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Biological *in silico* effects of ω-3 and its derivates during pregnancy and postpartum

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Abstract

Pregnancy is a crucial time for the development of fetal health, so the mother's diet must be healthy and balanced. Omega-3 is one of the most important nutrients during this time, as it has beneficial effects for both the mother and the fetus. The aim of this article is to evaluate the in silico biological effects of omega-3 and its derivatives during pregnancy and postpartum, using online software and molecular docking tools. These have been shown to be beneficial to the fetus, especially in the first trimester of pregnancy.

Keywords: DHA; EPA; *in silico*; molecular docking; pregnancy.

1. Introduction

Pregnancy is a crucial period for the development of fetal health, and maternal nutrition must be healthy and balanced, to optimize the health of both, reduce the risk of complications during childbirth and prevent the development of some maternal-fetal pathologies (Trotta *et al*. 2021), such as hypertensive disorders of pregnancy (HDP), endocrinopathies and heart disease (Araújo *et al.* 1996). Food intake and maternal reserves comprise the only source of nutrients received by the fetus. Thus, maternal nutrition is one of the main determinants of fetal development (Trotta *et al*. 2021).

Essential nutrients include polyunsaturated fatty acids (PUFAs), such as omega-3 (ω-3), which are essential in the diet (Harris and Back 2015). They are also important for oxygen transport, energy storage, cell membrane constitution, regulation of inflammatory processes and cell proliferation, especially in the third week of pregnancy when fetal brain growth reaches its peak (physiological omphalocele) (Golding *et al.* 2009, Castro Rodrigues *et al.* 2020).

Omega-3 fatty acids primarily include plant-derived alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). They are found in fish oil and microalgae (Costa and Rosa 2010, Park *et al.* 2015) as well as in selected vegetable oils such as flaxseed, canola and soybeans (Greenberg *et al*. 2008, Zhang *et al*. 2019)

Adequate consumption of these omega fatty acids is very important during pregnancy to ensure fetal growth and brain and cognitive development. In addition, studies show that ω-3 can play a key role in the duration of pregnancy, in the prevention of symptoms of postpartum depression and in reducing the inflammatory

response in children, especially in the third trimester of pregnancy (Golding *et al*. 2009).

Before birth, all ω-3 and ω-6 (omega-6) ingested by the fetus comes from the maternal circulation by placental transfer (Salari *et al.* 2021). This mechanism is called diffusion. However, since the fetal demand is quite high, passive diffusion alone cannot meet the demand, so specific carriers called proteins are required to assist in this process (Gil-Sánchez *et al*. 2012). These proteins are fatty acid binding proteins of the placental plasma membranes (pFABPpm) and fatty acid binding proteins (FABPs) (Lager *et al.* 2016). The first is expressed in the region of the placenta facing the maternal circulation (Pan *et al.* 2015). The second is focused on the fetus (Duttaroy 2009), among which the FABP7 stands out for being related to the brain (Cunnigham and MacDermott 2009).

Systems biology integrates computational tools to analyze databases that contain information about functional interactions associated with macromolecules (Silva 2007). Using these tools, the discovery process becomes more efficient and research cost and time are reduced by up to 50% (Souza 2012). Among these tools, *in silico* assays stand out.

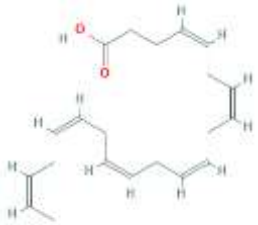
Therefore, the aim of the study was to verify the *in silico* prediction of ADME/Tox properties (absorption, distribution, metabolism, excretion and toxicity) and bioavailability of DHA and EPA and their interactions with placental transport proteins by molecular docking.

2. Material and Methods

2.1 In silico test

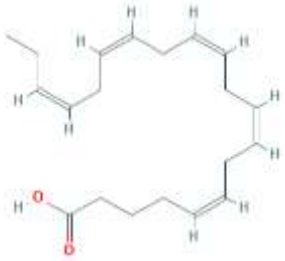
For the *in silico* test, the canonical SMILES of DHA and EPA were taken from PubChem (https://pubchem.nc.bi.nlm.nih.gov/) as shown in Table 1 and 2, respectively. Then, these canonical SMILES were copied into the computerized analysis tools (software).

Table 1. 2D structure and canonical SMILES of DHA.

