CHEMISTRY AND IMMUNOHODULATORY PROPERTIES OF VERNONIA CINEREA (L.) LESS., LINNAEA (ASTERACEAE) (An Immunological Basis for the use of Vernonia cinerea in Ayurveda)

ΒY

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CHEMISTRY AND IMMUNOMODULATORY PROPERTIES OF VERNONIA CINEREA (L.) LESS., LINNAEA(ASTERACEAE)

SALPAHANDI RAMYA PUSHPARANI DE SILVA

ABSTRACT

Vernonia cinerea is a small herb which is extensively used in oriental traditional medicine. It belongs to the family Asteraceae (Compositae).

The results of a field survey carried out by us indicates that through out Sri-Lanka, *Vernonia cinerea* is used mainly in inflammation related diseases such as hepatic, respiratory and rheumatic diseases.

The interference of different extracts of the Vernonia cinerea with different functions of the human immune system was investigated. All extracts expressed dose dependent inhibitory effects. A soxhlet methanol extract showed the strongest inhibition of chemiluminescence (CL) production by polymorpho nuclear leucocytes. A water extract of the marc of methanol extract showed the most potent action on classical pathway(CP) and alternative pathway(AP) mediated haemolysis by complement.

Those extracts were therefore considered to be the most rational choice for activity guided purification of active constituents.

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An activity guided fraction of methanol extract of Vernonia cinerea based on the inhibition of respiratory burst activity of activated PHNL yielded highly active phenolic compounds. Thev were identified 8.5 luteolin. luteolin-7-0-BDglucoside, $luteolin-4'-0-\beta Dglucoside$, chlorogenic acid, [3,5], [3,4], [4,5] dicaffeoyl quinic acid and methyl caffeate by HPLC, UV, 1H NMR, 2D NMR, 13C NMR and mass spectroscopy.

Control experiments have shown that the inhibition of respiratory burst is not due to cytotoxicity except in the case of luteolin-7-0-glucoside. Scavenging of oxygen radicals by the caffeoyl quinic acids was negligible compared with luteolin, and luteolin-4'-O-glucoside. Therefore our results are in favour of a specific interference with the functioning of activated PMNL by caffeov, quinic acids and to a lesser extent by luteolin and luteolin-4'-Oglucoside.

Scavenging of O₂- may also play a fundamental role in the <u>in</u> <u>vivo</u> anti-inflammatory activity of luteolin and luteolin-4'-O-glucoside. However the luteolin-7-O-glucoside showednon specific cytotoxicity towards PMNL in this assay.

New TLC systems were developed for the rapid identification of these compounds. Their quantitative analysis by HPLC was studied with view to developing standards for the quality of Ayurvedic preparations containing Vernonia cineres.

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The modulation of CL production by stimulated PMNL by the isolated and some structurally related compounds was analysed. It was shown that activity of these compounds is due to the presence of caffeoyl moiety.

An aqueous extract of the marc from a methanol extract of Vernonia cinerea was fractionated, guided by anti complementary activity. Strong classical pathway(CP) anti complementary activity was exhibited by a >300kD fraction. Its action on the alternative pathway(AP) was less marked. Immunomechanistic studies revealed that the inhibition was not caused by complement consumption, chelation of Ca^{2+} or by direct action on target erythrocytes. Studies with C_x depleted sera suggest probable sites of action are C2 and C3. C, and C4 appear to be not affected.

The protein dye binding¹ method indicates a high protein content and Dubois method² indicates the presence of saccharides in the >300kD fration. It was acid hydrolysed and analysed for monosaccharides and amino acids by TLC and HPLC. Further fractionation of this >300kD fraction by gel filtration yieled mainly four bands, suggesting that it is a mixture of glycoproteins with some impurities.

Our results suggest that the use of Vernonia cinerea in Ayurveda in the treatment of inflammation related diseases may have an immunological basis.

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