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MAY ANTIEPILEPTIC DRUGS CAUSE LETHAL, SUBLETHAL, TERATOGENIC EFFECTS AND MORPHOMETRIC ALTERATIONS ON EMBRYOS AND LARVAE OF ZEBRAFISH (*DANIO RERIO*)? A SYSTEMATIC REVIEW

Drogas antiepiléticas podem causar efeitos letais, subletais, teratogênicos e alterações morfométricas em embriões e larvas de zebrafish (*Danio rerio*)? Uma revisão sistemática

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ABSTRACT: **Objective:** The research was carried out for clarify, through a systematic review, the evidence of lethal, sublethal, teratogenic effects and morphometric changes caused by gabapentin, phenobarbital, oxcarbazepine and lamotrigine on embryos and larvae of zebrafish. **Methods:** The research strategy involved the participation of two researchers who independently evaluated the methodological quality of each papers, available in Pubmed, Science Direct, Google Scholar, Scopus and CAPES journals databases. **Results:** The research strategy found 07 papers in Pubmed, 228 papers in Science Direct, 2639 papers in Google Scholar, 330 papers in Scopus and 67 papers in CAPES journals totaling 3271 potentially relevant studies in the last 05 years. The 3269 studies were eliminated. **Conclusion:** According to the results, it was observed that only 2 papers have analyzed any lethal, sublethal or teratogenic effects on zebrafish larvae and larvae. Among them, only gabapentin, phenobarbital and lamotrigine were found in the literature. No studies were found approaching oxcarbazepine. And no work was done in the literature analyzing the morphometry in zebrafish larvae exposed to any antiepileptic.

Keywords: Zebrafish. Gabapentine. Phenobarbital. Oxcarbazepine. Lamotrigine.

RESUMO: **Objetivo:** A pesquisa foi realizada para analisar, por meio de uma revisão sistemática, as evidências dos efeitos letais, subletais, teratogênicos e alterações morfométricas causadas pela gabapentina, fenobarbital, oxcarbazepina e lamotrigina em embriões e larvas do peixe-zebra. **Métodos:** A estratégia de pesquisa contou com a participação de dois pesquisadores que avaliaram de forma independente a qualidade metodológica de cada artigo, disponível nas bases de dados de periódicos Pubmed, Science Direct, Google Scholar, Scopus e CAPES. **Resultados:** A estratégia de pesquisa encontrou 07 artigos no Pubmed, 228 artigos no Science Direct, 2.639 artigos no Google Scholar, 330 artigos no Scopus e 67 artigos em periódicos da CAPES totalizando 3.271 estudos potencialmente relevantes nos últimos 05 anos. Os 3269 estudos foram eliminados. **Conclusão:** De acordo com os resultados, observou-se que apenas 2 artigos analisaram efeitos letais, subletais ou teratogênicos sobre as larvas e larvas do peixe-zebra. Dentre eles, apenas gabapentina, fenobarbital e lamotrigina foram encontrados na literatura. Nenhum estudo foi encontrado abordando a oxcarbazepina. E nenhum trabalho foi feito na literatura analisando a morfometria em larvas de peixe-zebra expostas a qualquer antiepiléptico.

Palavras-chave: Peixe-zebra. Gabapentina. Fenobarbital. Oxcarbazepina. Lamotrigina.

