UNIVERSIDADE FEDERAL DO PAMPA

DIENIFFER DE OLIVEIRA ESPINOSA

EFEITO DO ÓLEO DE PEIXE SOBRE A VIA DAS QUINURENINAS EM UM MODELO DE DEPRESSÃO INDUZIDO PELO HORMÔNIO ADRENOCORTICOTRÓFICO EM CAMUNDONGOS.

Itaqui
2016

DIENIFFER DE OLIVEIRA ESPINOSA

EFFECT OF FISH OIL ON THE KYNURENINE PATHWAY IN A DEPRESSION MODEL INDUCED BY ADRENOCORTICOTROPIC HORMONE MICE

Trabalho de Conclusão de Curso apresentado ao Curso de Ciência e Tecnologia de Alimentos da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Bacharel em Ciência e Tecnologia de Alimentos.

Orientador: Cristiano Ricardo Jesse

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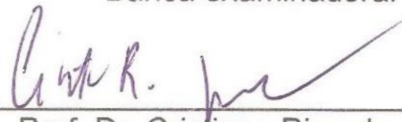
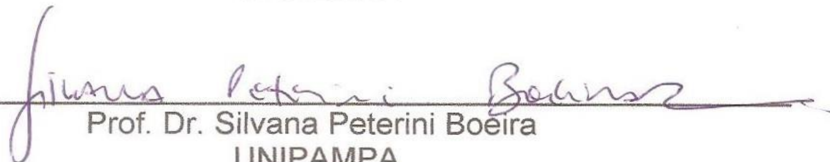


Prof. Dr. Cristiano Ricardo Jesse
Orientador
UNIPAMPA



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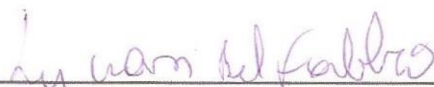

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“Eu aprendi que são os pequenos acontecimentos diários que tornam a vida espetacular!”

William Shakespeare.

RESUMO

A depressão é um transtorno cada vez mais presente na população, na qual sua fisiopatologia e tratamento vêm sendo estudados através de diversos modelos animais. Estudos demonstram que a depressão e as vias inflamatórias estão relacionadas, onde ocorre a indução da enzima indoleamina 2,3-dioxigenase (IDO) por citocinas inflamatórias, em que a ativação dessa enzima resulta na degradação do triptofano através da via das quinureninas, a qual resulta na formação de vários metabólitos neuroativos. O estresse parece ser um dos principais fatores ambientais que predispõem um indivíduo à depressão, onde tem um impacto profundo sobre o desenvolvimento de psicopatologias que afetam diversos processos fisiológicos como endócrino, imunológico e sistema nervoso central. Em resposta ao estresse, o hipotálamo secreta o hormônio liberador de corticotropina (CRH), o qual estimula a adeno-hipófise a liberar o hormônio adrenocorticotrófico (ACTH) na corrente sanguínea. Estudos recentes vem mostrando que a deficiência nutricional dos ácidos graxos poli-insaturados da família ômega-3 está diretamente relacionada à prevalência e severidade da depressão. A suplementação com os ácidos graxos durante períodos críticos do desenvolvimento do sistema nervoso central (SNC), são essenciais para a maturação cortical. Assim o presente estudo visou avaliar o efeito do óleo de peixe sobre a via das quinureninas em um modelo de depressão induzido pelo hormônio ACTH. A administração do óleo de peixe conseguiu reverter diversos parâmetros como a atividade da IDO, KMO, GSH, e os produtos formados na via das quinureninas (KP) demonstrando que o óleo de peixe é eficaz na redução de danos causados pelo hormônio adrenocorticotrófico (ACTH) em camundongos.

Palavras-Chave: Via da quinurenina, ACTH, depressão, estresse oxidativo

ABSTRACT

Depression is an increasingly present disorder in the population, in which the pathophysiology and treatment have been studied by various animal models. Studies show that depression and inflammatory pathways are related, which is the induction of indoleamine 2,3-dioxygenase (IDO) enzyme by inflammatory cytokines, wherein activation of this enzyme results in the breakdown of tryptophan by means of quinureninas, which results in the formation of several neuroactive metabolites. Stress seems to be one of the main environmental factors that predispose an individual to depression, which has a profound impact on the development of psychopathology that affect various physiological processes such as endocrine, immune and central nervous system. In response to stress, the hypothalamus secretes corticotropin releasing hormone (CRH), which stimulates the adeno-pituitary to release adrenocorticotrophic hormone (ACTH) into the bloodstream. Recent research has shown that nutritional deficiency of omega-3 family of polyunsaturated fatty acids is directly related to the prevalence and severity of depression. Supplementation with fatty acids during critical periods of the development of the central nervous system (CNS), are essential for cortical maturation. Thus the present study aimed to evaluate the effect of fish oil on the path of quinureninas in a model of depression induced by the hormone ACTH. Administration of fish oil could reverse several parameters such as the activity of IDO, KMO, GSH, and the products formed on the path of quinureninas (KP) showing that fish oil is effective in reducing damage caused by adrenocorticotropic hormone (ACTH) in mice.

Keywords: Via the kynurenine, ACTH, depression, oxidative stress

LISTA DE FIGURAS

Figure 1- Effect of fish oil (10 mg / kg) in depressive-like behavior in TST test (A), OFT (B) and SPT (C, D) in the groups treated with ACTH (12:45 mg / kg) and imipramine (10 mg / kg). 19

Figure 2 - Effect of fish oil (10 mg / kg) on serum corticosterone levels during administration of ACTH (00:45 mg / kg) and imipramine (10 mg / kg) compared with the control group. 20

Figure 3 - Effect of treatment with fish oil (10 mg / kg) on the administration of ACTH (12:45 mg / kg) and imipramine (10 mg / kg) in the levels of TRP (A), KYN (B), KYNA (C) 3-HK (D) and QA (E) in the hippocampus of mice. 21

Figure 4 - Effect of treatment with fish oil (10 mg / kg) on ACTH administration (0.45 mg / kg) and imipramine (10 mg / kg) on activity of IDO (A), KMO (B) and KAT (C) in the hippocampus of mice. 22

Figure 5 - Effect administration of fish oil (10 mg / kg) of treatment with ACTH (0.45 mg / kg) and imipramine (10 mg / kg) in the levels of RS (A), GSH (B) and Na +, K + ATPase (C) in the hippocampus of mice. 23

