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Docosahexaenoic Acid and Tualang Honey Improve Brain Oxidative Status in Chronic Stress Rat Model

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Abstract

The main objective of this study was to investigate the protective effects of docosahexaenoic acid (DHA) and Tualang honey (TH) and their combination on several parameters of oxidative stress of the brain following exposure to chronic stress. Five groups of male Sprague Dawley rats (10 animal per group) were allocated in which Group 1 received normal saline and used as control; Group 2 was exposed to stress and received normal saline; Group 3 was exposed to stress and reviewed DHA (450 mg/kg body weight BD); Group 4 was exposed to stress and received TH (1 g/kg body weight BD), and Group 5 was exposed to stress and received combination of DHA and TH. Animals were exposed to chronic stress from 9 am to 2 pm daily for 4 weeks. The following oxidative parameters were measured: total antioxidant status (TAS), glutathione, thiobarbituric acid reactive substances (TBARS) and protein carbonyl. The results showed that DHA, TH and their combination significantly reduced (p<0.05) stress-induced elevation of corticosterone and TBARS levels and concurrently caused a significantly increased (p<0.05) in TAS level. With regard to glutathione and protein carbonyl, only TH and combination of DHA+TH significantly reduced (p<0.05) the oxidised glutathione and protein carbonyl. In all parameters, there was no significant difference between Group 5 in comparison to Group 3 and Group 4 indicating combination of DHA and TH was not superior to consuming DHA and TH alone. In conclusion, TH and to lesser extent DHA may protect the brain against oxidative stress induced by exposure to chronic stress but consuming these substances together does not give synergistic effect.

Keywords: Oxidative stress, Honey, Docosahexaenoic acid, Chronic stress, Brain

Introduction

Docosahexaenoic acid (DHA) is one of the omega-3 fatty acids and at the same time belong to much larger category of polyunsaturated fatty acid. DHA, mainly derived from marine animals, is
particularly abundant in the brain and is a major component of membrane phospholipids and therefore plays paramount role in neural transport and synaptic functions (Tanaka et al., 2012). DHA is well known for it extremely important role in foetal and infant brain growth and development. DHA has been shown to be beneficial in the prevention or treatment of various human diseases such as diabetes, cardiovascular, cancers and many neurodegenerative diseases which are believed to be mediated by its anti-inflammatory and antioxidant properties (Ghasemi Fard et al, 2019). As important component of membrane phospholipid, DHA in involve in modulating antioxidant signalling pathways (Oppedisano et al, 2020). In vitro study has shown that DHA was able to reduce reactive oxygen species, increased enzymatic antioxidant level and reduced lipid peroxidation in chemical -induced cell oxidative damage model (Clementi et al, 2019). Similarly, in a vivo study demonstrated that rats fed with DHA had higher antioxidant enzymes and reduced expression of membrane lipid peroxidation as measured by thio-barbituric acid substances (Oppedisano et al, 2020).A few study indicated that omega 3 fatty acid may have a role in alleviating the damaging effects of chronic stress. For instance, Pérez et al., (2013) showed that omega-3 fatty acids supplementation prevented the stress-induced elevation of corticosterone and impaired learning. Therefore, it is hypothesized that DHA could prevent or reduce the extent of oxidative stress occurring in the brain following exposure to chronic stress.

Tualang honey (TH) is a one of the popular type of local honey in South East Asia including Malaysia. It is a type of multifloral honey produced by the Apis dorsata bees which build colony on branches of Tualang trees (Koompassia excelsa) (Khalil et al., 2012). TH has been shown to exhibits numerous therapeutic properties, including antimicrobial, anti-inflammatory, antitumour, antioxidant, antidiabetic as well as neuroprotective properties (Ahmed and Othman, 2013). TH has been shown to have more free radical scavenging and antioxidant activities than other local and commercially available honey in Malaysia (Kishore et al., 2011). Also, TH can potentially protect against the harmful effects of stress on our body. A study demonstrated that TH honey protected the brain against the stress-induced increase of proinflammatory cytokines (Asari et al., 2019) and improved pregnancy outcome in rats exposed to chronic stress (Haron et al., 2014). Therefore, we hypothesized that TH, through its antioxidant property could prevent or lessen the extent of oxidative stress induced by exposure to chronic stress.