Compound	**DHA**
Structure	
IUPAC name	(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid
Canonical SMILES	CCC=CCC=CCC=CCC=CCC=CCC=CCCC(=O)O

Source: Nacional Center for Biotechnology Information (NCBI) (2021).

Table 2. 2D structure and canonical SMILES of EPA.

Compound	EPA
Structure	
IUPAC name	(5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid
Canonical SMILES	CCC=CCC=CCC=CCC=CCC=CCCCC(=O)O

Source: Nacional Center for Biotechnology Information (NCBI) (2021).

The software used were Molinspiration (https://www.molinspiration.com/cgi-bin/properties), OSIRIS Property Explorer (https://www.organic-chemistry.org/prog/peo/), ProTox-II (http://tox.charite.de/protox_II/), and pkCSM (http://biosig.unimelb.edu.au/pkcsm/), all available online and free of charge.

Molinspiration was used to assess bioavailability; OSIRIS for predicting toxicity; ProTox-II, to measure hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity; and pkCSM, to observe pharmacokinetic and toxicological properties.

Molinspiration calculates the physicochemical properties relevant to the solubility and permeability of a given compound, such as. The octanol/water partition coefficient (logP), the topological polar surface area (TPSA), the number of atoms (nAtom), the number of hydrogen bond acceptors (nON), the number of hydrogen bond donors (nOHNH), the number of violations (nviolations), the number of rotatable bonds (nRot), and the molecular weight (MW) (Lipinski *et al*. 1997), which are related to Lipinski's Rule, also known as Lipinski's Rule of Five (Lipinski 2004).

2.1 Molecular docking

The docking procedure was performed to verify the interactions between the FABPs protein and the pFABPpm protein with the ligand DHA and the ligand EPA.

The 3D structures of the proteins studied in the simulation were obtained from the Protein Data Bank (PDB) database (https://www.rcsb.org/). The codes for these proteins are PDB ID: 1FE3 (FABP7) (Balendiran *et al.* 2000) and PDB ID: 1G5W (pFABPpm) (Lucke *et al.* 2001). The latter code was used as the basis for its generation in the SWISS-MODEL program (https://swissmodel.expasy.org/).

Subsequently, these molecules were analyzed in AutoDock Tools (ADT) (http://autodock.scripp.edu/) and the gridbox was created using the DeepSite program (https://playmolecule.com/deepsite/), preparing it for the next step.

The computational code applied in the simulations was the AutoDock Vina (ADV) (Trott and Oslon 2010),

which is a set of computational simulation software that allows the modeling of structures, in particular the analysis of the 3D geometric and energetic coupling between a protein and a ligand by molecular docking. Using this software, the interaction between proteins and ω-3 was evaluated to verify the affinity between the structures.

The analysis of the 3D results of the simulations was performed using the PyMOL™ 1.7.x software (Delano 2004) and the regions of docking interactions were verified using the LigPlus program (Trott and Oslon 2010).

The result is given in conformations, where each one has affinity, which is the binding energy (FEB) between the receptor and the ligand (Shityakov and Forster 2014) and is expressed in kcal/mol. It is considered favorable when it is represented negatively, that is, < 0 kcal/mol (Raschka 2014).

Another relevant parameter for the analysis is the RMSD (root mean squared deviation of atomic positions), which gives the upper and lower limits of the conformations performed and is expressed in angstroms (Å) (Quiroga and Villarreal 2016). The analysis parameters are maximum 2 Å and ≠ 0 (Lee *et al.* 2012).

3. Results and Discussion

In silico tests are performed using computer models formulated by numerical models and without human interaction (Travassos and Barros 2003). They are widely used to define the chemical-biological space of compounds incorporating various information such as prediction of molecular descriptors (Moda 2007). They can provide information on the toxicity, metabolism, absorption and excretion of a particular molecule or substance.

3.1 In silico test

DHA and EPA were analyzed *in silico* using the software described above, the results of which are shown in the following tables.

Table 3 shows the physicochemical properties of DHA and EPA by the Molinspiration software.

Table 3. Physicochemical properties and bioavailability of DHA and EPA using the Molisnpiration software.

	Compounds	
Parameters	**DHA**	**EPA**
logP	5,68	5,40
TPSA	37.30 $Å^2$	37.30 $Å^2$
nAtom	24	22
MW	328.50g/mol	302.46g/mol
nON	2	2
nOHNH	1	1
nviolations (Lipinski)	1	1
nRot	14	13
Volume	355.11	327.70

logP = octanol/water partition coefficient; TPSA = topological polar surface area; nAtom = number of

atoms; MW = molecular weight; nON = number of hydrogen bond acceptors; nOHNH = number of hydrogen bond donors; nviolations = number of violations; nRot = number of rotatable bonds.

