STUDY OF THE EDIBLE RUMEX INDURATUS FROM CÔA VALLEY IN HEPATOCARCINOMA CELL LINES

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Hepatocellular carcinoma (HCC) is the most common form of liver cancer (about 90%) which remains a worldwide health challenge due to its incidence growth and yet scarce and not specific treatments.HCC is characterized by a metabolic and oxidative stress which induces a prolonged pathological inflammation and cell damage, and also an evident increase in the production of reactive oxygen species (ROS) which will lead to an increase of cellular lipid peroxidation and of hepatic enzymes. Available treatments are not very effective being usually adapted from other illnesses. Plants are considered very important sources for the discovery of new compounds to prevent and treat diseases (1). There are several natural drug leads presently used in chemotherapy including in HCC. *Rumex induratus* is native in Iberian Peninsula and spontaneous in northwest Portugal where it is used in local cuisine. Its extracts have significative antioxidant activity (2). This work aimed to add value to this species focused on the evaluation of the antitumoral activity of different extracts.

Extractions of *Rumex induratus* (collected in Vale do Côa, Portugal, between March and May 2021) were made in water, ethanol 80% and ethanol 100% and tested in HCC *in vitro* models using HepG2, HuH7 and Hep3B cells lines. Metabolic activity, cellular morphology, cellular death and the quantification of reactive oxygen species (ROS) (superoxide anion and hydrogen peroxide) were assayed. A reduction in the metabolic activity was dependent on the extract and its concentration: it was observed that the infusion was more cytotoxic and Hep3B the most sensitive cell line. As for cellular morphology, in HuH7 was observed the condensation of the nucleus after treatment with infusion, and in Hep3B the hydroethanolic extract led to cellular contraction. Cellular death evaluated by the membrane potential revealed that HepG2 and HuH7 suffered from exposure to infusion and hydroethanolic extracts, while Hep3B was not so sensitive. As for ROS quantification, a growth pattern in the concentration of the superoxide anion and a decrease in peroxides were detected with an increase in the concentration of *R. induratus* extracts.

Although not so robust, results of this study still revealed an evident influence of *R. induratus* extracts in the cellular activity and intracellular ROS production in the studied hepatocellular carcinoma cell lines. It is understandable that these biologic activities are directly associated to the existent phenolic compounds which antioxidant potential allows the understanding that the extracts led to an increase in membrane potential in all cell lines, with HepG2 presenting the highest values. In conclusion, the plant extract provides intracellular differences that can lead to the triggering of apoptosis in tumor cells. Nevertheless, future assays will allow to understand the mechanism of action of *R. induratus* extracts in HCC and to potentiate its use.

References

1) Llovet, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Pikarsky, E., Roayaie, S., Lencioni, R., Koike, K., Zucman-Rossi, J. & Finn, R. S. (2021) Hepatocellular carcinoma. Nature Reviews, 7 (6), 1-28. https://doi.org/10.1038/s41572-020-00240-3
2) Guerra, L., Pereira, C., Andrade, P. B., Rodrigues, M. Â., Ferreres, F., De Pinho, P. G., Seabra, R. M., & Valentão, P. (2008). Targeted metabolite analysis and antioxidant potential of Rumex induratus. Journal of Agricultural and Food Chemistry, 56(17), 8184-8194.