Ceratonia Siliqua Methanolic Extract on 6-OHDA Zebrafish Model

Antiacetylcholinesterase and Anxiolytic Profile

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Anxiety and depression are the most common mental disorders that increase morbidity in the world, and recent data (World Health Organization - WHO) show that over 322 million people suffer from depression and anxiety, especially women being more exposed than men. 6-Hydroxydopamine (6-OHDA) is a catecholaminergic neurotoxin which is formed endogenously in patients diagnosed with Parkinson's disease and has two mechanisms of action. Thus, it easily forms free radicals and is a potent inhibitor of the mitochondrial respiratory chain complexes I and IV. The treatments administration of 6-OHDA (160 ìM) and Ceratonia siliqua L. methanolic extract (0.1 mg/mL, 0.3 mg/mL and 1.0 mg/mL) in Danio rerio specimens affected the acetylcholinesterase activity (AChE) and anxiolytic profile in close correlation with the dose of administered extract, the group with significant differences being the treated one with 6-OHDA alone.

Keywords: 6-hydroxydopamine, acetylcholinesterase, anxiety, carob

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In the last years, most of research studies were focused on the discovery of natural compounds with pharmacological properties to exploit them in the prevention and treatment of many serious illnesses. On the other hand, it is known that the disruption of the balance between pro-oxidant and anti-oxidant systems results in oxidative disasters in the respective organisms [1, 2].

During normal cellular respiration, both metallic catalysis processes and electron transport at the membrane level, reactive oxygen species (ROS) are generated such as: hydrogen peroxide, hydroxyl radical, hypochloric acid, superoxide anion, oxygen singlet, lipid peroxides and hypochlorite [3-5]. ROS induces imbalances in DNA, RNA, proteins, amino acids and unsaturated lipids and, most often, results in the establishment of diseases such as diabetes, cancer, cardiovascular and neuro-degenerative diseases [6]. Thus, in order to eliminate ROS [1], various endogenous and exogenous antioxidant systems such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) are involved, exogenous intake of antioxidants representing an extra benefit, in the fight against ROS accumulation [7].

The literature data highlights, on the one hand, the major influence of oxidative stress on mood disorder and / or depression [1, 8] and, on the other hand, the role of biological active substances from the various plant products with pharmaco-dynamic action in the prevention and treatment of psychiatric disorders, thus avoiding side effects such as agitation, insomnia, weight gain, restless legs etc., due to allopathic medication administration [9, 10].

Ceratonia siliqua L. is one of the most widespread medicinal plants on the Mediterranean soil belonging to the *Leguminoseae* family, the *Caesalpinaceae* sub-family [11], being a dioecious species with some hermaphroditic forms; thus male, female, and hermaphrodite flowers are generally borne on different trees [12].

It seems that the *Ceratonia siliqua* L. tree is still an insufficiently used crop, even though the pods are now used for a multitude of more innovative purposes: for animal feed as carob powder [13], in human food due to the high content of pulp carob in soluble sugar, respectively as cocoa replacer [14], as stabilizing and gelling additives in the pharmaceutical and food industry due to the presence of galactomannans in the seeds endosperm [15, 16], as well as for ethanol obtaining [17].

The carob contains a multitude of phytoconstituents such as flavonoids, phenolic compounds, tannins, anthocyanins, alkaloids, glycosides, minerals and proteins [18]. In addition, the literature on the field [1] have shown that the carob extracts have a high antioxidant potency and exert antibacterial, anti-hypercholestrolemic [19], antidiarrheal [20], antifungal, antiinflammatory, antidiabetic, hepatoprotective [21] and antiproliferative effects [22-25].

Custódio et al. 2011 [26] reported the reduction in the viability of different human cell lines by using carob pulp extracts. In addition, relatively recent data [27] indicated the possibility of using *Ceratonia siliqua* in the treatment of neurodegenerative diseases due to the rich content of this plant in bioactive compounds and with significant therapeutic potential [28].

The present paper aims to test the effects of 6-OHDA on the one hand, and on the other hand, of *Ceratonia siliqua* L. methanolic extract in a dose of 0.1 mg/mL, 0.3 mg/mL and 1.0 mg/mL, respectively on the activity of acetylcholinesterase and anxiety state in the *Danio rerio* experimental model.