As shown in Table 3, results were obtained with a partition coefficient of 5.68, a number of atoms 24, a molecular weight of 328.50 g/mol, a number of rotations of bonds 14, and a volume of 355.11 for DHA and a coefficient of partition of 5.40, a number of atoms 22, a molecular weight 302.46 g/mol, a number of rotations of bonds 13, and a volume of 327.70 for EPA. The topological polar surface area, number of hydrogen acceptors, number of hydrogen bond donors and number of violations were the same for both components: 37, 30, 2, 1, 1, respectively.

Based on the results found in this prediction using Molinspiration, the values of DHA and EPA were above the recommended value, indicating low hydrophilicity. The logP value should not exceed five, indicating low hydrophilicity to high logP values, resulting in malabsorption or permeation (Pereira 2013).

According to Lipinski's Rule of Five (Lipinski *et al*. 1997), a bioactive molecule to be absorbed by passive diffusion must have a logP value of less than 5, MW must not exceed 500 daltons (Da) and must not have more than 5 nOHNH functional groups and 10 nON groups. This rule was formulated to evaluate similarity or to determine whether a chemical compound with a particular biological or pharmacological activity has properties that make it a likely drug for oral use in humans (Lipinski 2004). Thus, the molecule only needs to violate one of these parameters to be a drug candidate. In Table 3, it can be seen that one of the rules has been violated for the two compounds, namely that the logP value is greater than 5.

The software OSIRIS Property Explore predicts the risk of toxic effects such as mutagenic, tumorogenic, irritant, reproductive effects and cytotoxic. The results found are described in Table 4.

Table 4. Toxicity properties of DHA and EPA by the OSIRIS software.

		Compounds	
	Parameters	**DHA**	**EPA**
Toxicity	Mutagenic	●	●
	Tumorogenic	●	●
	Irritant	●	●
	Reproductive effective	●	●
	Cytotoxicity	●	●

● low risk ● high risk

The prediction results of OSIRIS are evaluated and color coded. Properties with a high risk of adverse effects, such as mutagenicity or intestinal malabsorption, are shown in red. While green color indicates drug compliant behavior (OSIRIS Property Explorer). According to the results of this prediction, a low risk was found for all the parameters analyzed.

This software determines the abundance of the sought fragments in the structures of more than three thousand commercial drugs. Based on the assumption that marketed drugs are largely free of toxic effects, each fragment was considered a risk factor if it occurs frequently as a substructure of harmful compounds but never or rarely in marketed drugs (OSIRIS Property Explorer).

According to studies conducted in Australia and Iran, supplementation with ω-3 fatty acids can treat male infertility by significantly improving sperm motility and DHA concentration in seminal plasma of infertile men (Hosseini *et al*. 2019).

In addition, research shows that consuming adequate amounts of DHA and EPA is associated with a reduction in the risk of developing several diseases, including neurological, cardiovascular, and cancer. Since DHA inhibits proliferation, angiogenesis, metastasis and promotes cell death in various cancer models, it been shown to be efficient in reducing the risk of developing various tumor types. Moreover, these lipid compounds have shown cardioprotective, vasodilatory and antiallergic properties in preclinical studies (Schunck *et al.* 2018).

Studies also indicate that DHA plays an important role in inhibiting oxidative stress caused by proinflammatory genes and in inhibiting apoptosis in the brain and retina (Bazan 2006, Innis 2007a, 2007b). In addition, ω-3 reduces inflammation and fatty infiltration in the liver of patients with nonalcoholic fatty liver disease (NAFLD), which helps prevent patients from progressing to cirrhosis and liver failure (Scorletti and Byrne 2018).

ProTox-II predicts toxicity parameters, including median lethal dose (LD50), toxicity class, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity, as well as in predicting the OSIRIS program.

This software considers as reliable those results that have a p>0.7. The results found are listed in Table 5.

Table 5. Toxicity properties of DHA and EPA by the ProTox-II software.

