SHORT COMMUNICATION

Anxiolytic-like effect of chrysophanol from *Senna cana* stem in adult zebrafish (*Danio rerio*)

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ABSTRACT

The aim of this study was to evaluate the anxiolytic-like effect of chrysophanol (CHRY), isolated from hexane extract of *Senna cana* stem and its possible mechanism of action. CHRY was obtained through chromatographic treatments and its identity was confirmed by uni and bidimensional RMN\(^1\)H and RMN\(^{13}\)C. Adult zebrafish (n = 6/group) were treated (with CHRY (4.0 or 12.0 or 40.0 mg/Kg; 20 μL; intraperitoneally) and submitted to acute toxicity and open field tests. Subsequently, other groups (n = 6 each) received CHRY for the analysis of its effect on the Light & Dark Test. The participation of the GABAergic system was also assessed using the diazepam (GABA\(_A\) receptor agonist) and flumazenil (GABA\(_A\) receptor antagonist). CHRY was considered non-toxic, it did not reduce the locomotor activity, and showed an anxiolytic-like effect. This effect was reduced by pre-treatment with flumazenil. The results suggest that CHRY is an anxiolytic-like agent mediated via the GABAergic system.

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1. Introduction

The genus Senna (Fabaceae) are found in the Brazilian northeastern semiarid region and are traditionally used in folk medicine to treat colds, with laxative, cytotoxic, leishmanicidal and anti-inflammatory activities having been described, among others. Senna cana (Nees & Mart.) H.S. Irwin & Barneby is a species that belongs to the Leguminosae family, popularly known as ‘São João’ and occurs mainly in the Brazilian Savannah (Cerrado) and rupestrian fields, showing antioxidant and anti-acetylcholinesterase activities for hexane leaf extract (Monteiro et al. 2018).

Currently, treatment protocols to control anxiety and reduce stress are focussed on attenuating the effects of these disorders. Therefore, the search for anxiolytic drugs has become an important area of research. Benzodiazepines, barbiturates and tricyclic antidepressants have been used for a long time to treat anxiety disorders. Benzodiazepines act through the benzodiazepine receptors present in the GABAA pentameric complex. The most commonly used drug is diazepam. However, the clinical uses of benzodiazepines are limited by their side effects, such as psychomotor impairment, sedation, myorelaxation, ataxia, amnesia, and physical and psychological dependence (Kaur et al. 2016; Vincenzi et al. 2017).

In the last decade, the use of zebrafish (Danio rerio) has emerged as a promising predictive model of drugs with anxiolytic potential (Hamilton et al. 2017; Moradi-Afrapoli et al. 2017; Ferreira et al. 2019). This model is considered a low-cost alternative, of easy handling and maintenance when compared to other animal models, such as rodents (Magalhães et al. 2017). This study aimed to evaluate the anxiolytic-like effect of chrysophanol and its possible mechanism of action through the adult zebrafish (Danio rerio) model. This compound provides protection against neurotoxicity and has antiepileptic action (Santos et al. 2011), indicating that it may be important in the advancement of selective and targeted natural anxiolytic agents development.

2. Results and discussion

Chrysophanol (CHRY) was obtained like yellow crystals, from the hexane extract of Senna cana in significant amount, in addition to another anthraquinone phycion, triaccontanoic acid, and two triterpenes, friedelin and lupeol. The analysis of the MS and RMN spectra of $^1$H and $^{13}$C of the yellow compound isolated from the hexane extract of Senna cana stem allowed us to identify it as 1,8-dihydroxy-3-methyl anthraquinone, widely known as chrysophanol (Figure S1), reported in this plant for the first time. Thus, because of the several properties demonstrated by this compound, such as neuroprotection, anticancer, antibacterial, antiviral, antioxidation, antipsoriatic and blood lipid regulation, (Kuo et al. 2020), it was evaluated its anxiolytic effect to help broaden the biological action spectrum of this molecule. Therefore, establishing S. cana as a new source of this compound, and as a natural agent with anxiolytic properties.

The behavioural study of zebrafish may be useful for the screening of pharmaceutical effects, and it is considered a potential model for pharmacology, genetics and neuroscience studies (Hong and Zha 2019). The scototaxis protocol (preferably light/dark) is used to assess the anxiolytic-like effects of pharmacological agents in adult zebrafish (Danio rerio), as well as performed by Maximino et al. (2010), da Silva et al.
(2020), Gonçalves et al. (2020), Ferreira et al. (2021) and Alexandre et al. (2021). For the pharmacological tests used, three small doses have been used, based on other tests previously reported (Soares et al. 2019). CHRY (4.0 or 12.0 or 40.0 mg/Kg; i.p.) and diazepam (40.0 mg/Kg; 20 μL; i.p.) increased (**p < 0.001 vs. naive or vehicle) the permanence in the light zone in the Light & Dark Test (Figure S2) (Arellano-Aguilar et al. 2015).

The flumazenil is a specific antagonist of the GABA<sub>A</sub> channel, considered an excellent tool for GABA<sub>A</sub> receptor studies, as it antagonises the effects of benzodiazepine drugs, including anxiolytic, sedative and hypnotic effects, as well as performed to as previously shown by Ferreira et al. (2019), Lira et al. (2020), da Silva et al. (2020) and Ferreira et al. (2021). In this work, flumazenil reduced (p < 0.001) the anxiolytic-like effect of CHRY (4.0 mg/Kg; 20 μL; i.p.) and diazepam (4.0 mg/Kg; 20 μL; i.p.) (Figure S4) CHRY (4.0 or 12.0 or 40.0 mg/Kg; 20 μL; i.p.), and diazepam (40.0 mg/Kg; 20 μL; i.p.) increased the time span of the fish in the light zone, suggesting that the anxiolytic effect can be attributed to this biocompound (Figure S3). The reversal of anxiolysis (Figure S4) through the pre-treatment with flumazenil suggests a possible involvement of the GABA<sub>A</sub> receptor in the anxiolytic effects of Chrysophanol.

The zebrafish have been used in several studies as an alternative method to the use of rodents in toxicity testing of natural products (do Nascimento et al. 2018; Bezerra et al. 2021; Widelski et al. 2021) and extracts (Batista et al. 2018; Ferreira et al. 2019; Hacke et al. 2020; Lira et al. 2020). There was no mortality of the animal treated with CHRY (4.0 or 12.0 or 40.0 mg/Kg; 20 μL; i.p.), and, thus, we can suggest that this compound does not exhibit toxicity as pointed out by Ferreira et al. (2021) testing other compounds. CHRY in these doses used did not decrease the locomotor activity of adult zebrafish, significantly different (p < 0.01; p < 0.01; p < 0.05, respectively) from the group treated with diazepam (40.0 mg/Kg DZP; 20 μL i.p.), (Figure S2). The fact that CHRY did not interfere with the animals locomotor activities in the open field test precludes possible non-specific muscle relaxation effects induced by the substance (Batista et al. 2018).

3. Conclusion

This is the first study to evaluate the anthraquinone chrysophanol action in the treatment of anxiety using the adult zebrafish animal model. The data obtained in the present study showed that chrysophanol from *Senna cana* stem (CHRY) had an anxiolytic-like effect via the GABAergic system, and can be a safe and non-toxic natural anxiolytic agent source.

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Disclosure statement

No potential conflict of interest was reported by the authors.
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