Experimental part

Fish accomodation and treatment

After 10 days of accommodation in the laboratory, the *Danio rerio* specimens were divided into five experimental groups (control group, negative control - treated only with 6-OHDA 160 ìM and 3 variants treated with 0.1 mg/mL, 0.3 mg/mL and 1.0 mg/mL carob methanolic extract). The methanolic extract of *Ceratonia siliqua* L. was administered

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from 3 to 3 days during 9 days. Subsequently, in all batches, except for the control variant, treatment with 6-OHDA in a concentration of 160 ìM was applied for 60 minutes. Throughout the experiment, the fish were fed with Norvitall - fish food rich in vitamins, vegetable proteins, spirulin, lecithin, vegetable oils etc. At the end of the anxiety tests, the fish were used to determine AChE activity.

AChE assay activity

AChE was determined using acetylcholine as an artificial substrate, by spectrophotometric method with 2-dinitrobenzoic acid (DTNB) to form a yellow colored complex having a maximum absorption at the wavelength of 412 nm [29]. The enzymatic activity was expressed as nmol/min·mg protein.

Behavioral tasks

In our studies, a Logitech HD Webcam C922 Pro Stream camera recorded zebrafish behavior, and the videos analyzed using ANY-maze®software (Stoelting CO, USA).

Novel tank diving test (NTT)

The NTT is a specific test used for assessing anxiety in zebrafish as described by Levin et al. 2007 [30]. The animals were transferred individually to a trapezoid test tank, and swimming behavior was recorded for 6 min. The following parameters were evaluated: total distance in the tank (m); time spent in the top zone of the tank (s), time spent in the bottom zone of the tank (s), number of entries to the top of the tank and number of entries to the bottom of the tank.

Results and discussion

A first objective of our study was the determination the AChE activity in *Danio rerio* specimens treated with 6-OHDA and carob methanolic extract in concentrations of 0.1 mg/mL, 0.3 mg/mL and 1 mg/mL, respectively. From figure 1, it was observed that in the control group the AChE activity recorded an average threshold of 5.23±0.347 nmol/min·mg protein, whereas in the experimental variant treated only with 6-OHDA the enzyme reached a maximum activity of 11.352±0.379 nmol/min·mg of protein, a value exceeding 2.17 times to that recorded for the reference lot.

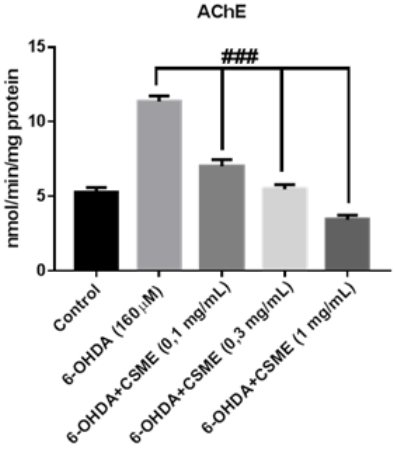

Fig. 1. Effects of *Ceratonia siliqua* methanolic extract (CSME, 0.1, 0.3 and 1.0 mg/mL) on the AChE specific activity in 6-OHDA (160 µM) zebrafish model. Data are expressed as mean ± S.E.M. (n = 10). ###p < 0.0001 vs. 6-OHDA group

Compared to the lot treated only with 6-OHDA, administration of the *Ceratonia siliqua* L. methanolic extract resulted in decreased AChE activity, in close correlation with the used extract dose. Thus, in the experimental variant, to which it was administered 0.1 mg/mL carob methanolic extract and then 6-OHDA an average activity of 7.01±0.449 nmol/min·mg was registered, and in the batches treated with carob methanolic extract in concentration of 0.3 mg/mL and 1.0 mg/mL, respectively, AChE recorded thereshold values increasingly lower (5.467±0.308 nmol/min·mg protein, respectively 3.415±0.298 nmol/min·mg protein).