Psychological stress is a widespread condition experienced by all human beings during all stages of human lifespan. Stress is also a state of threatened homeostasis of the body which results in physiological and biochemical changes that can affect many aspects of human health (McEwen, 2005). Stress is one of the known conditions that stimulate numerous intracellular pathways that ultimately cause the imbalance between oxidants (free radicals) and antioxidants in our body, a condition called oxidative stress (Dontha, 2016). Oxidative stress is a harmful process which can damage several cellular structures such as membranes, lipid, proteins and deoxyribonucleic acid (DNA) which if not strictly controlled can lead to induction of several chronic and degenerative diseases as well as accelerate the normal ageing process (Herbet et al., 2017). Fortunately, human body is equipped with a defence mechanism to counterbalance the effect of oxidants which can be divided into enzymatic and nonenzymatic mechanisms. Nonenzymatic antioxidants include endogenous glutathione which is one of the major soluble antioxidants found in cellular compartment as well as exogenous antioxidants such as vitamin C and beta carotene (Birben et al., 2012). Numerous studies have reported the ill effects of stress can be alleviated by exogenous antioxidants acting through various pathways to enhance resistance to stress. “Pharmacological interventions using exogenous antioxidants may be a promising stress management strategy for protecting against oxidative stress-induced cell damage” (Choi et al., 2017). Therefore, the present study aimed to investigate the stress-protective effects of these 2 substances, DHA and TH on several brain’s oxidative stress markers following exposure to chronic stress in rat stress model. This study also wished to assess if the combination of these substances would provide any synergistic protective effect on the brain.
Materials and Methods

Animals
Outbred male Sprague Dawley rats aged 5–6 weeks old were obtained from the Animal Research and Service Centre (ARASC) of the Universiti Sains Malaysia, Health Campus. The rats were housed in polypropylene cages in ARASC housing facility and given ad libitum access to standard rat chow and water except during the experimental period. This room was maintained on a 12:12 h light-dark cycle under standard laboratory conditions. The rats were acclimatised to the researchers and surrounding environment for one week prior to the start of the experiment. Experimental procedures were performed in strict adherence to the conditions approved by The Universiti Sains Malaysia Animal Ethics Committee (approval number: USM/IACUC/2017/(105)(846).

Study Design
The animals were matched by weight and randomly assigned into the five different experimental groups with 10 rats per group: Group 1 - control (C); Group 2 - stress (S); Group 3 - DHA plus stress (DHA); Group 4 - TH plus stress (TH) and Group 5 - DHA plus TH plus stress (DHA+TH). With the exception of Group 1, all other groups were exposed to prescribed stress regimen for consecutive 28 days period. DHA was administered at a dose of 450 mg/kg body weight twice daily via oral gavage. TH was administered at a dose of 1 g/kg body weight twice daily via oral gavage. The doses of DHA and TH have been shown to be effective to lower pro-inflammatory cytokines in similar experimental setting (Asari et al, 2019). Equal amount of normal saline was given to rats in the control and stress groups to make sure that all animals were placed under similar treatment condition during the experimental period. The body weight was recorded at the beginning of the study, once a week and at the end of the experimental period. TH (AgroMas) was procured from FAMA, Malaysia. “The honey was filtered, evaporated to 20 % (w/v) water content at 40 C and then sterilised by gamma irradiation (25kGy). DHA was purchased from General Nutrition Corporation (GNC), Pittsburgh, PA, USA”.

Stress Procedures
All rats except the control, were exposed to a combination of restraint stress (RS) and swim stress test (SST) in which the order which type of stress start first was randomly determined on the day of the experiment. RS was imposed by wrapping the animals with soft flexible plastic mesh with in which both ends were closed. The plastic mesh was adjusted to just fit the rats without causing any discomfort. The rats were restrained for 5 hours a day for 4 weeks. In SST, rats were individually placed in plastic containers filled with water (23-25 C) to a depth of approximately 30 cm for 15 minutes. During the test, animals were allowed to swim freely but were unable touch the bottom of the container either with their feet or tail. At the end of 15 minutes period, rats were dried with absorbent paper and return to their home cages. SST was employed in the present study as it was shown to minimise the habituation process commonly associated with RS method (16).