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1 INTRODUCTION

Drugs are naturally excreted by the body: unchanged or not. However, the presence of these drugs, released unchanged in aquatic environments, represents a serious risk when interacting with biological systems and thus causing changes in the development, growth and reproduction of organisms exposed to these substances. Aquatic ecosystems are constantly under pressure because of the human activities. The preservation as well as the remediation of aquatic ecosystems is essential for the maintenance of human and environmental well-being (MIKLOS *et al.*, 2018; SILVA *et al.*, 2018; SONG et al., 2018). And once in the environment, psychoactive drugs can trigger effects at different levels of biological organization, from the intracellular level to the ecosystem level (HAMILTON et al., 2017; KIRSTEN et al., 2018; MACLAREN, WISNIEWSKI and MACLAREN, 2018). Studies on ecotoxicology such as: the presence of antiepileptic drugs in the environment poses a serious risk when interacting with physiological systems. These substances can directly affect biosynthesis and neuronal metabolism, promoting changes in the development and reproduction of organisms exposed to them (ANDRADE et al., 2017). Studies on ecotoxicology as followed: the presence of antiepileptic drugs in the environment poses a serious risk when interacting with physiological systems. These substances can directly affect biosynthesis and neuronal metabolism, promoting changes in the development and reproduction of organisms exposed to them (ANDRADE et al., 2017). In addition, contamination of antiepileptic drugs in the environment from many sources can reach aquatic ecosystems and groundwater transported by drainage, leaching of rainwater, irrigation, drainage and can be absorbed by living organisms tending to bioaccumulate (ARAÚJO et al., 2016). Therefore, it is essential to control the presence of these compounds in the environment, as they pose a risk to human and environmental health. Besides that, early monitoring allows the identification of contaminations before there are higher levels of biological organisms affected (ARAÚJO et al., 2016). Such a task can be performed by biomarkers consisting of measurements of body fluids, cells or tissues that indicate, in biochemical terms, cellular, physiological or behavioral terms the presence of contaminants in the target organism, for example in Zebrafish. Therefore, these parameters represent tools capable of determining the magnitude of the effects of the mentioned pollutants (ARAÚJO et al., 2016). Aquatic ecosystems are constantly under pressure because of the human activities. The preservation as well as the remediation of aquatic ecosystems is essential for the maintenance of human and environmental well-being (MIKLOS et al., 2018; SILVA et al., 2018; SONG et al., 2018). And once in the environment, psychoactive drugs can trigger effects at different levels of biological organization, from the intracellular level to the ecosystem level (HAMILTON et al., 2017; KIRSTEN et al., 2018; MACLAREN, WISNIEWSKI and MACLAREN, 2018). And once in the environment,

psychoactive drugs can trigger effects at different levels of biological organization, from the intracellular level to the ecosystem level (HAMILTON et al., 2017; KIRSTEN et al., 2018; MACLAREN, WISNIEWSKI and MACLAREN, 2018).

Researches states that the zebrafish is rapidly gaining in popularity in translational neuroscience and behavioral research (LE et al., 2019; Shi et al., 2019; ALTENHOFEN et al., 2017; CUNLIFFE et al., 2015). Physiological similar to mammals, ease of genetic manipulation, sensitivity to pharmacological and genetic factors, robust behavior, low cost and potential for high-throughput screening contribute to the increasing use of Zebrafish models in this field (HILL et al., 2010; SANTOS et al., 2010). Thus, considering that: i) many neurotransmission systems have already been identified in Zebrafish, among them gabaergic (Kim et al., 2004); (ii) antiepileptics potentiate neural receptor function (iii) and that Zebrafish behavior can be easily observed and quantified in a controlled environment (Boxall, 2004; Chou; Talalayi, 1984; EPA, 2002). Zebrafish has been widely used in several studies, including toxicology. This is due to the fact of its size, its rapid external development and low maintenance cost (Scholz, 2013). Zebrafish also has mammalian-like neurophysiology (Aillon et al., 2009). That is why studies with zebrafish are a rapid tool for assessing epilepsy as well as the teratogenicity potential of these drugs. And, despite current studies on its teratogenic effects of antiepileptics, there are few studies comparing effects for each drug, and none comparing all the drugs analyzed here, together. A monitoring program conducted on the Rhine River (Germany) over a decade showed regular detection of carbazepine, with an annual mean of concentration of 100 ng/L. These results support the idea that the presence of carbazepine in the environment may represent a real threat (SANTOS, 2010). Such a task can be performed by biomarkers consisting of measurements of body fluids, cells or tissues that indicate, in biochemical terms, cellular, physiological or behavioral terms the presence of contaminants in the target organism, for example in Zebrafish. Therefore, these parameters represent tools capable of determining the magnitude of the effects of the mentioned pollutants (ARAÚJO et al., 2016).