LISTA DE ABREVIATURAS

3-HK: 3-hydroxy-L-kynurenine
ACTH: adrenocorticotropic
CRF: corticotropin releasing factor
DHA: docosahexaenoic acid
EPA: eicosapentaenoic acid
GSH: glutathione
HPA: hypothalamic-pituitary-adrenal
IDO: indoleamine 2,3-dioxygenase
KAT: kynurenine aminotransferase
KMO: kynurenine monooxygenase
KP: kynurenine pathway
KYNA: kynurenic acid
KYN: kynurenine
OFT: open-field test
QA: quinolinic acid
ROS: reactive oxigen species
RS: reactive species
SPT: Sucrose preference test
TRP: tryptophan
TST: tail suspension test

SUMÁRIO

1. **Introduction** 13
2. **Materials and methods** 14
   - **2.1. Animals** 14
   - **2.2. Reagents** 14
   - **2.3. Experimental design** 14
   - **2.4. Behavioral assessment** 14
     - **2.4.1. Tail suspension test (TST)** 14
     - **2.4.2. Open field test (OFT)** 15
     - **2.4.3. Sucrose preference test (SPT)** 15
   - **2.5. Biomarkers of hormonal and neurochemical alterations** 15
     - **2.5.1. Analysis of TRP, KYN, KYNA, 3-HK and QA concentrations by high performance liquid chromatography (HPLC)** 15
     - **2.5.2. Determination of IDO, KMO and KAT activities** 16
     - **2.5.3. Serum corticosterone assay** 17
   - **2.5 Tissue preparation** 17
   - **2.6 Biochemical Determinations** 17
     - **2.6.1 Non-proteinthiols (NPSH) content** 17
     - **2.6.2 Reactive oxygen species (ROS) levels** 18
     - **2.6.8 Na^+,K^+-ATPase activity** 18
   - **2.7 Protein determination** 18
   - **2.8 Statistical analysis** 19
3. **Results** 19
     - **3.1.1. Tail suspension test (TST)** 19
     - **3.1.2. Open field test (OFT)** 20
     - **3.1.3. Splash test (SPT)** 20
     - **3.2.3. Determination of serum corticosterone level** 20
     - **3.2.4 Hippocampal determinations** 21
4. **Discussion** 24
5. **Conclusion** 27
6. **References** 28
7. **Anexos** 32

Effect of fish oil on the kynurenine pathway in a depression model induced by adrenocorticotropic hormone mice.

Dieniffer de Oliveira Espinosa, Cristiano Ricardo Jesse.

Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas – LaftamBio Pampa – Universidade Federal do Pampa, CEP 97650-000, Itaqui, RS, Brazil.

1. Introduction

Depressive disorders are evidenced by various psychological, behavioral and physiological changes, and they anhedonia, feelings of hopelessness, sleep and appetite disturbances, and cognitive changes [1]. Where a number of stressful events often precede the initiation of an affective episode, indicating that stress plays an important role in the development of human depression [2].

Depression can result in deficiencies of neurotransmitters such as serotonin and norepinephrine, as well as structural and morphological abnormalities in the hippocampus, it was possible to identify these changes through the brain imaging study [3,4].

Studies show that inflammatory pathways and depression are related, where there is induction of indoleamine 2,3-dioxygenase enzyme (IDO) by pro-inflammatory cytokines, wherein activation of IDO results in the breakdown of tryptophan by the route of quinureninas, which ends up limiting the synthesis of serotonin [5].

During neuroinflammation 95% of the essential amino acid tryptophan (Trp) is catabolized via the route of kynurenine (KP), leading to the formation of several neuroactive metabolites as quinurenico acid (KYNA), 3-hydroxykynurenine (3-HK), and quinolinic acid (QA), which have been proposed to play important roles in brain physiology and pathology because of their possible negative contribution to the oxidative stress [6,7].

Oxidative stress is characterized by pro- and antioxidant imbalance, with a predominance of oxidants, where the oxidative modification may affect the activity of Na / K-ATPase through different mechanisms and functional changes [8,9].

In the presence of stress the hypothalamus is stimulated to release the corticotropin-releasing hormone (CRH), which stimulates the secretion of adrenocorticotropic hormone (ACTH), which in turn stimulates the synthesis and release of glucocorticoids in the bloodstream, where excess of glucocorticoids can produce physiological effects such as hypertension, ulcers and other changes in metabolismo [10,11].

Studies have shown that patients with depression have omega-3 deficiency, both in plasma and in the composition of erythrocytes, as well as the imbalance in the ratio of omega-3 / omega-6 constituting the pathological condition [12].

Facing constant prevalence of depression affecting a significant portion of the population, the need arises to search for compounds that may help treat depression, which may potentiate the effect of antidepressants or reduce side effects caused by drugs where the oil fish has been shown to be a good candidate to meet these requirements.

Therefore this study aims to investigate the effect of fish oil on depression and neurochemical mechanisms in adult mice subjected to the model induced by adrenocorticotropic hormone (ACTH).

2. Materials and methods

2.1. Animals

Experiments wereperformed using male C57BL/6 mice (25-35 g, 3-6 months old). Animals were maintained at 22-25ºC with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 7:00 a.m., experimental manipulations were carried out during light phase of the day. All efforts were made to minimize animal suffering and to reduce the number of animals used. The procedures of this study were conducted according to the guidelines of the Committee on Care and Use of Experimental Animals Resources and with the approval of Ethical Committee for Animal Use (CEUA protocol #025/2014) of the Federal University of Pampa, Brazil.

2.2. Reagents

All reagentes used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Experimental design

The animals were treated with ACTH or vehicle (0.45 mg/kg per day, subcutaneously, s.c.) during 14 days. On the tenth day of ACTH treatment, they were given imipramine (10 mg/kg, intraperitoneally, i.p. positive control), or fish oil (10 mg/kg per day, per oral, p.o.) totaling fourteen days of treatment. On the fifteenth day, all animals were subjected to behavioral tests and hormonal and neurochemical analysis.

2.4. Behavioral assessment

2.4.1. Tail suspension test (TST)

Antidepressant-like effects were measured using TST, with minor alterations [13]. Each mouse was suspended by its tail using adhesive tape placed approximately

1 cm from the tip of the tail, and hung approximately 30 cm above a table. The animals were suspended for a period of 6 min, and the duration of immobility was scored manually during the last 4-min interval of the test (activity in the first 2 min was discarded because animals predominantly try to escape during this period).

2.4.2. Open field test (OFT)

An open field test was conducted as previously described. To verify the effects of imipramine, amitriptyline, 1-MT or ACTH administration on locomotor activity. Each mice was tested for 5 min OFT (Insight model EP 154C) 24 hs after the last treatment. The parameter observed included the distance (unit: mm) [14].

