Abstract

Coreopsis tinctoria Nutt. is a small aromatic annual and widely distributed plant that belongs to the Asteraceae family. Its use in traditional medicine has been described as early as the XVII century in China, where its decoction has been used for diarrhoea. Other medicinal properties have been added by North American Indians and include treatment of internal pains, bleeding, to strengthen blood and as an emetic. In Portugal, an infusion of the flowering tops has been described to have antidiabetic proprieties, applied to diabetes type 2 treatment.

There are some published phytochemical studies on the genus Coreopsis but the information on this species chemical composition, until recently, was scarce. Flavonoids of chalcone, aurone and flavanone structure have been described in C. tinctoria flowering tops although there was no available information on the chemical constitution of bioactive extracts.

Flavonoids are polyphenols well known for their antioxidant and cytoprotective activity that have also been shown to act at several levels in the glucose metabolism, which may be of relevance in the prevention and treatment of diabetes and its complications.

Thus the present study aimed to: i) search for new antidiabetic lead molecules able to reduce both glycemia and tissue damage; ii) chemically characterize the bioactive extracts, and; iii) study the antidiabetic profile of extracts and metabolites of C. tinctoria as this species lacked any kind of pharmacological and toxicological data.

The aqueous extract of C. tinctoria flowering tops was prepared following the traditional recipe. Fractionation by chromatography followed, and three major compounds were isolated and identified by spectroscopic methodologies. Other thirteen phenolic compounds were identified by HPLC-DAD-MS/MS. Through this technique, reproducible methods for quantitative analysis of the main compounds in bioactive extracts were also developed.

Further on, to evaluate whether C. tinctoria had a beneficial effect on diabetes some in vivo assays were performed. In order to test for acute antihyperglycemic activity, the aqueous extract was administered to normal (euglycemic) rats at different concentrations and blood glucose levels were monitored by an oral glucose tolerance
None of concentrations tested have shown significant lowering blood glucose effect compared to control group.

In order to understand other possible glucose lowering effects, a streptozotocin-induced glucose-intolerant rat model was developed. *C. tinctoria* aqueous extract (500 mg/Kg) was then daily administrated during three weeks which resulted in significant recovery of glucose tolerance. The active extract was quantified in terms of its main compound, the chalcone marein.

As means to determine whether flavonoids were the bioactive compounds, a flavonoid-rich AcOEt fraction (125 mg/Kg), with the same marein content as the bioactive aqueous extract, was prepared and administered to the glucose-intolerant model. Results indicated glucose tolerance regain after only two weeks of treatment, an effect that was maintained over the remaining experimental period. Moreover, lipase values normalized indicating re-establishment of pancreatic function.

In addition, the oral treatment with either *C. tinctoria* aqueous extract or AcOEt fraction caused no hepatotoxicity. Regarding cytotoxicity, *C. tinctoria* extracts were tested *in vitro*, using a pancreatic mouse insulinoma cell line (MIN6). Cell viability tests were performed and no cytotoxic effects were observed at the concentrations tested (up to 3 mg/mL).

Possible insulin-secretagogue action of the bioactive *C. tinctoria* extracts was evaluated in two ways: i) using MIN6 cells disposed as monolayers through static incubation method or; ii) in a more sensitive model, using MIN6 cells disposed in three dimensional structures (pseudoislets) using a perifusion technique. Both yielded data that seems to indicate that *C. tinctoria* extracts do not act increasing insulin secretion.

*C. tinctoria* extracts and pure compounds (marein and flavanomarein), known to be good antioxidants, were then tested for their potential protective effects in MIN6 cells challenged with pro-oxidant tert-Butyl-Hydroperoxide (tBHP) or cytokines. Results indicate that *C. tinctoria* extracts and pure compounds are effective cytoprotectors, which seems to be due to inhibition of the apoptotic pathway, and not through a decrease on superoxide radical production.

Overall, these results support the conclusion that phenolic compounds present in *C. tinctoria* flowering tops extracts promote recovery of pancreatic function and glucose intolerance possibly by an anti-apoptotic mechanism of injured pancreas, suggesting *C. tinctoria* traditional use in diabetes therapy.
Keywords: Asteraceae; *Coreopsis tinctoria*; flavonoids; marein; flavanomarein; glucose tolerance; antihyperglycemia; beta-cell; cell viability; apoptosis; diabetes.