1.1 Materials and Methods

Initially, the search strategy involved the participation of three researchers that evaluated the methodological quality of each papers, available full text in Pubmed, Science Direct, Google Scholar, Scopus and CAPES journals databases. The lethal, sublethal and teratogenic effects of gabapentin, lamotrigine, phenobarbital and oxcarbamazepine on zebrafish embryos were defined as the focus of the investigation. The outcomes sought in the search, judged to be clinically relevant were the following: The lethal endpoints analyzed were coagulation (Cg), tail not detached (Tnd), no somite formation (Nsf), no heart-beat (Nhb), lack of hatching (Lh) and mortality (Mo) (%)

(LAMMERet al., 2009). The dead animals were removed every day (Yekti, Hsu and Wang, 2014). The sublethal developmental endpoints analyzed were the formation of somites (Fs), development of eyes (De), heartbeat/blood circulation (Hbc), heartbeat frequency (Hf), increased pigmentation (Ip), pericardial edema (Pe), Number of heartbeats (Nh) (bpm/min). Lastly, the endpoints of teratogenicity were malformation of the head (Mhd), malformation of heart (Mh), tail deformation (Td), spine deformation (Sd), yolk sac edema (Yse), inflated swim bladder (Isb), growth retardation (Gr) and coagulation points (Cp) (LAMMERet al., 2009). The following parameters were analyzed to evaluate the morphometric alteration: The body length (Bl) (μm), head-width (Hd) (midbrain) measurement (μm) (Nakayama, Johnstone, Manica, 2012), ocular distance (OD) using the distance between the inner edge of the two eyes (μm) (similar to the inner intercanthal distance in humans) (ALTENHOFEN et al., 2017) and eye diameter (ED) (μm) (Dzieciolowska et al., 2017) were evaluated. In the search strategy, in order to avoid the loss of studies that eventually had a description of such outcomes only in the complete papers without reporting in the abstract, no words referring to outcomes of interest were placed. The search strategy used was the following for all databases: ''Zebrafish AND Anticonvulsants'', ''Zebrafish AND Gabapentin'', ''Zebrafish AND Lamotrigine'', ''Zebrafish AND Phenobarbital'', ''Zebrafish AND Oxcarbamazepine'', ''Zebrafish AND Morphometry AND Antiepileptics''. After the completion of the search, whose deadline was 05/01/2020, all summaries were evaluated. The studies described by design of lethal, sublethal and teratogenic effects of gabapentin, lamotrigine, phenobarbital and oxcarbamazepine on zebrafish embryos were selected to search the full text. No abstracts were searched for that could lead to unpublished studies on the basis of journals or in progress or those studies in which they were available only in dissertation banks or theses. The included studies had their references reviewed to search for other possible relevant studies. The method chosen to compile the available evidence was the meta-analysis.

1.3 Results

The search strategy found 07 papers in Pubmed, 228 papers in Science Direct, 2639 papers in Google Scholar, 330 papers in Scopus and 67 papers in CAPES journals totaling 3271 potentially relevant studies. The 3269 studies eliminated in this stage are detailed (figure 1), 2 papers are included according to the eligibility criteria, Table 1. The outcomes that were objectified in our work as tail not detached (Tnd), lack of hatching (Lh), development of eyes (De), increased pigmentation (Ip), Number of heartbeats (Nh) (bpm/min), malformation of the head (Mhd), malformation of heart (Mh), tail deformation (Td), spine deformation (Sd), yolk sac edema (Yse), inflated swim bladder (Isb), growth retardation (Gr) and coagulation points (Cp) were not possible to be contemplated in this review. Was not possible to be contemplated in this review. Furthermore, no studies were found

on any lethal, sublethal and teratogenic effects on oxcarbazepine. The only points that could be observed were: coagulation, no somite formation, heartbeat/blood circulation, pericardial edema, coagulation points, malformation of the head, malformation of heart, tail deformation, spine deformation, yolk sac edema, inflated swim bladder, growth retardation Table 2.

Figure 1. Flowchart and criteria for selecting and including articles in Pubmed, Science Direct, Google Scholar, Scopus and CAPES journals to identify endpoints: lethal, sublethal, teratogenic and morphometric effects of zebrafish exposure to gabapentin, phenobarbital, oxcarbazepine and lamotrigine.