2.4.3. Sucrose preference test (SPT)

Anhedonia was measured by preference for a sucrose solution over water, using a two-bottle free choice method [15]. Each animal was presented simultaneously to two bottles, one containing 1% sucrose solution (w/v), and the other containing tap water. Blunted sucrose intake, in this test, is proposed to reflect impaired sensitivity to reward and model anhedonia, a core symptom of major. Tap water and 1% sucrose solution were placed inpremeasured bottles in the cages, and fluid intake was monitored for 24h. Before the start of the protocol the mice were exposed only to a 1% sucrose solution for 24h aiming the adaptation to sucrose solution. Mice were private food and water for about 20h before each sucrose preference test. On day 0 (baseline) and on day 28 of the experiment the animals were exposed to the SPT. Sucrose preference was evaluated via the sucrose uptake rate, namely, the ratio of volume of sucrose consumption to the volume of sucrose consumption plus tap water consumption (sucrose preference = sucrose consumption/(sucrose consumption + water consumption) × 100%).

2.5. Biomarkers of hormonal and neurochemical alterations

2.5.1. Analysis of TRP, KYN, KYNA, 3-HK and QA concentrations by high performance liquid chromatography (HPLC)

TRP, KYN, KYNA, 3-HK and QA levels were measured in hippocampus samples using HPLC. The mobile phase contained 50 nM glacial acetic acid, 100 mM zinc acetate and 3% acetonitrile dissolved in double- distilled NANOpure water HPLC grade H_2O. The pH was adjusted to 4.9, using 5M NaOH. Hippocampal tissue was

sonicated in 1 ml of mobile phase containing 7% perchloric acid spiked with 50 ng/20 µl of N-methyl 5-HT as internal standard. Hippocampal homogenates were centrifuged at 20,000 rpm for 20 min and the supernatants were placed into new eppendorf tubes, using a syringe fitted with a 0.45 µm filter (phenomenex). 20 µl of the filtered supernatant was injected using a Waters autosampler and a Reverse Phase analytical column (Kinetex™ Core Shell Technology column with specific area of 4.6 mm and particle size of 2.6 µl Phenomenex) was used for separation of metabolites. A PDA-UV detector (Shimadzu SPD-M10A VP), calibrated to integrate at 230 and 250 nm, as well as a fluorescent detector (Shimadzu RF- 20A XS prominence fluorescence /0 detector), set to excitation wavelength 254 nm; emission wavelength 404 nm, were used to detect the metabolites. Chromatographs were generated by CLASS-VP software (Shimadzu). Results are expressed as nmol/h/mg protein.

2.5.2. Determination of IDO, KMO and KAT activities

IDO, KMO and KAT activities were determined as described previously, with minor modifications [16]. To the IDO activity, hippocampal homogenate of mice (50 µl) were incubated with 200 µl of assay buffer (400 mM L-tryptophan, 20 mM ascorbate, 10 µM methylene blue, 100 µg catalase, in 50 mM potassium phosphate buffer pH 6.5) at 37 ^{0}C for 1 h. The reaction was stopped by adding 50 µl of 10% sulfosalicylic acid solution (SSA) and then incubated for an additional 30 min at 50 ^{0}C to hydrolyze N-formylkynurenine to L-kynurenine. The reaction mixture was then centrifuged at 13,000g for 10 min at 4 ^{0}C and filtered through 0.2 mM filter tubes at 13,000g for 5 min. KYN was analyzed by HPLC, as previously described [17]. The results are expressed as nmol KYN/h/mg protein.

To measurement the KMO activity, the hippocampus tissue was homogenized 1:5 (wt/vol) in ultrapure water and further diluted 1:5 (vol/vol) in 100 mM Tris–HCl buffer (pH 8.1) containing 10 mM KCl and 1 mM EDTA. Eighty µl of the tissue preparation were incubated for 40 min at 37°C in a solution containing 1 mM NADPH, 3 mM glucose-6-phosphate, 1 U/ml glucose-6 phosphate dehydrogenase, 100 µM kynurenine, 10 mM KCl and 1 mM EDTA in a total volume of 200 µl. The reaction was stopped by the addition of 50 µl of 6% perchloric acid. Blanks were obtained by adding the specific enzyme inhibitor Ro 61-8048 (100 µM) in the incubation solution. After centrifugation (16,000 x g, 15 min), 20 µl of the supernatant were applied to HPLC to measure 3-HK.

The KAT activity was made using the method previously described [18].Briefly, the hippocampus was harvested and homogenized in distilled water. After centrifugation (12 000 g, 10 min), KAT activity was measured in a total volume of 200 µl, containing 80 µl of supernatant fluid, 150 mm Tris-acetate buffer, pH 7.4, 2 lm kynurenine, 1 mm pyruvate and 80 lm pyridoxal-5-phosphate. Samples were incubated for 24 h at 37 ºC, and the reaction was terminated by adding 50% (w/v) trichloroacetic acid. After successive washes with 0.1 m HCl and distilled water,KYNA was eluted from the column with 2 X 1 mL of distilled water and quantified by HPLC.

2.5.3. Serum corticosterone assay

Blood was collected on ice and separated in a refrigerated centrifuge at 4 ºC (4000g for 10 min). Serum was stored at –20 ºC until assays were performed. Serum corticosterone levels were measured using a commercial kit based on enzyme immune assay (ELISA) [19]. Corticosterone plasma levels were expressed as nmolg/ml.

2.5 Tissue preparation

24h after the FST, blood was colleted by cardiac puncture. Mice were euthanized for decapitation and hippocampus and prefrontal cortex were dissecated and homogeneized (1:10) in Tris-HCl 50mM, pH 7.5. The homohenate was centrifugated at 2,4009G for 5 min and supernadant fraction (S1) was used for the analysis of oxidative, biochemical and neurotropic parameters.

2.6 Biochemical Determinations

2.6.1 Non-proteinthiols (NPSH) content

Reduced glutathione (GSH) levels in the hippocampus and prefrontal cortex were measured as non-protein thiols (NPSH), based on the protocol developed [20]. In this protocol, 500µL of S1 was precipitated with 500µL of 10% trichloroacetic acid. The samples were centrifuged at 2,4009G for 10 min. An aliquot (100µL) of supernatant was added in a system containing 1M potassium phosphate buffer (850µL), pH 7.4, and 10mM 5,5´-dithio-bis(2-nitrobenzoic acid) (DTNB) (50µL), in a final volume of 1mL. The color reaction was measured at 412nm. NPSH levels were expressed as nmol GSH/g tissue.