Tissue Sample Preparation
At the end of the experimental period, rats were sacrificed by decapitation. Following decapitation, the trunk blood was collected into a 20 ml tube and allowed to clot naturally at room temperature and then centrifuged at 1000xg for 20 minutes. The supernatants were aliquoted into 1.5 ml tubes and stored in deep freezer (-80 Celsius) until analysis. Meanwhile, the brains were removed from the skulls, weighted immediately and then washed with ice-cold saline. Cerebrum was then separated from the cerebellum and brainstem. The right cerebrum was homogenised in sodium phosphate buffer (0.1 M, pH 7.4) using a motor-driven tissue homogeniser fitted with a Teflon pestle at 900 rpm for ninety second to produce 10% (w/v) homogenates. The homogenates were
centrifuged at 3000xg for fifteen minutes at 4°C, following which the supernatants were aliquoted into 1.5 ml centrifuge tubes and stored at -80 Celsius until further use.

**Corticosterone Analysis**
Serum corticosterone was quantified using a commercial enzyme immunoassay kit supplied by Cayman Chemical (USA) following the manufacturer’s protocol. The resulting concentration of serum corticosterone was expressed as ng/ml using a corticosterone standard curve.

**Total Antioxidant Capacity**
Total antioxidant capacity (TAC) was measured in the brain tissue homogenate using a commercial kit produced by Cayman Chemical (USA) following the manufacturer’s protocol. The principle is based on the ability of antioxidants in tissue samples to inhibit the oxidation of 2,2'-azino-di-3-ethylbenzothiazoline sulphonate (ABTS) by metmyoglobin. The ability of antioxidants in the sample to prevent ABTS oxidation is compared with that of Trolox and the results were quantified as nmol/gram wet tissue.

**Thiobarbituric Acid Reactive Substances**
The extent of brain lipid peroxidation was determined using a thiobarbituric acid reactive substances (TBARS) assay as described previously by Chatterjee et al. (2000). Briefly, 100 ul of 10% brain tissue homogenate or standard was added to the reaction mixture tubes containing 0.2 ml of 8.1 % (w/v) sodium dodecyl sulphate, 1.5 ml of 20% (v/v) acetic acid (pH 3.5), 1.5 ml of 0.8% (w/v) thiobarbituric acid and 0.7 ml of distilled water. The mixture tube was then vortexed and kept in water bath at 95°C for 60 minutes. After cooling with an ice-bath for 5 minutes, the tube was centrifuged at 1000xg for 10 minutes at room temperature. The supernatant was collected and the absorbance was measured spectrophotometrically at 532 nm. Sample values were quantified from a standard curve using 1,1,3,3-tetraethoxypropane as an external standard. Data were expressed as nmol/g wet tissue.

**Protein carbonyl**
In the present study, the level of protein carbonyl was measured using a commercial kit produced by Cayman Chemical (USA) following the manufacturer’s protocol. This assay protocol is based on the reaction of 2,4-dinitrophenylhydrazine (DNPH) with protein forming corresponding hydrazones which can be easily quantifiable at 370 nm absorbance. The results were quantified as nmol/gram wet tissue.

**Glutathione**
Glutathione exists in two forms, one as antioxidant reduced glutathione (GSH) and the other as oxidized form known as glutathione disulfide (GSSG). In the present study, concentration of total glutathione and GSSG were measured separately using commercial kit supplied by Cayman Chemical (USA) following manufacturer’s protocol. This kit utilizes an optimized enzymatic GR recycling method for quantification of glutathione. Each brain tissue homogenate sample was assessed in duplicates and sample concentrations were determined from total glutathione and GSSG standard curves. The amount of reduced glutathione (GSH) was obtained by subtracting GSSG from total glutathione. The levels of GSH and GSSG were expressed as nmol/g wet tissue. The ratio of GSH over GSSG (GSH: GSSG) was calculated and used to indicate redox status.

**Statistical analyses**
Statistical analysis was carried out using ANOVA. If ANOVA analysis achieved significance, multiple Turkey’s post hoc tests were performed. P value of less than 0.05 were considered significant. Data are presented as means ± standard error of the means (SEM).
Results and Discussion

**Body Weight Gain and Brain Weight**
Analysis of body weight on the first day of the experiment showed no statistically significant difference between all groups (data not shown). In the present study, body weight change was calculated by subtracting the initial body weight from final body weight. Analysis of body weight change showed significant difference between the control group and stress group and TH group ($p < 0.05$) (Table 1). Moreover, rats in the DHA and DHA+TH groups gained significantly more weight than rats in the stress group ($p < 0.05$). Regarding the brain weight, there was no significant difference in the brain weight between all groups (Table 1).

