilated, and in normal day/night cycle. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Ethical approval was obtained for the study from the Research Ethics Committee, Faculty of Medicine, Ain Shams University (FMASU, REC, Cairo, Egypt). The rats were fed a diet constructed in our laboratory according to the normal nutritional dietary requirement (59% of food intake from carbohydrates, 7% from fat, 21% from protein, 13% from minerals and ash) (Hallfrisch et al., 1979). The period of the present study was 2 weeks during which animals were allocated into four groups, 7 rats each:

Group I: Control group (control): Freely moving rats all through the 2 weeks study period.

Group II: Extra virgin olive oil-supplemented group (OO): OO was given in a daily dose of 300 ?l/kg by gavage 5 days a week for 2 consecutive weeks (Nassef, 2017) (Positive control).

Group III: Restraint stress group (RS): rats in this group were kept freely moving in the 1st week then were subjected to restraint stress 1 hour/ day for 5 consecutive days in the 2nd week.

Group IV: Extra virgin olive oil-supplemented, restraint stress group (OO+RS): OO was administered as in group II. In the 2nd week, rats in this group were subjected to RS as in group III.

Restraint stress model: Restraint rats were put in a specially built size-manipulable cabin (dimensions of 8 x 6 x 10 cm, one rat/cabin), 1 hour/day for 5 consecutive days at room temperature (20-24°C). Rats were deprived from food and water during this period. The restraint was
performed at the same time of the day between 10-12 am.

Forced swim test (FST) was performed for the assessment of depression-like symptoms. It was performed as described by Detke et al. (1997). The test was performed in the animal room and no acclimation period was necessary. Rats were taken from their home cage, placed individually in transparent tanks filled with water up to certain height such that animal is supposed to swim. Four rats were tested at a time, each in a separate tank, for 6 minutes, then were placed back in their home cages. Rats from different groups were represented in each session. This minimized the effect of changes in environmental circumstances. Also, the four rats per session prevented the extension of the experiment into many hours and thus resulting in a situation in which rats were tested at same time of the circadian cycle. The sessions were recorded with a smart digital video camera (Samsung, WB1100F), data were uploaded to a lab top and then analyzed blindly. The behavior of interest was to calculate the immobility time expressed in seconds (mobility is considered any movements other than those necessary to balance the body and keep the head above the water). A manual cumulative stopwatch was used to measure the time spent mobile.

Intestinal motility was assessed by counting the fecal pellets during the swimming session (FPs). Fecal pellets were also counted in restraint cages (FPr).

Experimental procedures: On the day of sacrifice, rats were weighed, anesthetized by intraperitoneal injection of sodium pentobarbital (25 mg/kg). A midline abdominal incision was made, and the abdominal aorta was exposed and cannulated with a polyethylene catheter. Blood sample was collected and centrifuged at 3000 rpm for 15 minutes. Serum was collected in aliquots and kept frozen at −80°C for determination of cortisol. The brain was then removed from skull. The membrane covering the brain was removed with the help of fine forceps. The brain stem was then taken out using spatula and stored at −80°C for determination of neurochemical and oxidant-antioxidant state. Frozen brain stem samples were homogenized in phosphate buffer saline (pH 7.4) using an electric homogenizer. The supernatants were carefully collected after centrifugation for 15 min at 3000 rpm.

Biochemical analysis:
1. **Serum cortisol** was determined by ELISA method using Rat Cortisol ELISA Kit supplied by Mybiosource, USA (Watts and Tindall, 1988).

2. **Serotonin**: The neurochemical analysis was performed to estimate concentrations of serotonin in the brain stem of rats by Rat 5 Hydroxytryptamine (5-HT) ELISA Kit (My Bio Source, USA). The method was performed according to Aronson et al. (1995).

3. **Glutathione peroxidase** (GPx) in the brain stem homogenate was performed by the UV method using Glutathione Peroxidase Kit supplied by Biodiagnostic, USA (Paglia and Valentine, 1967).

4. **Lipid peroxide** (malondialdehyde) in the brain stem homogenate was determined by the colorimetric method using Lipid peroxide (Malondial-
dehydrate) Kits supplied by Biodiagnostic, USA (Ohkawa et al., 1979).

**Statistical analysis:** Statistics were done using statistical package for the social sciences (SPSS) program (SPSS Inc., version 20). All data were expressed as mean ± standard error of mean (SEM). Statistical significance for data was determined using a one-way analysis of variance (ANOVA) with post-hoc test. Significance was calculated by LSD (least significant difference), and the level of significance was accepted as P <0.05.

**RESULTS**

**Changes in immobility time:** In the present study, restraint stress group showed positive antidepressant effect as indicated by the significant decrease in the immobility time compared to control group (P<0.05). OO+RS group demonstrated more antidepressant effect compared to RS group (P<0.05), and compared to control group (P<0.001). Unstressed OO supplemented group also showed significant antidepressant effect compared to control group (P<0.001 Table 1).