2.6.2 Reactive oxygen species (ROS) levels

To determine ROS levels in the hippocampus and prefrontal cortex, 40 µL of S1 were diluted (1:50) in 10mM Tris–HCl (pH 7.4) and incubated with 10µL of 2′,7′-dichlorofluorescein diacetate (DCHF-DA; 1mM), at 37°C for 30 minutes. The RS levels were determined by a spectrofluorimetric method described in the literature [21]. In summary, DCHF-DA is enzymatically hydrolyzed by intracellular esterases to form non fluorescent, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. The DCF fluorescence intensity emission was recorded at 520nm (with 480nm excitation) 30 minutes after the addition of DCHF-DA. The ROS levels were expressed as arbitrary unit (AU).

2.6.8 Na^+,K^+-ATPase activity

Na^+,K^+-ATPase activity in the hippocampus and prefrontal cortex was measured according in the literature [22], with modifications. Briefly, the assay medium consisted of 50mM Tris-HCl buffer, pH 7.4; 125mM NaCl, 20mM KCl, 3mM $MgCl_2$ and 50µl of S1 in the presence or absence of ouabain (0,1mM), in a final volume of 450µL. The reaction was started by the addition of adenosine triphosphate (ATP) to a final concentration of 30mM. After 30 min at 37°C, the reaction was stopped by the addition of 250µL of 10% (w/v) trichloroacetic acid. Resulting solution was centrifugated at 2,4009G for 5 min. An aliquot (500µL) of supernatant was removed and used for the quantification of inorganic phosphate (Pi). Appropriate controls were included in the assays for non-enzymatic hydrolysis of ATP. The amount of Pi released was quantified by the colorimetric method described in the literature [23]. Specific Na^+,K^+ -ATPase activity was calculated by subtracting the ouabain-insensitive activity from the overall activity (in the absence of ouabain) and expressed in nmol Pi/mg protein/min.

2.7 Protein determination

Protein concentration was measured by the method described in the literature [24], using bovine serum albumin as the standard.

2.8 Statistical analysis

The data distribution was verified by applying the Kolmogorov-Smirnov test. The results are presented as means ± standard error medium (SEM). Comparisons between the experimental and the control groups were performed by one-way analysis of variance (ANOVA), followed by Tukey post hoc test or two-way ANOVA, followed by Bonferroni post hoc test, when appropriate. A value of $P < 0.05$ was considered to be statistically significant. All tests were carried out using the GraphPad software 5.0 (San Diego, California, USA).

3. Results

3.1. Behavioral assessment

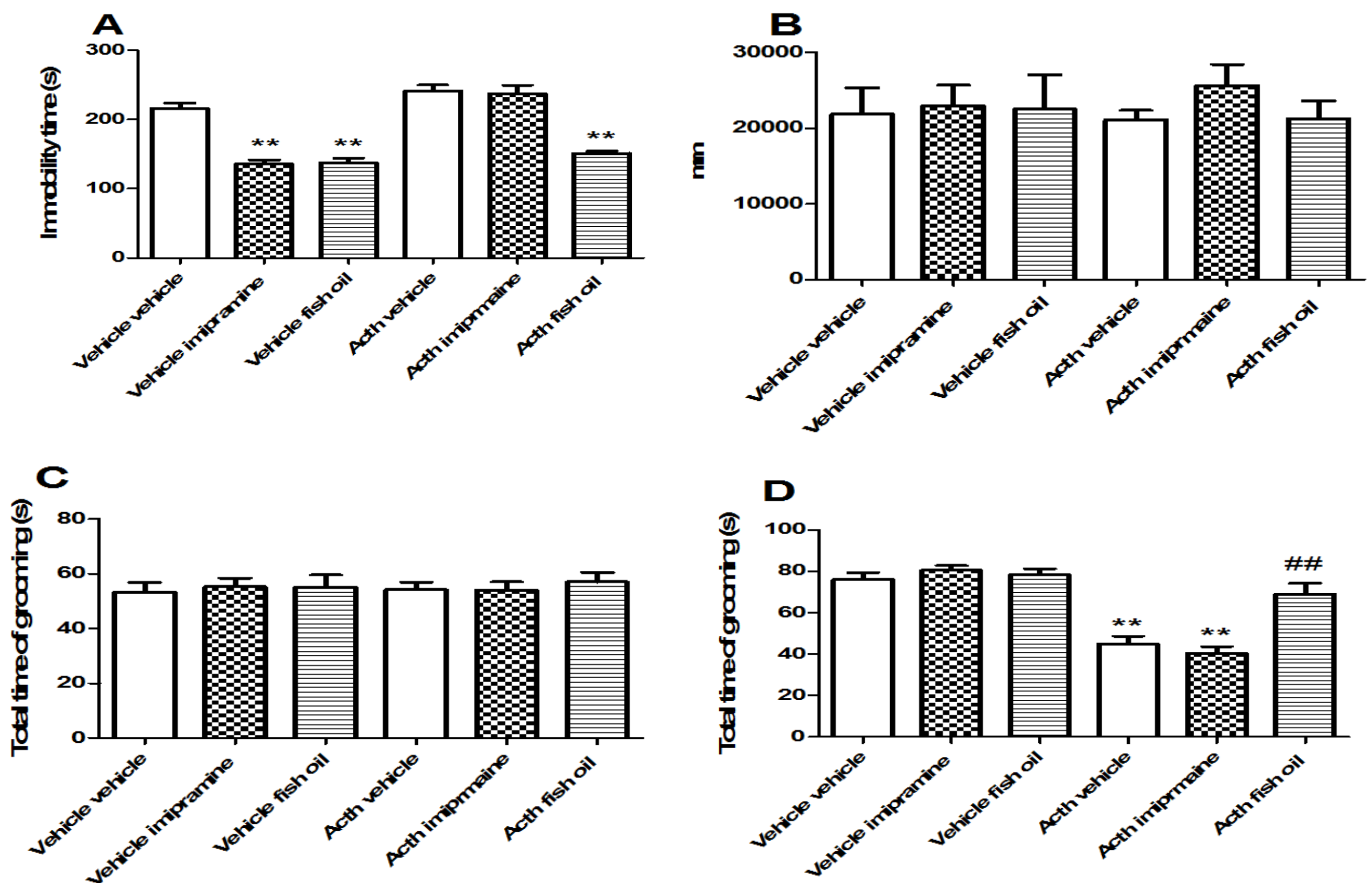

Figure 1- *Effect of fish oil (10 mg / kg) in depressive-like behavior in TST test (A), OFT (B) and SPT (C, D) in the groups treated with ACTH (12:45 mg / kg) and imipramine (10 mg / kg).*

3.1.1. Tail suspension test (TST)

Two-way ANOVA of depressive-like behavior in the TST did not reveal significant ACTH/tricyclic antidepressants interaction ($F(1,36) = 15.56$; $p<0.22$). It was observed a main effect of ACTH ($F(1,36) = 62.24$, $p< 0.001$), and imipramine (F (1,36)

= 15.65, *p*< 0.001) (Fig. 1A). Mice treated with fish oil showed a significant interaction (F (3,36) = 25.23; *p*< 0.001) (Fig. 1A). Post hoc comparisons demonstrated that fish oil reduced the immobility in mice that received ACTH, promoting antidepressant-like effect.