		Compounds			
	Parameters	**DHA**	**p***	**EPA**	**p***
Toxicity	LD_{50}	10.000 mg/kg	1.0	10.000 mg/kg	1.0
	Toxicity class	VI	1.0	VI	1.0
	Hepatotoxicity	ND	0.57	ND	0.54
	Carcinogenicity	ND	0.64	ND	0.63
	Immunotoxicity	No	0.99	No	0.99
	Mutagenicity	No	0.90	No	0.95
	Cytotoxicity	No	0.70	No	0.71

LD50 = median lethal dose; *Reliable results were not detected p>0.7; ND = not detected, because the value of p<0.7.

This software has six toxicity classes according to LD50, as shown in Table 6. The LD50 is the dose required to cause the death of 50% of a test population in mg/Kg (ProTox-II).

Table 6. ProTox-II software toxicity class.

Class	Consequence	LD50
I	Fatal by ingestion	≤ 5 mg/Kg
II	Fatal by ingestion	5 – 50 mg/Kg
III	Toxic if swallowed	50 – 300 mg/Kg
IV	Harmful if swallowed	300 – 2.000 mg/Kg
V	May be harmful if swallowed	2,000 – 5,000 mg/Kg
VI	Non-toxic	> 5,000 mg/Kg

Source: ProTox-II (2021).

From the results obtained, the LD50 of both components is 10,000 mg/Kg, as well as the toxicity class VI, which means that they are non-toxic and have no hepatotoxic and carcinogenic profiles.

The ADME/Tox properties of the compounds DHA and EPA were performed using the pkCSM software, the results of which are described in Table 7.

Table 7. ADME/Tox properties of DHA and EPA using pkCSM software.

		Compounds	
	Parameters	**DHA**	**EPA**
Absorption	Caco2 permeability	High	High
	Intestinal absorption	Good	Good
	Skin permeability	Good	Good
	P-glycoprotein substrate (Pgp)	No	No
Distribution	VDss	Low	Low
	BBB permeability	Does not permeate 100%	No
	CNS permeability	Yes	Yes
Metabolism	CYP1A2 inhibitor	Yes	Yes
	CYP3A4 substrate	Yes	Yes
	CYP2C9 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2D6 substrate	No	No
Excretion	Total clearance	2,264 mL/min/Kg	2,155 mL/min/Kg
Toxicity	Mutagenic	No	No
	Hepatotoxicity	Yes	Yes
	Skin sensitization	Yes	Yes

Source: pkCSM (2021).

DHA and EPA showed high permeability in Caco-2 gastrointestinal cells, with good permeability in the intestine and skin permeability, but not complete permeability the blood-brain barrier (BBB), which is due to its bioavailability.

DHA, in combination with DHA-derived NPD1 (neuroprotection D1), promotes enhanced neurogenesis and angiogenesis, reduces ischemic tissue damage and decreases BBB (Belayev *et al.* 2011, Eady *et al.* 2012).

In agreement with the above data, experimental studies have shown that DHA significantly improves histopathological findings after ischemic stroke. This highlights its neuroprotective capacity and attenuation of behavioral (Belayev *et al.* 2015, Hong *et al.* 2015).

During the initial phase of the life cycle, from embryogenesis to the second year of life, there is the greatest expansion of the brain mass and the main events involved in the formation of the Central Nervous System (CNS). Factors that can disrupt these processes have significant effects on brain tissue and, consequently, on cognitive performance (Innis 2014).

Therefore, an adequate prenatal supply of ω-3 is crucial prerequisite for the ideal development of neural functions (Janssen 2014).

The clinically most important cytochrome P450 (CYP450) enzymes are CYP1A2, CYP2D6, CYP2C9, CYP2C19, and CYP3A4 and are involved in the metabolism of more than 90% of drugs (Lynch and Price 2007). The most important among them is CYP3A4, which is involved in the synthesis of lipids such as cholesterol. Among these enzymes, CYP1A2, CYP2D6 and CYP3A4 may show changes in their activities depending on the week of pregnancy (Tracy *et al.* 2005).

Furthermore, lipid mediators formed from ω-3 fatty acids play an important role in renal regulation of hemodynamic processes (An *et al.* 2009).

3.1 Molecular docking

Molecular docking allows the study of chemical structures and possible orientations that a given molecule may adopt within a binding site of a bioreceptor or between two macromolecules (pharmacophore model), that is, it is used to verify the affinity of a ligand to a given binding site (Lipinski 2004).