**Changes in intestinal motility:** Intestinal motility increased in the RS group and in the OO group compared to control group, but did not reach significant levels. This increase reached significant levels (P<0.005) in the combined OO+RS rats compared to the control. Intestinal motility was also found to be significantly increased in the OO+RS compared to the RS (P<0.05), as indicated by fecal pellets counted during the restraint session, supporting the idea that OO has increased the intestinal motility (Table 1).

**Table (1):** Immobility time (Imm), fecal pellets counted during the swimming session (FPs) and fecal pellets counted during the restraint session (FPr) in the different studied rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>OO</th>
<th>RS</th>
<th>OO+RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imm (S)</td>
<td>309.2±5.96</td>
<td>250.3±11.0 a</td>
<td>285.0±3.87a</td>
<td>256.3±7.8ab</td>
</tr>
<tr>
<td>FPs</td>
<td>2.29±0.5</td>
<td>3.0±0.4</td>
<td>3.0±0.8</td>
<td>5.29±0.5 ab</td>
</tr>
<tr>
<td>FPr</td>
<td>--------</td>
<td>--------</td>
<td>2.7±0.3</td>
<td>3.9±0.4 b</td>
</tr>
</tbody>
</table>

a: Significant change when compared to control group.
b: Significant change when compared to RS group.

**Changes in biochemical and neurochemical parameters:** Biochemical and neurochemical tests demonstrated that the stress hormone cortisol significantly increased in restraint stress group compared to control group (P<0.05). OO supplemented restraint stress group showed significant decrease in cortisol compared to restraint stress group (P<0.05, Table 2).

Results also showed significant increase in the synthesis of serotonin in the brain.
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stem of RS rats compared to control group (P<0.001). OO supplemented restraint stress group showed significant decrease in serotonin synthesis compared to restraint stress group (P<0.001, Table 2). Regarding oxidant antioxidant state, RS group showed significant decrease in GPx and significant increase in MDA compared to control group (P<0.001). The balance was recovered back in the OO+RS group which showed significant increase in GPx and significant decrease in MDA compared to RS group (P<0.001, Table 2).

Table (2): Serum cortisol, serotonin (5-HT), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels in the brain stem tissues in different studied rat groups.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>Control</th>
<th>OO</th>
<th>RS</th>
<th>OO+RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (ng/ml)</td>
<td>0.37±0.03</td>
<td>0.46±0.05</td>
<td>6.04±2.21</td>
<td>1.10±0.09</td>
</tr>
<tr>
<td>5-HT (ng/mg ptn)</td>
<td>2.8±0.7</td>
<td>2.6±0.6</td>
<td>11.9±1.9</td>
<td>4.0±0.8</td>
</tr>
<tr>
<td>GPx (umol/mg ptn)</td>
<td>72.3±4.9</td>
<td>69.2±7.1</td>
<td>24.6±3.8</td>
<td>51.4±2.1</td>
</tr>
<tr>
<td>MDA (nmol/mg ptn)</td>
<td>6.4±1.2</td>
<td>7.2±1.2</td>
<td>84.5±16.5</td>
<td>23.0±4.0</td>
</tr>
</tbody>
</table>

a: Significant change when compared to control group.
b: Significant change when compared to RS group.

There was a significant positive correlation between fecal pellets counted during the swim session and fecal pellets counted during the restraint session (r=0.6, P<0.01) in the data obtained from RS and OO+RS groups, indicating that the increase in intestinal motility during FST was associated with an increase in intestinal motility during restraint session (Figure 1).

There was a significant negative correlation between fecal pellets counted during the swim session and immobility time during the FST (r=−0.46, P<0.05) in the data pooled from the four studied groups. This indicated that the positive antidepressant effect was associated with enhanced intestinal motility (Figure 2).
Serum cortisol showed significant positive correlation with MDA production in the brain stem tissue ($r=0.62$, $P<0.01$) and significant negative correlation with GPx production in the brain stem tissue ($r=-0.66$, $P<0.01$) in the data pooled from the four studied groups. This correlation pointed to the relation between cortisol hormone as a stress hormone and the oxidative stress in the brain stem neurons (Figure 3).

Similarly, brain stem serotonin showed significant positive correlation with MDA production in the brain stem tissue ($r=0.61$, $P<0.001$) and significant negative correlation with GPx production in the brain stem tissue ($r=-0.65$, $P<0.001$) in the data pooled from the four studied groups. This correlation pointed to the relation between serotonin neurotransmitter and the oxidative stress in the brain stem neurons (Figure 4).
DISCUSSION

The results obtained from the forced swim test of the 5 days-scheduled restraint rats showed significant decrease in the immobility time which was interpreted as an elevation of the mood and positive antidepressant behavior. Platt and Stone (1982) reported that chronic restraint stress elicited a positive antidepressant response on the forced swim test and was quantitatively similar to that produced by the antidepressant drug desmethylimipramine (norepinephrine uptake inhibitor). Although restraint stress model was used to induce depression (Sun et al., 2015), the current work showed that it produced antidepressant response in FST. Likewise, some people become depressed when exposed to stress, while others do not, and stress does not produce depression in some human and animal studies (Jans et al., 2007). The neurochemical investigation revealed significant increase in serotonin level in the brain stem of those rats, which explains the mood elevation.