3.1.2. Open field test (OFT)

Fig. 1B shows the effect of ACTH, imipramine and fish oil treatments on the performance in OFT. Two-way ANOVA revealed that total distance in this test did not change at all groups: ACTH (F (1,36) = 1.57, p< 0.22), imipramine or fish oil treatments (F (1,36) = 0.05, p< 0.95). Facing this test can be possible to note that treatments (ACTH, imipramine or fish oil) did not cause excitement or sedation in animals.

3.1.3. Splash test (SPT)

Two-way ANOVA showed significant main effect for ACHT [F(1.32)=9.62, p<0.01] and treatments [F(3.32)=3.05, p<0.05] and no significant ACHT X treatments interaction [F(3.32)=2.89, p=0.0505] in the total time of grooming in the SPT. Results of post hoc indicated that after ACTH the animals displayed significant decrease in the total time of grooming compared to control animals (vehicle + vehicle). The administration of fish oil prevented the decrease in the total time of grooming compared to OB group (OB + vehicle) (Fig. 1C and 1D).

3.2.3. Determination of serum corticosterone level

Corticosterone levels increased in ACTH/vehicle group when compared with control (vehicle/vehicle). In fish oil group, the treatment reverses this increase in serum (Fig. 2).

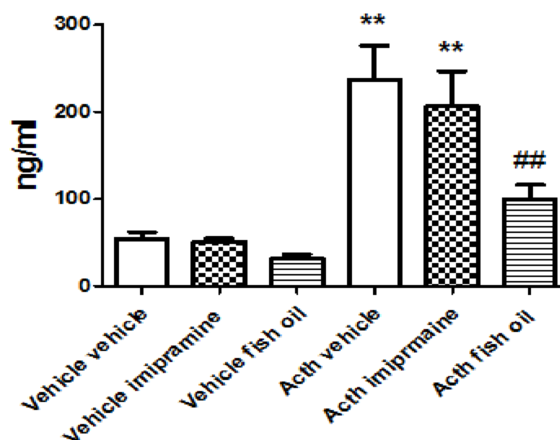

Figure 2 - *Effect of fish oil (10 mg / kg) on serum corticosterone levels during administration of ACTH (00:45 mg / kg) and imipramine (10 mg / kg) compared with the control group.*

3.2.4 Hippocampal determinations

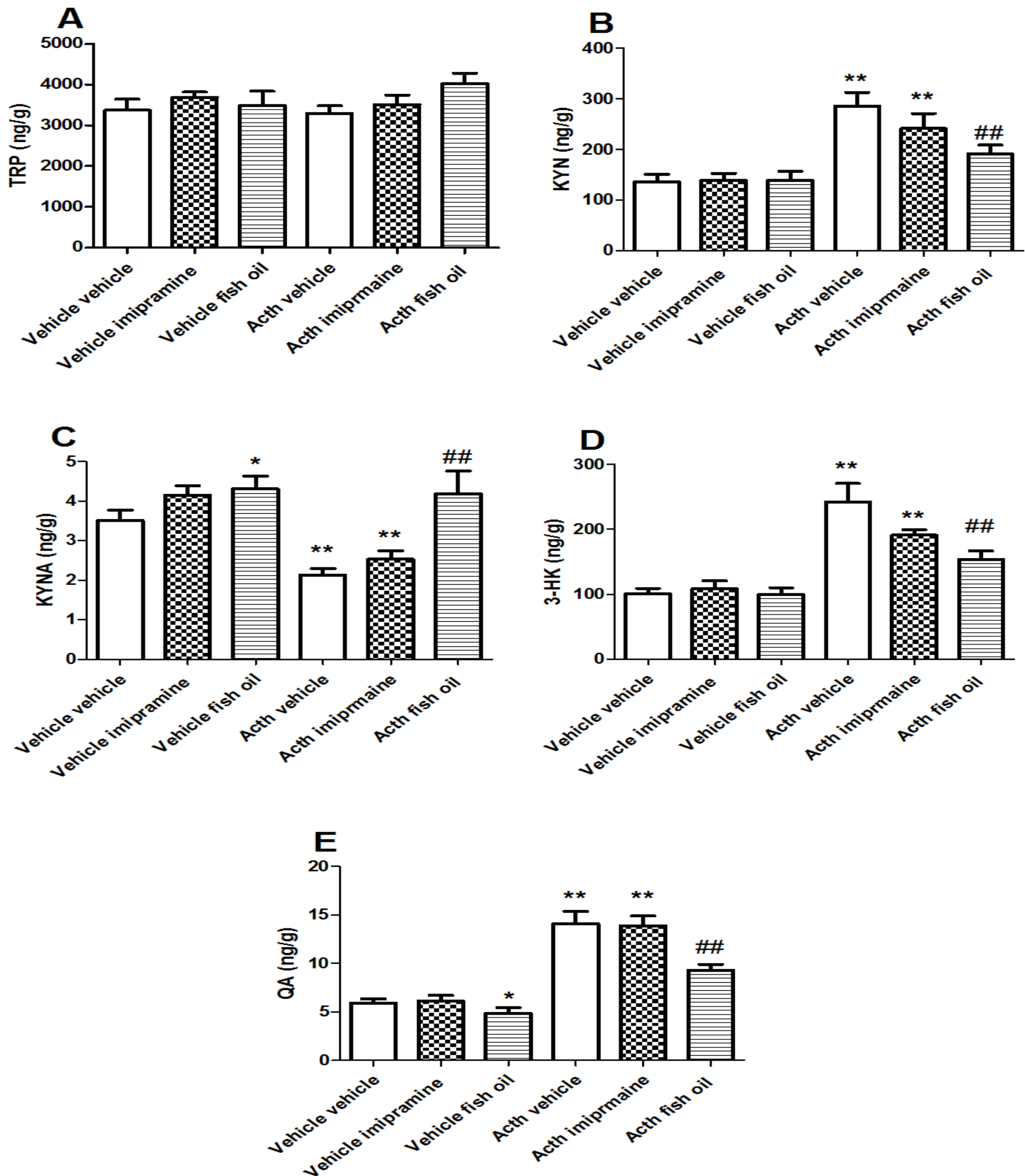

Figure 3 - *Effect of treatment with fish oil (10 mg / kg) on the administration of ACTH (12:45 mg / kg) and imipramine (10 mg / kg) in the levels of TRP (A), KYN (B), KYNA (C) 3-HK (D) and QA (E) in the hippocampus of mice.*