As for the proteins used for molecular docking, pFABPpm are exclusively expressed in the placental region exposed to maternal circulation (Pan *et al.* 2015). Among them, pFABPpm 5 stands out to facilitate the transport of DHA to the BBB (Pan *et al.* 2015).

FABPs, on the other hand, may be responsible for cytoplasmic translocation of free fatty acids (FFA) to sites of esterification, β-oxidation or into the fetal circulation (Duttaroy 2009) and are able to bind specifically to nuclear transcription factors such as the PPAR (peroxisome proliferator activated receptor) in the cell nucleus (Storch and Corsico 2008). The FABPs described in placenta are FABP1 or liver FABP (L-FBP), FAB3 or heart FABP (H-FABP), FABP4 or adipocyte FABP (A-FABP), FABP5 or keratinocyte FABP (K-FABP) and FABP7 or brain FABP (B-FABP) (Cunningham and McDermott 2009). FABP7 was chosen for the study because it is associated with the brain.

The values of the central coordinates for the docking box (gridbox) determine the active site of each protein and are shown in Table 8.

Table 8. Central coordinates of box (gridbox) in Deepsite software.

Protein	Central coordinates [X, Y, Z]
pFABPpm	[-3,5; 4,5; -3,1]
FABP7	[96,6; 72,9; -41,0]

The interaction between the ligands (DHA and EPA) with the above proteins was observed using the AutoDock Vina software and the analysis of the 3D results of the docking interaction regions performed using the PyMOL™ and LigPlus programs can be found below. The results found by AutoDock Vina are shown in Table 9.

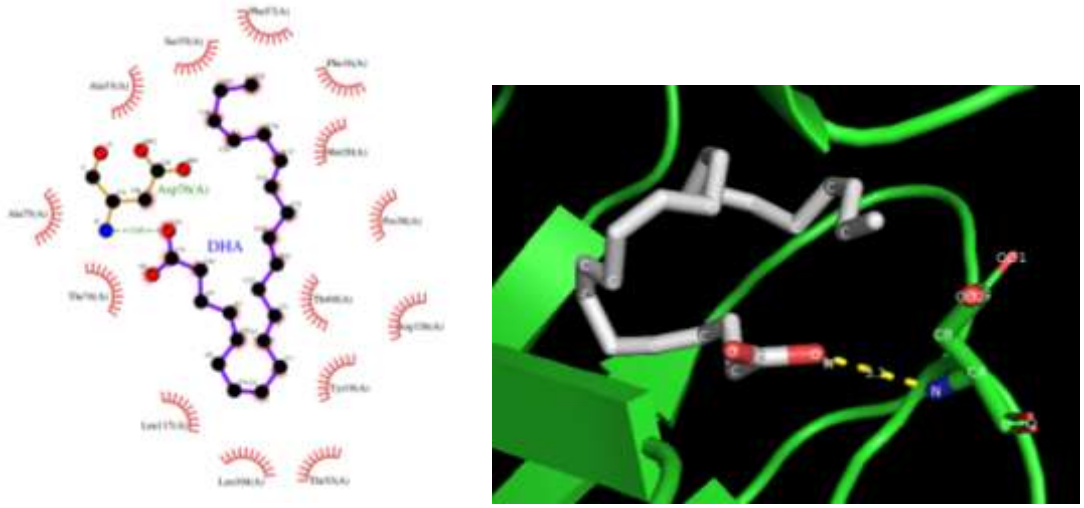
Table 9. Affinity values (FEB) and RMSD for the best fit configurations for each system studied.

Complex		FEB (kcal/mol)	RMSD (Å)
Protein	Binder		
pFABPpm	DHA	- 5,8	1,963
	EPA	- 5,7	1,673
FABP7	DHA	- 7,0	1,935
	EPA	- 6,8	1,522

The pFABPpm-DHA complex had configuration 2 as being the most stable, FEB of – 5.8 kcal/mol and an RMSD of 1.963 Å, whereas the pFABPpm-EPA complex had configuration 3 the most stable, with FEB of – 5.7 kcal/mol and RMSD of 1.673 Å. The data obtained by LigPlus and PyMOL, regarding the interactions with the pFABPpm protein are shown in Figure 1 and Figure 2.

Figure 1: pFABPpm-DHA complex: (a) 2D diagram of the system interaction type; and (b) 3D representation of the best fit configuration.