In addition, the literature data [31, 32] emphasizes that in patients diagnosed with Alzheimer's disease, irreversible

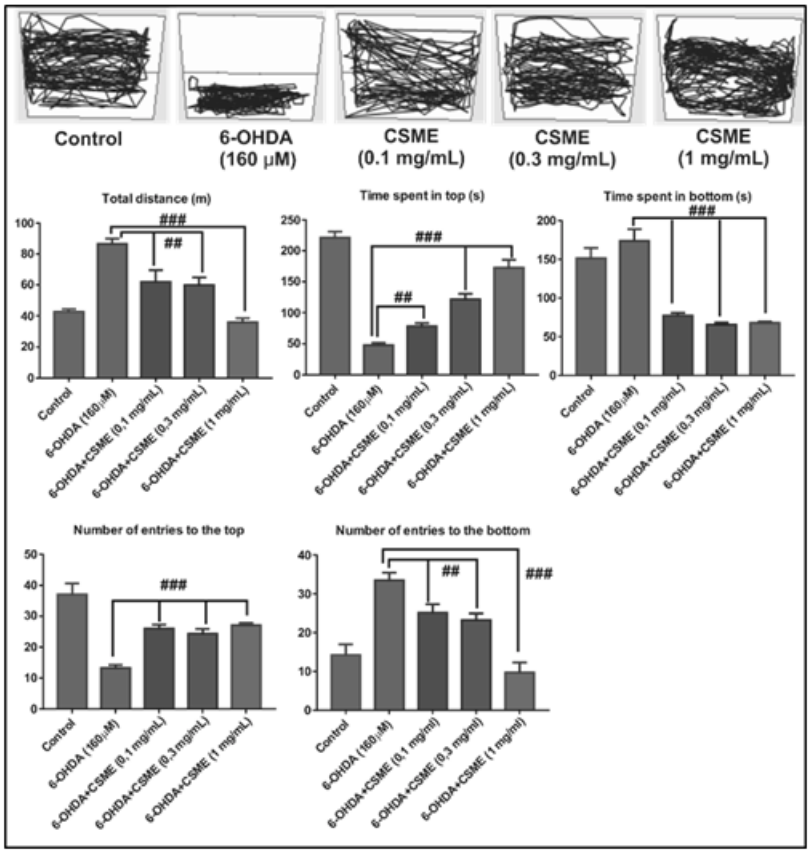

Fig. 2. Effects of *Ceratonia siliqua* methanolic extract (CSME, 0.1, 0.3 and 1.0 mg/mL) on the total distance (m); time spent in the top (s), time spent in the bottom (s), number of entries to the top and number of entries to the bottom of the tank in 6-OHDA (160 µM) zebrafish model in the novel tank diving (NTT) test. Data are expressed as mean ± S.E.M. (n = 10). Representative locomotion tracking patterns of all groups are also presented. ##p < 0.001 and ###p < 0.0001 vs. 6-OHDA group

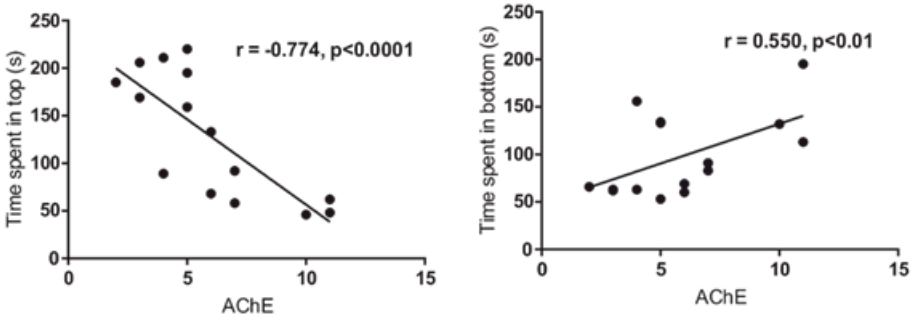

Fig. 3. Pearson's correlation between time spent in top vs. AChE and time spent in bottom vs. AChE

brain disorder characterized mainly by cholinergic deficits, oxidative stress and mitochondrial dysfunctions [33], the target is to inhibit AChE activity in order to maintain acetylcholine levels in the neuronal synapses to obtain beneficial effects in patients with Alzheimer's disease [29].

In the NTT test (fig. 2), one-way ANOVA revealed significant effect of treatment on the total distance in the tank ($F(4, 45) = 19.14$, $p < 0.001$), on the time spent in the top ($F(4, 45) = 63.97$, $p < 0.0001$), on the time spent in the bottom ($F(4, 45) = 30.04$, $p < 0.0001$), on the number of entries to the top ($F(4, 45) = 20.09$, $p < 0.0001$) and on the number of entries to the bottom of the tank ($F(4, 45) = 16.38$, $p < 0.0001$).