Two-way ANOVA demonstrated no significant main effect for ACTH [F(1.24)=0.0024, p=0.9612], treatments [F(3.24)=0.57, p=0.6385] and ACTH X treatments interaction [F(3.24)=0.37, p=0.7771] in the TRP levels in the HP (Fig. 3A).

Newman-Keuls test revaluated that ACTH induced a significant elevation in the KYN, 3-HK and QA levels (Fig. 3B, 3D and 3E) in the HP compared to control group. The treatment with fish oil promoted the prevention of the increase in the KYN, 3-HK and QA levels in the HP occasioned by ACTH when compared to control group.

Results demonstrated that ACTH occasioned a significant decrease in the KYNA levels in the HP compared to control group. The treatment with fish oil prevented the decrease in the KYNA levels in the HP resulting from ACTH treatment (Fig. 3C).

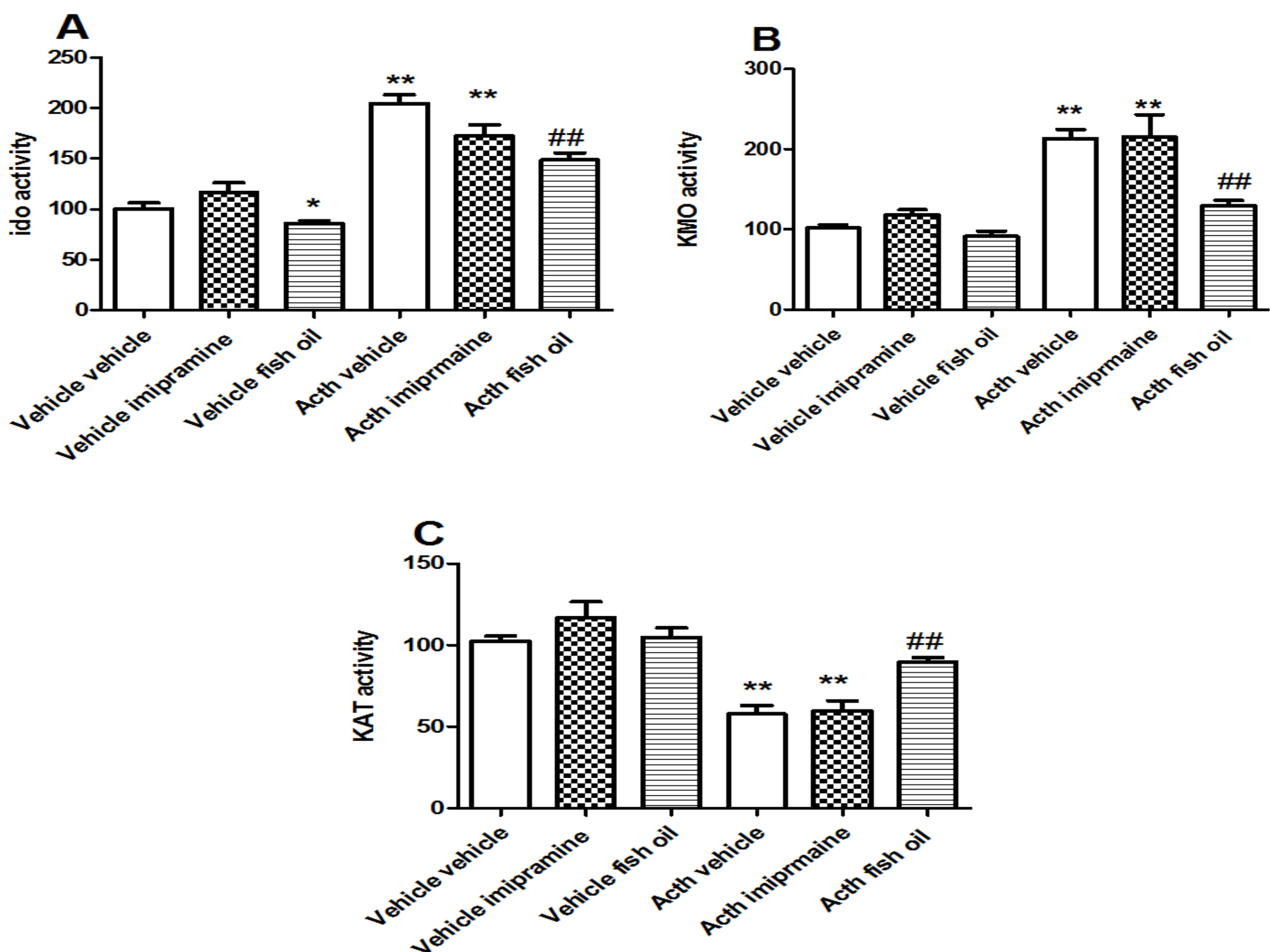

Figure 4 - *Effect of treatment with fish oil (10 mg / kg) on ACTH administration (0.45 mg / kg) and imipramine (10 mg / kg) on activity of IDO (A), KMO (B) and KAT (C) in the hippocampus of mice.*

Post hoc test showed that ACTH occasioned a significant increase in the IDO and KMO activities in the HP compared to control group. The treatment with fish

attenuated the increase in the IDO and KMO activities in the HP resulting from ACTH treatment (Fig. 4A and 4B).

Two-way ANOVA of KAT activity in HP demonstrated a significant ACTH x treatments interaction ($F_{1.16} = 10.13$; $p < 0.01$) and a significant effect of ACTH ($F_{1.16} = 24.12$; $p < 0.001$). Post hoc comparisons revealed that ACTH significantly increased KAT activity in HP of mice compared to control group. Fish oil treatment prevented the increase of KAT activity caused by ACTH (Fig. 4C).