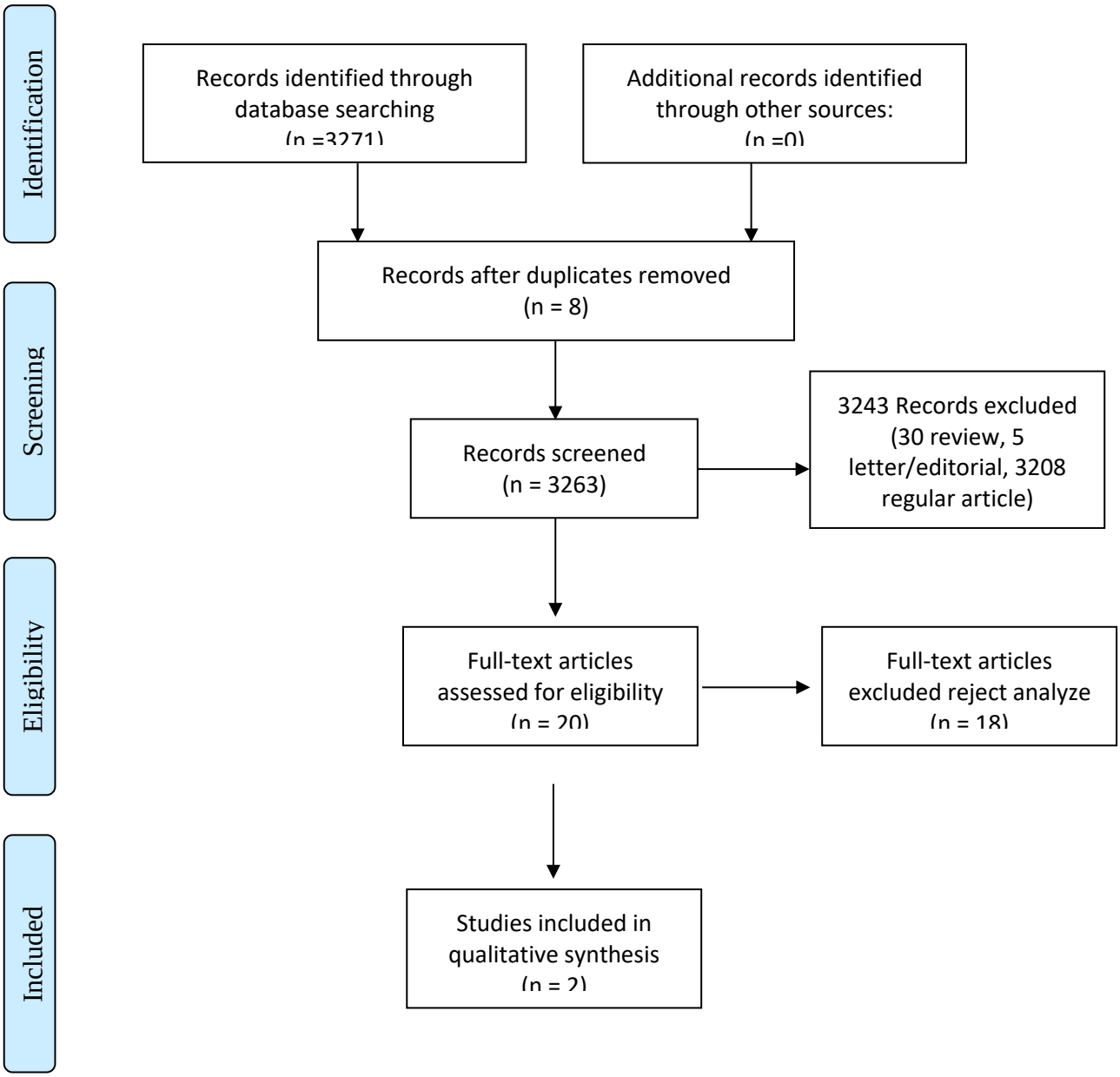

Table 1 - Demonstration of the articles that integrate the systematic review

Author/Date	Objective	Endpoint analyzed	Results
Li et al, 2018	To investigate the effects of GAB on the early development of zebrafish and on its antioxidant system.	Heartbeat rate, body length, swimming frequency and dry weight.	The GAB causes malformation of embryos such as hemagglutination and pericardial edema. Compared to the control group, a significant increase ($p < 0.05$) of heartbeat rates was found at GAB concentrations exceeding 50 mg L^{-1}, while the swimming frequency was clearly increased upon exposure to GAB at a concentration of 100 mg L^{-1} ($p < 0.05$). Additionally, the development of the zebrafish embryo was also negatively impacted after exposure to GAB as demonstrated by significantly decreased body lengths. Exposure to GAB at concentrations exceeding 50 mg L^{-1} significantly influenced the development of zebrafish, leading to malformation of organs and abnormal movements. Although it was observed no significant developmental effects of GAB at environmentally relevant concentrations (0.1 and 10 mg L^{-1}).
MARTINEZ et al., 2018	To evaluate the teratogenic and anticonvulsant effects of six different AEDs: carbamazepine, levetiracetam, lamotrigine, phenobarbital, phenytoin and valproic acid.	Hatching, mortality and body axis of zebrafish embryos.	Lamotrigine and phenytoin could be appropriate non-teratogenic and efficient anticonvulsant options for epilepsy treatment.

2 Discussion

Epilepsy consists of a neurological disorder that can reach 3% of the world population and may be associated with anxiety and depression (SCHMIDT and SILLANPAA, 2012; TELLEZ-ZENTENO et al., 2007). It is characterized by recurrent seizures and its origin may be related to the increase of excitatory receptors and decrease of gamma-aminobutyric acid (GABA) (SIEBEL et al., 2011; FISHER et al., 2005; RAHN et al., 2014). Many antiepileptic drugs are proven to be effective, but also have proven side effects (LÖSCHER and SCHMIDT, 2011). In addition, antiepileptics are administered to women with epilepsy and of childbearing age and even pregnancies, so it is essential