Administration of the 6-OHDA (160 µM) induced anxiety, as revealed by significant decrease of the time spent in the tope zone of the tank ($p < 0.0001$) and increase of the time spent in the bottom zone of the tank ($p < 0.0001$) as compared to control group. The anxiogenic profile of the 6-OHDA is also confirmed significant decrease of the number of entries in the tope zone of the tank ($p < 0.0001$) and significant increase of the number of entries in the bottom zone of the tank ($p < 0.0001$). Treatment with CSME prevents effects induced by 6-OHDA in a dose-dependent manner, especial on the high dose were observed. The locomotion tracking pattern in the control group was demonstrated by regular swimming all over the tank. The 6-OHDA-treated group exhibited high levels of anxiety, as evidenced by abnormal tracking pattern, whereas administration of the CSME attenuated 6-OHDA-induced abnormal tracking pattern nearly like control group.

Evaluation of Pearson correlation coefficient (r) revealed significant correlations between time spent in top vs. AChE ($r = -0.774$, $p < 0.0001$) and time spent in bottom vs. AChE ($r = 0.550$, $p < 0.01$). our results clearly demonstrated that the anxiolytic profile of CSME in 6-OHDA-induced model are correlated with decrease of the AChE activity (fig. 3).

Conclusions

Our results revealed that AChE activity was significantly increased in the brain from the adult zebrafish ($p < 0.0001$), while treatment by CSME attenuated the increase AChE activity ($p < 0.0001$) as compared to 6-OHDA-treated group. Thus, CSME reduced cholinergic deficits that are related to improving anxiety-like behavior in zebrafish, as observed in the NTT tank.