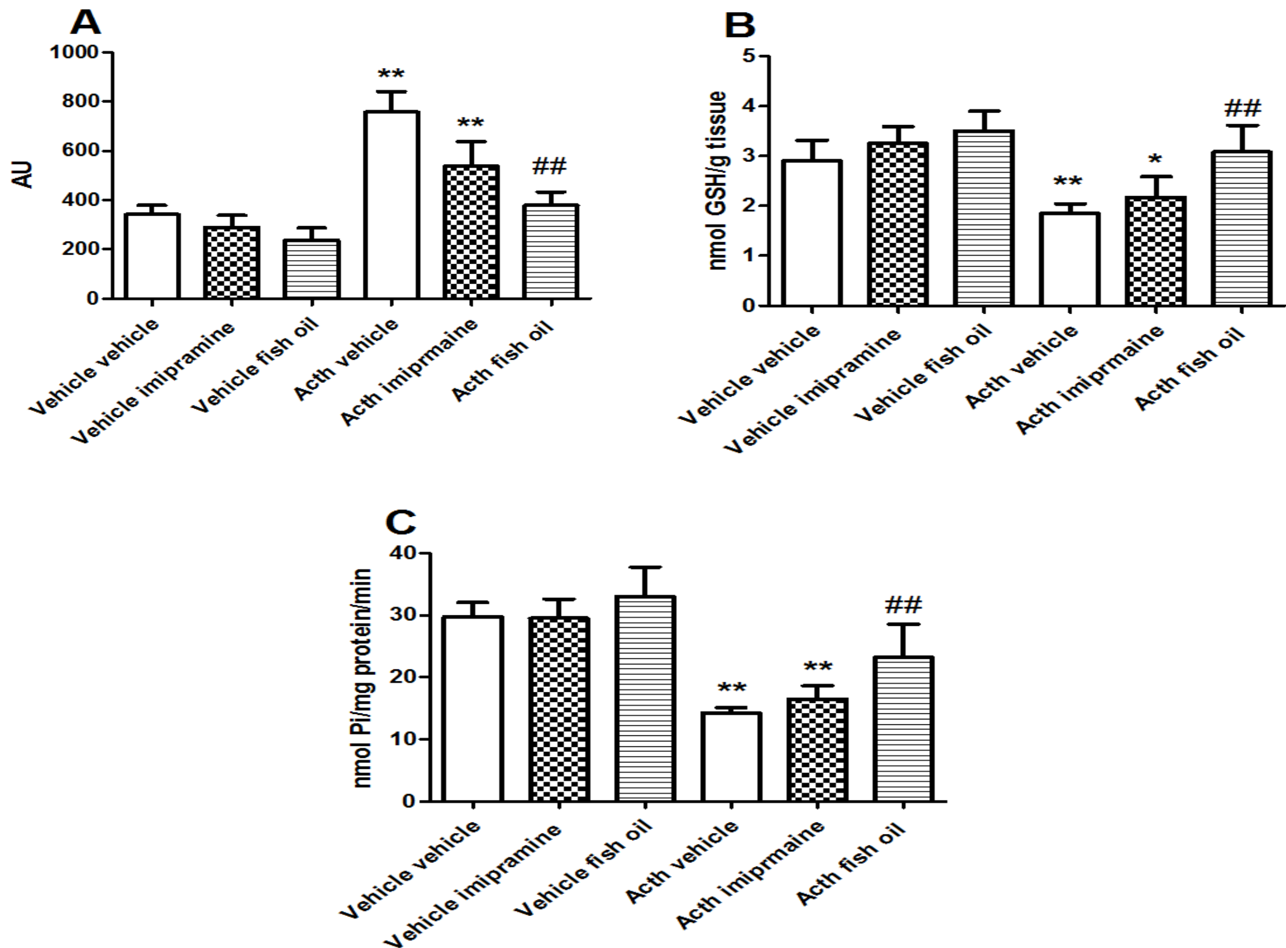

Figure 5 - *Effect administration of fish oil (10 mg / kg) of treatment with ACTH (0.45 mg / kg) and imipramine (10 mg / kg) in the levels of RS (A), GSH (B) and Na +, K + ATPase (C) in the hippocampus of mice.*

Statistical analysis of RS levels in hippocampus revealed a significant ACTH X treatments interaction ($F_{1.16} = 12.52$; $p < 0.01$) and a significant effect of ACTH ($F_{1.16} = 47.23$; $p < 0.001$). Post hoc comparisons demonstrated that ACTH significantly increased RS levels in hippocampus of mice compared to control group. Fish oil treatment prevented the increase of RS levels caused by ACTH (Fig. 5A).

Statistical analysis of GSH levels in hippocampus revealed a significant ACTH x treatmetns interaction ($F_{1.20} = 6.20$; $p < 0.05$) and a significant effect of fish oil ($F_{1.20} = 10.24$; $p < 0.05$) and ACTH ($F_{1.20} = 20.26$; $p < 0.05$) (Fig. 5B). Post hoc comparisons demonstrated that ACTH significantly depleted NPSH levels in hippocampus of mice compared to control group. Treatment with fish oil reversed the depletion of NPSH levels induced by ACTH.

Two-way ANOVA showed a significant interactions (ACTH x treatments) [F (2,44) = 8.34, p< 0.01] in Na^{+},K^{+}-ATPase activity in the hippocampus of mice. Results showed that animals exposed to ACTH presented a significant inhibition in Na^{+},K^{+}-ATPase activity when compared to control group [$p < 0.05$]. The treatment with fish oil prevented the inhibition in Na^{+},K^{+}-ATPase activity when compared to stress group (Fig. 5C).

4. Discussion

Several evidence indicates that depression may develop as a result of cytokines and inflammatory mediators of action in the brain. In which the major mechanism linking the immune system with the mood appears to be the activation of the enzyme IDO and the resulting conversion of the amino acid tryptophan (TRP) in kynurenine and its underlying neurotoxic metabolites, free radicals, 3-hydroxy-kynurenine (3HK) and N-methyl-D-aspartate (NMDA), quinolinic acid receptor agonist (QA) [25,26]. As the quinurenico acid (KYNA), an inhibitor and neuroprotective metabolite that is formed in a side arm of the pathway of kynurenine (KP) degradation of TRP and which seeks a series of receptors, so that these compounds primarily derived glial may modulate the glutamatergic neurotransmission, which is involved in mood disorders [27]. However few studies have assessed the changes suffered during the tryptophan metabolism during the depression, so this study aimed to assess the levels of key enzymes IDO, KMO and KAT involved in the metabolism of tryptophan through the pathway of kynurenine (KP), along with their products and by-products TRP, KYN, KYNA, 3-HK and QA which are formed during the route as well as cell damage markers by ROS, the hippocampus through the action of the hormone ACTH, and the possible protective action oil of fish on these parameters.