to study toxicity, malformations and even growth retardation (FRIEDMAN and FRENCH, 2012). Not only are antiepileptic drugs teratogenic, they are also risk factors for congenital malformation. An estimation of important congenital malformations, such as: cardiac alterations, neural tube defects and facial fissures in epileptic women may reach 10%. This means that pregnant women may have an increase in congenital malformations two to four times more than the general population. In contrast, data on the effects of these drugs are still unclear (METE et al, 2016). According to this, there are drugs that have been prescribed as GAB, PB, OX and LTG that can cause teratogenic effects (MEADOR and LORING, 2016). Moreover, contamination of antiepileptic drugs in the environment from many sources can reach aquatic ecosystems and groundwater transported by drainage, leaching of rainwater, irrigation, drainage and can be absorbed by living organisms tending to bioaccumulate (ARAÚJO et al., 2016).Therefore, it is essential to control the presence of these compounds in the environment, as they pose a risk to human and environmental health. In addition, early monitoring allows the identification of contaminants before there are higher levels of biological organisms affected (ARAÚJO et al., 2016).

Pollution of aquatic ecosystems and the degradation of water resources result in the scarcity of drinking water. Thus, it is necessary to develop innovative, efficient technologies in the removal of emerging pollutants, including drugs, in contaminated waters (Bittencourt et al., 2017, SANTOS et al., 2016, SANTOS et al., 2016). Consequently, the use of animal models in psychiatry is of great importance to perform preclinical evaluations of several psychotropic drugs, through the animal tests to produce information regarding the biochemical effects of these drugs on specific targets (ARAÚJO et al., 2016, KALUEFF et al., 2013, SNEDDON, 2015). The study is part of the main psychiatric instrument, mainly in the search for new and better treatments (DAMASKI et al., 2011, GEBAUER et al., 2011, LIMA, 2011). And for this it is important to use the acute toxicity tests that are used as legal parameter of regulation of water quality, effluent and sediment. Conama Resolution 344/04 instituted ecotoxicological tests in cases of sediment disposal to be dredged when the concentration of some substances may pose a risk. Conama Resolution 357/05 instituted the use of ecotoxicological tests as both a parameter of water quality and effluent (ANDRADE et al., 2017).

Lethal, sublethal, teratogenic, and morphometric alterations on embryos and larvae of zebrafish were observed in zebrafish embryos exposed to gabapentin (GA), phenobarbital (PH), lamotrigine (LA) and oxcarbazepine (OX).