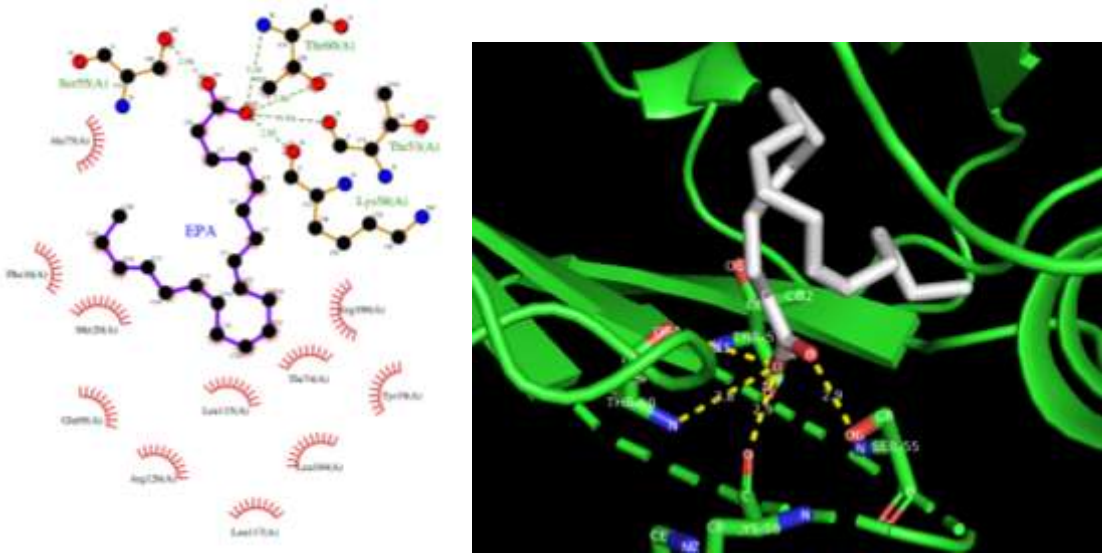
(a) (b)



(a) 2D diagram representing the types of bonds between the pFABPpm-DHA complex and the aminoacids; (b) 3D diagram representing the best fit configuration between the pFABPpm-DHA complex.

Figure 2: pFABPpm-EPA complex: (a) 2D diagram of the system interaction type; and (b) 3D representation of the best fit configuration.

(a) (b)



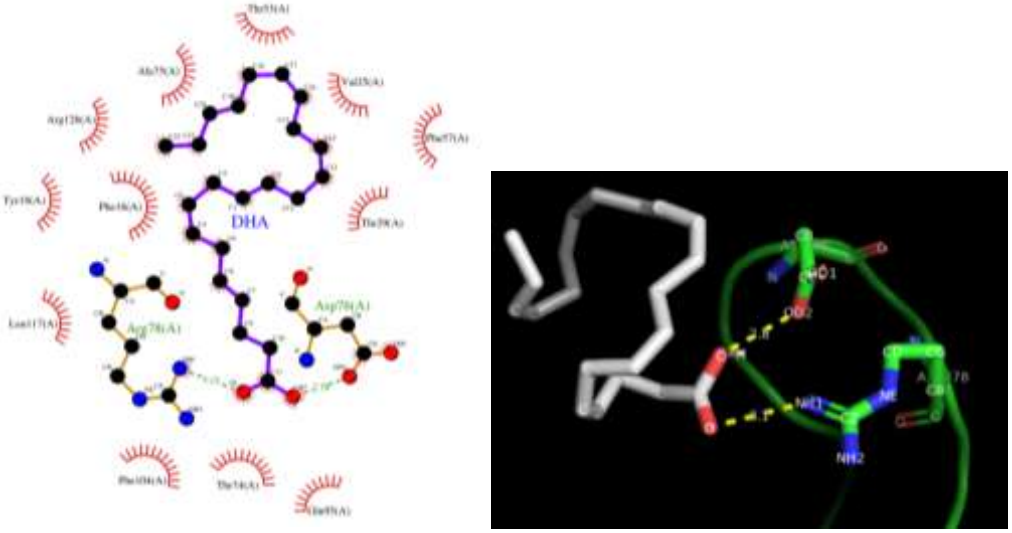
(a) 2D diagram representing the types of bonds between the pFABPpm-EPA complex and the aminoacids; (b) 3D diagram representing the best fit configuration between the pFABPpm-EPA complex.