References

1.LAKKAB, I., EL HAJAJI, H., LACHKA, N., LEFTER, R., CIOBICA, A., EL BALI, B., LACHKAR, M., Journal of Functional Foods, **54**, 2019, p. 457.
2.DUMITRU, G., TODIRASCU CIORNEA, E., SANDU, I., HRITCU, L., Rev. Chim. (Bucharest), **70**, no. 1, 2019, p. 202.
3.HALLIWELL, B., GUTTERIDGE, J.M.C., Molecular Aspects of Medicine, **8**, 1985, p. 89.
4.WOJTUNIK-KULESZAA, K.A., ONISZCZUKA, A., ONISZCZUKB, T., WAKSMUNDZKA-HAJNOSA, M., Biomedicine & Pharmacotherapy, **78**, 2016, p. 39.
5.DUMITRU, G., TODIRASCU CIORNEA, E., HRITCU, L., SANDU, I.G., Rev. Chim. (Bucharest), **69**, no. 5, 2018, p. 1194.
6.RAJENDRAN, P., NANDAKUMAR, N., RENGARAJAN, T., PALANISWAMI, R., GNANADHAS, E. N., LAKSHMINARASAIAH, U., NISHIGAKI, I., Clinica Chimica Acta, **436**, 2014, p. 332.
7.DUMITRU, G., DIRVARIU, L., BARBACARIU, C.A., MIRON, I., SANDU, I., TODIRASCU CIORNEA, E., Rev. Chim. (Bucharest), **69**, no. 11, 2018, p. 3146.
8.XU, Y., WANG, C., KLABNIK, J. J., O'DONNELL, J. M., Current Neuropharmacology, **2**, 2014, p. 108.
9.BERKEN, G.H., WEINSTEIN, D. O., STERN, W.C., Journal of Affective Disorders, **7**, 1984, p. 133.
10. ROTTACH, K.G., SCHANER, B.M., KIRCH, M.H., ZIVOTOFSKY, A.Z., TEUFEL, L.M., GALLWITZ, T., MESSER, T., Journal of Psychiatric Research, **43**, 2008, p. 70.
11. OZIYCI, H.R., TETIK, N., TURHAN, I., YATMAZ, E., UCGUN, K., AKGUL, H., GUBBUK, H., KARHAN, M., Scientia Horticulturae, 167, 2014, p. 149.
12. EL HAJAJI, H., LACHKAR, N., ALAOUI, K., CHERRAH, Y., FARAH, A., ENNABILI, A., EL BALI, B., LACHKAR, M., Arabian Journal of Chemistry, **4**, 2011, p. 321.
13. MANSO, T., NUNES, C., RAPOSO, S., LIMA-COSTA, M.E., Journal of Industrial Microbiology & Biotechnology, **2010**, 2010, p. 1367.
14. BENGOECHEA, C., ROMERO, A., VILLANUEVA, A., MORENO, G., ALAIZ, M., MILLAN, F., GUERRERO, A., PUPPO, M.C., Food Chemistry, **107**, 2008, p. 675.
15. NASAR-ABBAS, S.M., HUMA, Z.E., THI-HUONG, V., KHAN, M.K., ESBENSHADE, H., JAYASENA, V., Food Sci. Food Saf., **15**, 2016, p. 63.
16. CORREIA, P.J., SAAVEDRA, T., GAMA, F., DA GRAÇA MIGUEL, M., DE VARENNES, A., PESTANA, M., Scientia Horticulturae, **235**, 2018, p. 228.
17. TURHAN, I., BIALKA, K.L., DEMIRCI, A., KARHAN, M., Bioresource Technology, **101**, 2010, p. 5290.
18. EL BATAL, H., HASIB, A., OUATMANE, A., DEHBI, F., JAOUAD, A., BOULLI, A., Arabian Journal of Chemistry, **9**, 2016, p. S955.
19. MARTINEZ-RODRIGUEZ, R., NAVARRO-ALARCON, M., RODRIGUEZ - MARTINEZ, C., FONOLLA-JOYA, J., Nutr. Hosp., **28**, no. 6, 2013, p. 2107.
20. AZAB, A., Eur. Chem. Bull., **6**, no. 10, 2017, p. 456.
21. FARAG, M.A., EL-KERSH, D. M., Journal of Advanced Research, **8**, 2017, p. 379.
22. CUSTÓDIO, L., PATARRA, J., ALBERÍCIO, F., NENGE, N. R., NOGUEIRAE, J. M. F., ROMANO, A., Natural Product Research, **29**, 2015, p. 2155.
23. RTIBI, K., SELMI, S., GRAMI, D., AMRI, M., ETO, B., EL-BENNA, J., MARZOUKI, L., Biomedicine & Pharmacotherapy, **93**, 2017, p. 522.
24. GOULAS, V., HADJISOLOMOU, A., Food Science and Technology, **101**, 2019, p. 269.
25. GOULAS, V., STYLOS, E., CHATZIATHANASIADOU, M. V., MAVROMOUSTAKOS, T., TZAKOS, A.G., International Journal of Molecular Sciences, **17**, 2016, p. 1875.
26. CUSTODIO, L., FERNANDES, E., ESCAPA, A.L., FAJARDO, A., ALIGNE, R., ALBERÍCIO, F., NENG, N.R., NOGUEIRA, J.M.F., ROMANO, A., J. Agric. Food Chem., **59**, 2011, p. 7005.
27. LAKKAB, I., EL HAJAJI, H., LACHKAR, N., EL BALI, B., LACHKAR, M., CIOBICA, A., Journal of Complementary and Integrative Medicine, **15**, no. 4, 2018, p. 20180013.

28. DUMITRU, G., ABIDAR, S., NHIRI, M., HRITCU, L., BOIANGIU, R.S., SANDU, I., TODIRASCU CIORNEA, E., Rev. Chim. (Bucharest), **69**, no. 12, 2018, p. 3545.
29. POSTU, AP. A., NOUMEDEM, J.A.K., CIOANCA, O., HANCIANU, M., MIHASAN, M., CIORPAC, M., GORGAN, D.L., PETRE, B.A., HRITCU, L., J. Cell. Mol. Med., **22**, no. 1, 2018, p. 111.
30. LEVIN, E.D., BENCAN, Z., CERUTTI, D.T., Physiol. Behav., **90**, 2007, p. 54.
31. DINAMARCA, M.C., SAGAL, J.P., QUINTANILLA, R.A, GODOY, J.A., ARRAZOLA, M.S., INESTROSA, N.C., Mol. Neurodegener., **5**, 2010, p. 4.
32. CHANG, Y.T., CHANG, W.N., TSAI, N.W., HUANG, C.C., KUNG, C.T., SU, Y.J., LIN, W.C., CHENG, B.C, SU, C.M., CHIANG, Y.F., LU, C.H., Biomed Res Int., **2014**, 2014, p. 182303.
33. MOHAMED, T., SHAKERI, A., RAO, P.P.N., Eur. J. Med. Chem., **113**, 2016, p. 258.

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