In the tail suspension test (TST), the groups of mice treated with vehicle + imipramine and vehicle + fish oil had lower immobility time, whereas animals treated with ACTH + vehicle and ACTH + imipramine showed no significant differences when compared to the control group (vehicle), as the group of animals Treatise ACTH + fish

oil showed a reduction in the immobility time (Fig. 1A). In the open field test (OFT) there was no difference between the treated groups (Fig. 1B), already in borrifagem test sucrose (SPT), the groups of mice treated with ACTH decreased in total self-cleaning time compared to control group (vehicle), the animals treated with fish oil + ACTH showed avoid a reduction in self-cleaning time compared to the control group (Fig. 1C and 1D). These tests demonstrate that the administration of fish oil has a standard antidepressant action, since it has been shown to reduce the standard depressive behavior in the above test. Where there was evidence by studies suggesting the involvement of DHA in the regulation of emotional and locomotor state, as well as exploratory activity and cognitive functions in rodents [28].

In Figure 2 are shown corticosterone levels in the serum, the group treated with ACTH + vehicle showed an increase in corticosterone levels compared to the control group and in the group of mice treated with ACTH + fish oil is observed that fish oil reversed the increase of corticosterone in the serum. Studies have linked depression with a high level of cortisol in the blood due to hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA), largely due to a hypersecretion releasing factor corticotrophin (CRF) in which EPA can regulate axis dysfunction PAH associated with depression, reducing CRF and cortisol secretion [29]. So some animal studies report that the response to chronic stress can be modulated by omega-3, since their dietary deficiency has been shown to be harmful while enrichment has protected stress [30].

According to demostrados results in Figure 3, there was no difference in the levels of TRP in the hippocampus between groups of treated animals (Fig. 3A). The levels of KYN, 3-HK and QA showed an increase ACTH induced in the hippocampus (Fig. 3B, 3D and 3E), where the administration of fish oil demonstrated to prevent the increase compared with the control group. In Figure 3C, the group treated with ACTH showed a significant reduction in KYNA levels in the hippocampus compared to controls, in which the group treated with fish oil prevented the reduction in KYNA levels. Tryptophan is an amino acid widely known to be a precursor of serotonin. But the hyperactivity of IDO, leads to the accumulation of tryptophan metabolites, known as TRYCATs (kynurenine, kynurenic acid, xanthurenic acid, and quinolinic acid), which has neurotoxic activities (kynurenine / quinolinic acid) and neuroprotective (kynurenic acid), 3-HK compound is also neurotoxic due to its conversion to quinoniminas and reactive oxygen species [31,32]. The uncontrolled activity of IDO causes not on the tryptophan required for serotonin synthesis. So in this study, fish oil administration was satisfactory across the metabolites formed.

The groups of animals treated with ACTH showed a significant increase in the activity of IDO and KMO in the hippocampus, while KAT showed decreased activity, in which supplementation with fish oil reduced the activity of IDO and KMO and increased activity KAT (FIG. 4A, 4B and 4C). The increase of the enzyme IDO activity is associated with immune activation in viral, parasitic infections, and autoimmune disease, as well as some neurological diseases [33]. KMO is a key enzyme which converts KYN in 3HK and subsequently QA. Evidence suggests that KP is the primary route for QA production, where in a study, inhibition of KMO effectively improved QA neurodegeneration mediated in rodents [34]. There are studies on KP through the possible inhibition of KMO and overactivation of the KAT, wherein the ratio of metabolites are essences for the balance and performance of the cell, to provide new therapeutic opportunities such as development of new and powerful compounds as a promising outlook for brain neuroprotection [35].

In Figure 5 it is possible to note that ACTH increased RS levels in the hippocampus compared with the control group, and fish oil administration provided a prevention in the increase of RS caused by ACTH (Fig. 5A). The production RS may originate in response to various stimuli including: signaling through the plasmatic membrane receptors, such as hormones and pro-inflammatory cytokines [36-37]. Some QA studies indicated that forms a complex with iron, and the transfer of electrons from the oxygen of this complex results in the formation of reactive oxygen species which mediate lipid peroxidation [38,39], and a decrease in repairing the damage so as the antioxidant activity [40]. In this study, fish oil proved to be able to reduce RS formation in the hippocampus of mice.

GSH levels in the hippocampus (Fig. 5B) demonstrated a significant reduction in the group treated with ACTH + vehicle compared to the control group, where the administration of fish oil reversed the depletion of GSH levels induced ACTH. Studies report GSH as the main non-enzymatic antioxidant defense of the body, our data corroborate studies in animals supplemented with fish oil, which was observed decrease in oxidative damage [41], and increased availability of GSH, major antioxidant endogenous enzymatic system of humans [42].

The Na +, K + -ATPase suffered significant inhibition in mice treated with ACTH group compared to the control group, treatment with fish oil prevented the inhibition of Na +, K + -ATPase when compared to the group exposed to stress. The Na + K + ATPase is crucial for many cell functions such as maintaining the resting potential,

regulating cell volume, pH regulator, and directing the secondary active transport [43], where this study, fish oil has been shown to prevent inhibition of Na +, K + -ATPase.

5. Conclusion

Our data show that fish oil proved to be a viable alternative to supplement, where this study was able to reverse the damage caused by ACTH in several parameters analyzed, and combined with the use of antidepressant drugs can generate beneficial effects, but there has to need for further studies evaluating the effects of treatment with fish oil on the products and by-products formed along the pathway of kynurenine.

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7. Anexos

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Data da aprovação: **10/07/2014**

Período de vigência do projeto: De: **07/2014** Até: **07/2017**

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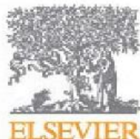

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TABLE OF CONTENTS

- **Description** **p.1**
- **Audience** **p.1**
- **Impact Factor** **p.1**
- **Abstracting and Indexing** **p.2**
- **Editorial Board** **p.2**
- **Guide for Authors** **p.4**

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Contact Information

Dr. Bernhard Hennig, Editor-in-Chief
The Journal of Nutritional Biochemistry
University of Kentucky
900 Limestone Street
Rm. 599, Wethington Health Sciences Building
Lexington, KY 40536-0200
E-mail address: JNB@uky.edu
Fax: 859-257-1811

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