**Serum corticosterone**
Analysis of corticosterone level showed that stress group significantly had higher level of corticosterone as compared to the control group ($p < 0.001$) (Table 1). This indicated that acceptable level of stress had been imposed to the rats throughout the experimental period. In addition, corticosterone level in the DHA group, TH group and combination DHA+TH group were significantly higher than that of the control group ($p < 0.001$), but significantly lower than the stress group ($p < 0.001$). Other comparisons were not significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Stress</th>
<th>DHA</th>
<th>TH</th>
<th>DHA+TH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>174.6 ± 7.0</td>
<td>125.0 ± 5.5*</td>
<td>152.7 ± 7.3#</td>
<td>138.5 ± 6.0’</td>
<td>151.9 ± 6.2#</td>
</tr>
<tr>
<td>Brain weight (g)</td>
<td>2.58 ± 0.053</td>
<td>2.49 ± 0.052</td>
<td>2.61 ± 0.067</td>
<td>2.44 ± 0.049</td>
<td>2.55 ± 0.68</td>
</tr>
<tr>
<td>Serum corticosterone (x 10³ ng/ml)</td>
<td>12.8 ± 1.0</td>
<td>35.2 ± 1.7*</td>
<td>29.3 ± 1.5’#</td>
<td>24.4 ± 1.7’#</td>
<td>24.9 ± 1.4’#</td>
</tr>
</tbody>
</table>

The values are expressed as means ± SEM. * $p < 0.05$ compared to the control group. # $p < 0.05$ compared to the stress group.

**Total Antioxidant Capacity**
Total antioxidant capacity (TAC) was measured to evaluate overall oxidative buffering capacity in the brain. Analysis revealed significant effect of stress on the TAC as revealed by significant reduction in TAC following stress exposure compared to the control ($p < 0.05$) (Fig. 1). There were also significant effects of DHA and TH as indicated by significantly increased in TAC in DHA ($p < 0.05$) and TH ($p < 0.001$) groups compared to the stress group. There was no significant change in TAC in combined DHA+TH group compared to the DHA and TH groups.
Figure 1. Total antioxidant capacity in the brain (nmol/g tissue) in various experimental groups (n = 10 rats per group). * Significant changes compared to the control group (p < 0.05). # Significant changes compared to the stress group (p < 0.05).

Lipid Peroxidation
The amount of lipid peroxidation was measured using TBARS as the marker. Analysis revealed significant effects of stress on the level of TBARS (p < 0.001) (Fig. 2). Stress exposure caused lipid peroxidation as indicated by significant higher TBARS in the stress group compared to the control group (p < 0.001). There was significant effect of DHA and TH on the level of TBARS as evident by the significant reduction of TBARS in t DHA (p < 0.05) and TH (p < 0.05) groups compared to the stress group. However, no significant change was observed in the combined DHA+TH group when compared to the DHA and TH groups.

Figure 2. The amount of thiobarbituric acid reactive substances (TBARS) in brain in various experimental groups (nmol/g tissue) (n = 10 rats per group). * Significant changes compared to the control group (p < 0.05). # Significant changes compared to the stress group (p < 0.05).
**Protein Oxidation**

Protein carbonyl is commonly used as a marker of protein oxidation in tissues under investigation. Analysis revealed that stress exposure significantly caused an increase in protein carbonyl compared to the control group \( (p < 0.05) \) (Fig. 3). There was significant effect of TH whereby TH significantly reduced the protein carbonyl level in comparison to the stress group \( (p < 0.05) \). Similarly, combination of DHA and TH significantly reduced protein carbonyl level in comparison to the stress group. However, administration of DHA failed to reduce the protein carbonyl level when compared to the stress group. No significant change was observed in the combined DHA+TH group when compared to the DHA and TH groups.

![Figure 3](image)

*Figure 3.* The level of protein carbonyl in the brain in various groups \( (\text{nmol/g tissue}) \) \( (n = 10\) rats per group). * Significant changes compared to the control group \( (p < 0.05) \). # Significant changes compared to the stress group \( (p < 0.05) \).