The increased serotonin synthesis in the brains of stressed rats could be attributed to the high serum cortisol which increased in stressed rats to face stress. This cortisol increases the availability of tryptophan, the limiting factor in the synthesis of serotonin in the neurons of brain stem (Jenkins et al., 2016). However, when cortisol levels remain high, 5-HT levels would be reduced (Lanzenberger et al., 2010). This explains why when stress is prolonged availability of brain serotonin may diminish and vulnerability to depression may increase (Jans et al., 2007). Restraint stress increased the production of MDA in the brain stem and reduced the antioxidant enzyme GPx, disturbing the reduction-oxidation (redox) state of the brain and generating a form of oxidative stress. This effect was reported in different stress models (Samarghandian et al., 2017).

When the stressed rats were supplemented with OO, it alleviated the stress effect as serum cortisol level was.

Figure (4): Correlation between serotonin and each of lipid peroxide malondialdehyde (MDA) and glutathione peroxidase (GPx) in the brain stem homogenate, lines of regression and the coefficients of determination ($R^2$).
lowered back to normal. This was associated with decreased availability of plasma tryptophan and concomitant reduction in serotonin synthesis in the brain stem neurons. In the present study, it was found that OO produced antidepressant effect as indicated by the decrease in the immobility time and increase in struggling time. This antidepressant effect could be explained by the antioxidant effect of OO as results also showed that OO supplementation decreased lipid peroxidation product (MDA) in the brain stem of rats, and provided the antioxidant enzyme GPx in their brain stem. The antidepressant effect of OO supplementation along with its effect on serotonin synthesis was previously obtained by Perveen et al. (2013) but with longer duration of OO supplementation (4 weeks instead of 2 weeks in the present study). However, the reduction in the immobility time and the decrease in serotonin level in brain stem were not correlated to each other, suggesting that the antidepressant effect of OO could be attributed to antioxidant effect and not mediated by serotonin synthesis. Controversial results were reported by Perveen et al. (2013) who attributed the antidepressant effect of olive oil to the increase in serotonin levels in the brain.

The current work also studied the intestinal motility. Both stress and OO increased intestinal motility, but did not reach significant levels. When they were combined together, the increase became significant from the control. This behavior was referred to as Maudsley reactive and Maudsley non-reactive (high and low defecation rate in the forced swimming test, respectively) (Wood et al., 2008). The tendency for increasing number of fecal pellets in restraint stress indicates increased intestinal motility. In previous studies, similar intestinal response to stress was reported by Nakade et al. (2007). This was also demonstrated in mice with even other type of stress like maternal separation (Murakami et al., 2017). The mechanism underlying this effect was attributed to a decrease in neurotensin receptors after restraint stress (Castagliuolo et al., 1996). Olive oil also enhanced the intestinal motility in rats of the present work and this could be mediated through gut hormones that play a role in gastrointestinal motility like GLP-1, neurotensin and CCK secretion (Mand?e et al., 2015).

Correlation study from the present work pointed to a relation between the antidepressant effect and the increase in the intestinal motility. The higher rate of defecation was associated with an increase in the struggling time in the FST. This action could be attributed, at least in part, to changes in serotonin neurotransmitter in brain stem, however, whether this effect is related to serotonin pathways in the gut remains to be elucidated. The present work showed that olive oil has antidepressant potential which could be mediated via its antioxidant not its neurochemical effects. However, the exact mechanism is still unclear since most of the present studies are retrospective studies carried on volunteers while the experimental studies are rare. Also, most of the investigations have been performed in small samples and data are mixed (Lang et al., 2015).

It could be concluded that restraint stress has antidepressant effect which is
mediated through serotonin. Olive oil also has antidepressant potential which could be mediated via its antioxidant not its neurochemical effects.

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**Funding:** The author declares that no funds exist.

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The effectiveness of olive oil in reducing the symptoms of depression in adult males was studied. The researchers observed a significant decrease in plasma cholesterol and triglyceride levels, as well as a noticeable reduction in malondialdehyde and superoxide dismutase activities in the blood, indicating the potential antiperoxidative effect of olive oil. The effectiveness varied depending on the olive oil concentration, with the highest concentration showing the best results. The study concluded that olive oil could be a potential natural treatment for depression in adult males. However, further research is needed to confirm these findings and explore the mechanisms behind the observed effects.