2.1 Lethal effects

The lethal endpoints analyzed were coagulation (Cg), tail not detached (Tnd), no somite formation (Nsf), no heart-beat (Nhb), lack of hatching (Lh) and mortality (Mo) (%) (LAMMERet al., 2009). The most recent works that drugs the heartbeat rate at 48 hpf is often used as an indicator

to assess adverse effects of pollutants are important to highlight being (LI et al., 2018; FRAYSSE, MONS and GARRIC, 2006). The heart is the first organ formed in the zebrafish. The heart can be visualized quickly due to transparent development of this animal. Its beat is regularized between 36 and 48 hpf nd this parameter is directly linked to the temperature (LI et al., 2018). Carbamazepine and valproic acid significantly decrease the heartbeat of zebrafish embryos according to the dose at which the embryos are exposed (PRUVOT et al., 2012). However, the use of gabapentin increased the frequency of zebrafish, as well as mammals suggesting GABA activated the circulatory system of zebrafish and this might be caused by the mechanism of GAB to the nervous system of zebrafish (KUKKAR et al.,2013) Furthermore, GABA toxicity tests show children who have become hyperactive and have aggressive behavior (WOLF et al., 1996). This strongly indicates GA has adverse effects on the development of zebrafish.

2.2 Sublethal effects

Sublethal developmental endpoints analyzed were the formation of somites (Fs), development of eyes (De), heartbeat/blood circulation (Hbc), heartbeat frequency (Hf), increased pigmentation (Ip), pericardial edema (Pe), Number of heartbeats (Nh) (bpm/min) (LAMMERet al., 2009). Pericardial edema is the most popular indicator in zebrafish toxicity test. (LI et al., 2018; FRAYSSE, MONS and GARRIC, 2006).

2.3 Teratogenic effects

Endpoints of teratogenicity were malformation of the head (Mhd), malformation of heart (Mh), tail deformation (Td), spine deformation (Sd), yolk sac edema (Yse), inflated swim bladder (Isb), growth retardation (Gr) and coagulation points (Cp) (LAMMERet al., 2009). A reducing tendency was observable on the body length of zebrafish by effect of GA (LI et al., 2018). Carbamazepine induced an increase of body length (MADUREIRA et al., 2012). However, exposure to GAB caused a concentration-dependent decrease of body length of zebrafish at 72 hpf (LI et al., 2018). Tail size is also an important indicator to assess toxicity of chemicals. Tail size decreases due to exposure of pollutants (FRAYSSE, MONS AND GARRIC, 2006). Normal hatching was observed, and the morphology was not altered in the animals exposed to PH and LA in concentrations up to 100 μM (MARTINEZ et al., 2018). Among the antiepileptic drugs analyzed LA is the most appropriate candidate since it performs in the right way as an antiepileptic drug at low doses and it does not present teratogenic or neurotoxic effects (MARTINEZ et al., 2018).

2.4 Morphometric alterations

Drugs directly or indirectly act on the neurotransmission system may alter behavior, modulate responses to sensory stimuli (MURPHY and SILLITO, 1991), sleep control, learning, and memory (SHAKED et al., 2008). And, these neuronal changes can be measured indirectly from morphometric analyzes of embryos in zebrafish (ALTENHOFEN et al., 2017). Morphometric analysis are specific non-invasive neuroimaging investigations to identify neuronal changes caused by the use of antiepileptics (LI et al., 2019). Yet, little attention is given to the event in the literature. This analysis can be done and is important in determining the toxicity of antiepileptics.

3 Conclusion

In this work, it was studied if antiepileptic drugs can cause lethal, sublethal, teratogenic, and morphometric alterations on embryos and larvae of zebrafish. The results of the studies show GA produces changes in the heart beats and morphology of these animals. It is given little attention to the effect on many zebrafish embryo effects have already been widely analyzed for other compounds, but little studied on GA, PH, LA and there is no work in the literature on lethal, sublethal and teratogenic changes caused by the use of OX. LA showed little or no alteration in the animals in all analyzes: lethal, sublethal and teratogenic. Besides, little attention is given to morphometric alterations on embryos and larvae of zebrafish caused by antiepileptics.

Conflict of interest

All authors declare that they have no conflict of interest.

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