

SUMMARY

Title: Antioxidant principles from Sri Lankan medicinal plants reputed to possess cardioprotective activity.

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Scientific background and Scope/ Objectives of the Project

Reactive oxygen species (ROS) such as superoxides, peroxides and hydroxyl free radicals are high energy molecules that cause damage derived from normal as well as pathologic conditions. Enzymatic and non-enzymatic mechanisms to detoxify them. There is a growing body of evidence suggesting the involvement of these reactive oxygen species in heart diseases including ischemia / reperfusion injury, diabetes mellitus associated cardiomyopathy, and adriamycin induced cardiomyopathy. In Sri Lanka number of plants have been used by Ayurvedic and other traditional medical practitioners to treat heart disease. In view of the beneficial effects of AO have on the myocardium, the question is whether the therapeutic action of these plant extracts on the heart is due their AO activity? If so what is the chemical nature of these AO principles with cardioprotective activity ?

Experimental methods and Results obtained: Crude aqueous extracts (CAE) of nine plant extracts were assayed for *in vitro* free radical scavenging activity using DPPH where scavenging of DPPH by plant extracts were monitored by measuring absorbance at 517 nm. *T. arjuna* and *C. fistula* showed such activity with $B_{50} = 8.3 \pm 0.3$ and 59.0 ± 2.7 respectively. None of the other extracts showed such activity. On hydroxyl mediated deoxyribose damage protection assays, CAE of *T. arjuna* and *V. negundo* demonstrated no activity from 0-20 ug/ ml but at higher concentrations, a pro-oxidant activity was observed. *C. fistula* showed a 30% protection against deoxyribose damage at 125ug/ml . Rest of the plant extracts investigated did not demonstrate this activity. *In vivo* lipid peroxidation in the rat heart was measured as thiobarbituric acid reactive substances (TBARS); *T. arjuna* and *C. fistula* reduced lipid peroxidation by $37.7 \pm 3.5\%$ and $11.6 \pm 3.5\%$ respectively compared to the control. Remaining 7 extracts did not show anti-lipoperoxidative activity. Total polyphenolic content of aqueous extracts measured using Folin-Dennis reagent showed a good correlation between the phenolic content and the *in vitro* free radical scavenging activity and *in vivo* anti-lipoperoxidative activities. Fractionation of *T. arjuna* to polysaccharide and non-polysaccharide fractions did not result in the enrichment of its *in-vitro* free radical scavenging activity. Ethylacetate fractions (EF) of *C. fistula* showed a 4-fold increase in *in vitro* free radical scavenging activity compared to crude aqueous extracts but no such enhancement was observed with *T. arjuna*. *T. arjuna* ethylacetate fractions showed 40% protection against deoxyribose damage compared to 156% increase in pro-oxidant activity observe for CAE at 125 ug/ml. Ethyl acetate fractionation of *C. fistula* also increased the protection against deoxyribose damage from 30 to 75% at 125 ug/ml. Further analysis of *C. fistula* ethyl acetate fraction (CasEF) using silica thin layer chromatography

(TLC) and spray reagents suggested the presence of flavanoids. Fractionation with LH-20 column chromatography and analysis with silica TLC gave four fractions: A, B, D and E. TLC analysis A ran as a single spot; on preparatory TLC, B separated to 3 bands, B1 to B3; D and E gave a complex patterns on TLC with spray reagents.

¹H NMR was attempted with A and B 1-3 fractions using DMSO-D₆ as the solvent which resulted in the water peak of the solvent obliterating the signal of the compounds.

Conclusions : Cardioprotective activities of *T. arjuna* and *C. fistula* are at least in part due to the antioxidant activity. These AO principles are probably polyphenolic in nature. In *C. fistula*, flavanoids may contribute to the antioxidant activity as well. Further, *C. fistula*, contains a complex array of compounds with antioxidant activity. Also, antioxidative action may not be the only mechanism of cardioprotection.

Publications

Papers

T C J Munasinghe, C K Seneviratne, M I Thabrew and A M Abeysekera. Antiradical, anti-lipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection. *Phytother. Res.* 15, 519-523, 2001.

Abstracts

1) T C J Munasinghe, C K Seneviratne, M I Thabrew and A M Abeysekera. Antiradical, anti-lipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection. *Proc. SLAAS*, E2-236, 2000. *Vol. 56*
p. 257

2) C K Seneviratne, T C J Munasinghe, M I Thabrew and A M Abeysekera. Anti-oxidant activity in plant extracts and cardiovascular adaptations. *Proc. VI of ISAM (International Society for Adaptive Medicine)*, Lyon, France, August 2000.

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