**Glutathione**

In order to gauge the activity of endogenous antioxidant in the brain, reduced glutathione (GSH), oxidised glutathione (GSSG) and the total amount of glutathione (GSH + GSSG) were determined. Analysis of GSH showed a significant reduction of GSH following stress exposure in comparison to the control group \( (p < 0.05) \) but other groups comparison was not significant (Table 2). With regard to GSSG, there was significant effect of stress on the level of GSSG. Stress exposure caused significant increase in GSSG in all experimental groups when compared to the control \( (p < 0.05) \). Analysis also revealed significant effect of TH on the GSSG level. TH treatment significantly reduced the GSSG level when compared to the stress group \( (p < 0.05) \). However, DHA treatment did not significantly change the GSSG level when compared to the stress group. Combination of DHA and TH caused significant reduction in GSSG in comparison to the stress group but not when compared to TH group and DHA group.

Regarding the ratio GSG:GSSG, analysis revealed significant effect of stress on the ratio (Table 2). Stress exposure significantly reduced the GSH:GSSG ratio in the stress group compared to the control \( (p < 0.001) \). There was also significant effect of TH on the ratio. TH treatment significantly increased the ratio in the TH group when compared to the stress group \( (p < 0.01) \). However, DHA treatment did not influence the GSH:GSSG ratio when compared to the stress group. Combination of DHA and TH caused significant increase in the ratio compared to the stress group but not when compared to the DHA group and TH groups. These findings suggested that TH but not DHA seemed to enhance glutathione activities in the brain.
The present study showed a significant reduction in TAC following exposure to chronic stress which is parallel to the finding by several other researchers (Fontella et al., 2005; Yin et al., 2018). Interestingly, a study has demonstrated that that maternal stress exposure during gestation can affect TAC of the mother as well as the offspring rats although the offspring were not directly exposed to the stress (Yin et al., 2018). Reduction in TAC during stress indicates the presence of low antioxidants in scavenging ROS resulting in higher formation of free radicals which in turn can damage the cells and tissues. Regarding the effect of TH, the present showed that TH significantly increased TAC in the brain which is in accordance with studies by Al-Rahbi et al. (2014) and Azman et al. (2018) employing social instability stress and noise stress respectively. TH used in the present study has been reported to contain a high amount of antioxidants such as phenolic acids and flavonoids which have strong free radical-scavenging activities (Khalil et al., 2012). Similarly, the present study showed that DHA treatment caused an increase in the TAC in the brain which is similar to several previous studies (Akmal and Roy, 2017; Hennebelle et al., 2012). In addition, the present study indicated that a combination of DHA and TH can increase TAC significantly, but the effect was similar to those observed when DHA or TH was consumed separately, in other words, consuming these substances simultaneously give no extra benefit.

Lipid peroxidation is one of the widely used markers to indicate oxidative injury in various pathological conditions (Zhu et al., 2006). The present study is in parallel with many other studies which showed chronic stress caused significant lipid peroxidation in the brain tissue (Sahin & Gümüşlü, 2007; Herbet et al., 2017). Oxidative degradation of lipid occurs when “free radicals attack lipids containing carbon-carbon double bond(s), especially polyunsaturated fatty acids” resulting in cascades of reactive lipid radicals which ultimately lead to serious damage to the cell membrane (Ayala et al, 2014). Brain membrane lipids are very rich in polyunsaturated fatty acids, which explained the susceptibility of brain tissue to lipid peroxidation (Zhu et al., 2006). In the present study, TH treatment seems to protect against stress-induced lipid peroxidation. This effect is mediated by antioxidant property of TH which prevented the generation of free radicals and enhances the activities of antioxidant enzymes in the body. The present study also indicated that DHA can reduce lipid peroxidation and hence can protect the brain against stress-induced oxidative stress. This finding is parallel to other studies investigating the effect of omega-3 fatty acids and DHA on the brain and other organs (Réus et al., 2018; Firat et al., 2017). This present study also shows that combination of DHA and TH is not superior than consuming each of these substances separately.

