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Safety Assessment of Leaves Extracts of *Vitis Labrusca* and *Vitis Vinifera L.* using Zebrafish Embryo Toxicity Test

<u>Biegelmeyer R.</u>^{a,b}, Rico E.P.^c, Dresch R.R.^a, Rambo D.F.^a, Bertelli, P.R.^a, de Oliveira D.L.^c, Henriques A.T.^a, Bassani V.L.^b

^aLaboratório de Farmacognosia and ^bLaboratório de Desenvolvimento Galênico, UFRGS, Porto Alegre, Brazil; ^cLaboratório de Sinalização Neural e Psicofarmacologia, UNESC, Criciúma, Brazil; ^dDepartamento de Bioquímica, UFRGS, Porto Alegre, Brazil; <u>renata.biegel@gmail.com</u>

Introduction: The cultivation of *Vitis* (Vitaceae) grape varieties is one of the most important economic activities in agribusiness in Brazil; especially in Rio Grande do Sul. Waste byproducts generated by the grape industry such as leaves have no destination [1]. In this sense, our research group has been working with leaves extracts of Vitis Labrusca and Vitis Vinifera L. varieties [2]. Considering that this extracts demonstrated potential pharmacological results, is important to investigate its toxicity. For toxicological screenings, zebrafish has been emerged as a powerful tool. The main advantage of using this alternative model in toxicity studies is the replacement and reduction of more laborious and expensive mammals [3]. In this context, the objective of this work was the investigation of toxicity parameters of aqueous extracts of Vitis Labrusca (Niagara rosada) and Vitis vinifera (Cabernet sauvignon) leaves using zebrafish as in vivo model. Methods: The Zebrafish Embryo Toxicity Test was conducted as established by OECD (Organisation for Economic Co-operation and Development) [4] using zebrafish embryo (Danio rerio) of heterogeneous "wild type" (short-fin phenotype). The parameters observed were: (i) coagulation of fertilized eggs, (ii) somite formation, (iii) tail development, and (iv) number of heartbeat per minute. Aqueous extracts of Niagara rosada and Cabernet sauvignon leaves were analyzed in a wide range of concentration (50-2000 µg/ml) until 96 hours pos-fertilization (hpf). Results and Conclusions: For aqueous extract of Niagara rosada leaves, in 24hpf we observed a significant reduction in heartbeat/min and lack of somite formation using concentration of 2000 µg/ml. After 48hpf, eggs were coagulated from concentration of 1000 μ g/ml, being the letal dose of 50% (LD₅₀)_{96hpf} = 466.17 ± 5.02. On the other hand, aqueous extract of Cabernet sauvignon leaves showed higher toxicity, because in 24hpf was observed coagulation of embryos treated with 2000 μ g/ml of the extract. In 48hpf, we verify mortality of embryos from concentration of 1000 µg/ml and for 500 µg/ml, a reduction in heartbeat/min and an abnormal development of tail. The LD_{50} in 96hpf obtained for aqueous extract of Cabernet souvignon leaves was $325.81 \pm 4.01 \,\mu$ g/ml. Both varieties present as major compound quercetin-3-O-glucuronide and also; rutin, quercetin-3-O-galactoside, quercetin-3-O-glucosid and trans-caftaric acid. However, anthocyanins are present only for Cabernet sauvignon. In summary, it was possible to conclude that anthocyanins can contribute significantly for toxicity of aqueous extracts of Vitis leaves.

References:

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