HELLEBORUS PURPURASCENS ALCOHOLIC EXTRACTS AFFECT CELL PROLIFERATION, AND ELICIT CHANGES AT THE EPIGENETIC AND PROTEOMIC LEVEL

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SUMMARY

Helleborus purpurascens phytopreparations are currently in use, but little information exists regarding their effects at the cellular and molecular level. This study reports the effects of H. purpurascens alcoholic extracts on cultured HeLa cell, in terms of proliferation and changes at the epigenetic and proteomic level.

H. purpurascens roots were extracted in absolute ethanol, by refluxing for 8 hrs. The obtained extracts were analyzed for composition, by thin layer chromatography (TLC), for total polyphenol content, by Folin-Ciocalteu colorimetric test, and for antioxidant capacity, by DPPH assay, determining radical scavenging activity. Extract effects on cell proliferation was assessed by MTS assay, while the effects at the molecular level were investigated by DNA sensitivity to endonucleases depending on cytosine nucleotide methylation, and by western blot for expression level of AP-1 transcription factor subunits.

The extracts contain at least eight major components as revealed under TLC. The polyphenol content is of ~15mg/100ml, while the antioxidant capacity is low (~2% of inactivation). H. purpurascens extracts reduced HeLa cell proliferation, in a concentration dependent manner, by about 25% maximum in our experimental conditions. Treatment of HeLa cells with H. purpurascens extract, for 4 hrs resulted in a concentration dependent reduction of DNA digestion by endonucleases Cse I, or Sma I. The same treatment of HeLa cells induced, as revealed by western blot, a decrease in the expression level of c-Jun, suggesting changes in the dimerization balance of AP-1 subunits. This effect is also dependent on extract concentration.

In conclusion, our results showed an inhibition of cell proliferation by H. purpurascens extract in ethanol. That effect is accompanied by DNA methylation meaning epigenetic effects, and changes in dimerization balance of AP-1 transcription factor by decreasing c-Jun expression level, suggesting the expression of a different set of genes elicited by HeLa cells treatment with the investigated extracts.

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