Oxidation of protein may cause both structural and functional alterations to proteins in our body which may overtime progress to various diseases. For instance, protein oxidation has been implicated in the pathophysiology of cancer, ageing, atherosclerosis and various degenerative
diseases (Barreiro, 2016). Therefore, any natural substances that can help to reduce protein oxidation may prevent or at least slow down the progression of these diseases. In the present study, TH has been shown to protect the brain from stress-induced increase in protein carbonyl. This is in accordance with a study by Azman et al, 2018 in noise stress rat model in which it demonstrated the ability of TH to reduce stress-induced elevation of protein carbonyl in both young and old rats. However, DHA failed to provide protection against stress-induced increase in protein carbonyl. Previous studies in animal model demonstrated the inability of DHA to influence the protein carbonyl level under normal non stress condition (Mariee and Abd-Ellah, 2011; Wander and Du, 2000). The present study also indicated that combination of DHA+TH group confers protective effect, but this action is most likely due to the action of TH not the DHA.

In the present study, chronic stress causes a compromise in antioxidant glutathione defence as indicated by the decrease in GSG level, increase in the GSSG level and reduction in GSH:GSSG ratio which are parallel to the findings of several previous studies (Atif et al., 2008, Sahin, & Gümüşlü, 2007). GSH is the most abundant non-protein thiol that buffers free radical in the brain tissue. GSH protects the cells from oxidative damage by reacting with free radicals to form oxidised glutathione (GSSG). GSH also “detoxifies hydrogen peroxide and lipid peroxides via the action of glutathione peroxidase” (Dringen et al., 2000). GSSG can be recycled back to GSH by the enzyme glutathione reductase using NADPH as a co-factor which completes the cycle. The ratio of GSH to GSSG often reflects cellular redox balance and is considered as the most sensitive indicator of oxidative stress (Dontha 2016).

With regard to TH, the present study showed that TH improved glutathione status in the body as indicated by lower level of GSSG and higher value of GSG:GSSG ratio in comparison to the stress only group. This finding is parallel with the work of other researchers (Azman et al., 2019, Al-Rahbi et al., 2014). In addition, it has been demonstrated that TH could influence the activity of several enzymatic glutathione biomarkers such as glutathione peroxidase and glutathione reductase (Al-Rahbi et al., 2014). Although not measured in the present study, the activities of these two enzymes are speculated to be affected by TH resulting in a reduction in GSSG and higher GSH:GSSG ratio. Regarding DHA, this study failed to show that it can influence the glutathione status in the brain following exposure to stress. A study in rat model of post-traumatic stress disorder demonstrated omega-3 fatty acids was able to normalise GSSG and GSH:GSSG ratio (Alquraan et al., 2019). However, a systematic review on omega-3 fatty acids indicated that while it can significantly affect some oxidative parameters such as total antioxidant capacity and lipid peroxidation, it has no effect of glutathione in the body (Heshmati et al., 2019).

Taken together, these set of results indicate that TH can protect against oxidative stress induced by chronic stress by increasing TAC and simultaneously mitigate oxidised glutathione to protect cells from harmful effects of free radicals. Besides possessing anti-stress activities, TH have been shown to exert antidepressant-like effect by restoration of hypothalamic-pituitary-adrenal (HPA) axis (Azman et al., 2019). Therefore, the other possible protective mechanism of TH during stress condition is by influencing HPA axis, specifically by supressing the influence of corticosterone and adrenocorticotropic hormone, thus reducing the adverse effect of stress on the body (Azman et al., 2019). The present study also indicated that DHA may have important role in preventing oxidative damage following exposure to chronic stress although not to the same extent as the TH. While DHA can influence the TAC and provide protection against lipid peroxidation, it lacks significant effects on glutathione status and protein carbonyl. A previous study showed that rats exposed to chronic stress improved learning, locomotor activity and improved personal behaviour after were given omega-3 fatty acids. On the other hand, rats chronically lacking DHA exhibited reduced locomotor activity as well as abnormal behavioural responses (Hennebelle et al., 2012; Pérez et al., 2013). Interestingly it has been shown that omega-3 fatty acids supplementation prevented behavioural and brain changes that occurred in adult life following exposure to stress during at early life stage (Réus et al., 2018).
Conclusion

It can be concluded that TH is able to reduce some aspects of oxidative damage following exposure to chronic stress. These effects possibly work by enhancing TAC well as improving the glutathione status in the brain. Regarding the DHA, while it improved TAC, it has no influence on glutathione status and therefore may be less efficacious to protect against oxidative damage induced by chronic stress. However, combination of DHA and TH present no synergistic effect on antioxidant activity in the brain.

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