In both pFABPpm complexes, a good affinity with the studied ligands was found, as well as similar FEB values. However, when we analyze the 2D diagram (Figure 2), it is verified the occurrence of a stronger interaction in the pFABPpm-EPA coupling, because in addition to the hydrophobic contact interactions, it has four hydrogen bonds (more intense intermolecular force) between the EPA ligand atoms and the protein chain aminoacids serine, threonine and lysine. Furthermore, in these connections, the interatomic distances were smller than 7 Å, which is considered relevant for the coupling process (Trott and Oslon 2010).

The FABP selected for the study was FABP7 because it has functions related to the brain. According to the data in Table 9, the FABP7-DHA coupling had FEB of – 7.0 kcal/mol and an RMSD of 1.935 Å, while FABP7-EPA had FEB of – 6.8 kcal/mol and an RMSD of 1.522 Å. In Figure 3 and Figure 4, the possible sites of interaction between the FABP7 protein and the DHA and EPA ligands, respectively, are observed. Both complexes showed good affinities with the ligands, as well as hydrophobic contact interactions and hydrogen bonds.

Figure 3: FABP7-DHA complex: (a) 2D diagram of the system interaction type; and (b) 3D representation of the best fit configuration.

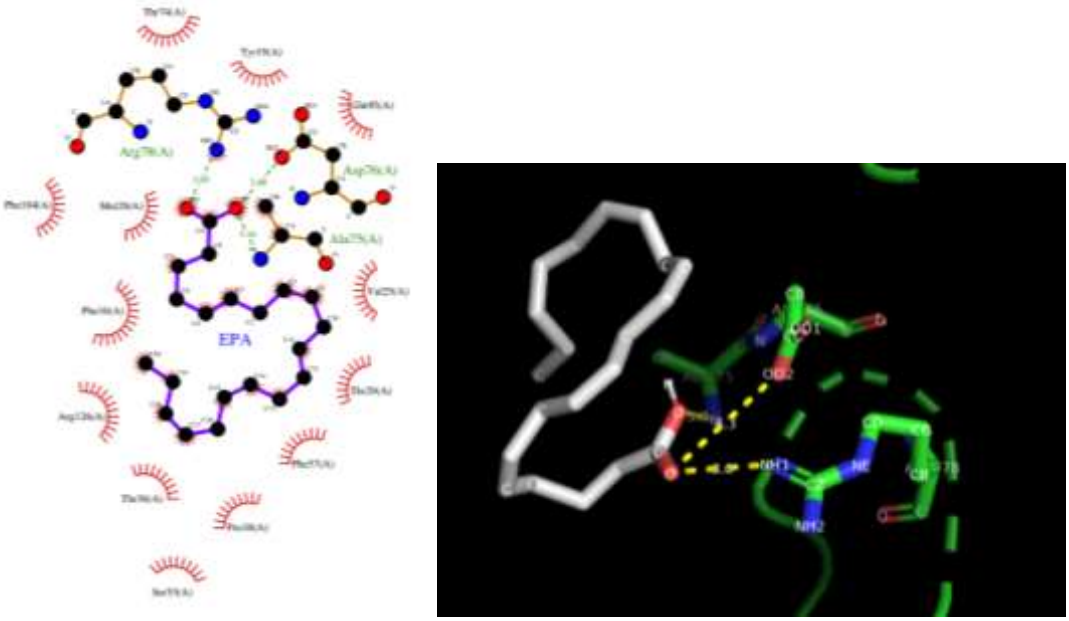
(a) (b)



(a) 2D diagram representing the types of bonds between the FABP7-DHA complex and the aminoacids; (b) 3D diagram representing the best fit configuration between the FABP7-DHA complex.

Figure 4: FABP7-EPA complex: (a) 2D diagram of the system interaction type; and (b) 3D representation of the best fit configuration.

(a) (b)



(a) 2D diagram representing the types of bonds between the FABP7-EPA complex and the aminoacids; (b) 3D diagram representing the best fit configuration between the FABP7-EPA complex.

5. Conclusion

Based on the results presented through the *in silico* tests suggest that DHA and EPA have positive factors for the formation and development of the fetus, especially in the first trimester of pregnancy, as this is when fetal brain development occurs, since they do not produced significant toxic effects on *in silico* predictions. Furthermore, it presented relevant pharmacophoric models demonstrating that the molecules studied can act in the regulation of lipid mediators.

As with any other test, these have their limitations, but they reduce the cost and research time by up to 50%, thus suggesting further research in area.

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