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Letter

Immunostimulatory Activity of Mangiferin, A Naturally Occurring Xanthone-C-Glucoside

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In connection with our work on immunomodulants from higher plants (1,2), we have examined the effect of mangiferin on the activation of thymocytes and splenic lymphocytes of mice. Mangiferin occurs widely in angiosperms and its concentration was found to increase considerably during the incidence of stress in the producer plants, e.g., when they suffer an injury or are invaded by pathogenic microorganisms (3–5). Mangiferin was also found to stabilize lysosomal membranes by inhibiting the release of acid phosphatases (6), to produce antiinflammatory activity in albino rats, and to provide marginal antitumor immunity to hosts against P-388 and L-1210 tumors (S. Ghosal, unpublished). These observations suggest immunostimulatory activity of mangiferin.

Mangiferin (I) was isolated from Mangifera *indica Linn.* as described before (4). It was dissolved in phosphate-buffered saline (PBS; pH 7.2, containing 0.15 *M* NaCl). The freshly prepared, membrane (0.45 μm)-filtered mangiferin solution was used for assay of lymphoblast transformation as before (2). Phytohemagglutinin (PHA) and concanavalin A (Con A), obtained from Sigma Chemical Co., USA, were used as the standard mitogens for the lymphoid cells. The lectins were dissolved in PBS, at the desired concentrations, membrane filtered, and stored at 0°C before use. The splenic lymphocytes and thymocytes were prepared (2) from the spleen and thymus of 12- to 16-week-old Swiss mice. Multiple assays were carried out for each dose of the test compound and of the mitogens.

Standard mitogens, PHA (2.5 μg/ml) and Con A (2.5 μg/ml), were used to assess the stimulatory activity of mangiferin on thymocytes and splenic cells. After 48 hr of exposure to mangiferin, significant proliferation of thymocytes and splenic lymphocytes was observed (Fig. 1). Furthermore, a dose-dependent relationship, at dose levels of 5–40 μg/ml, was observed in each case. In the case of the thymocytes, the peak response was observed at 5 μg/ml, while the peak response in the splenic lymphocytes was observed at 20 μg/ml. The differential specificity of mangiferin for the subpopulations of lymphocytes might be responsible for such a difference in sensitivity to thymocytes and splenic cells at low dose levels. Activation of thymocytes by mangiferin further suggested that the compound was capable of stimulating T cells independently of B cells. At higher doses (50–100 μg/ml), the incorporation of [^{3}H]thymidine into the cells was still higher than the base level and there was no toxicity as was suggested by the absence of any cell death. These observations were similar to the dose–response of Con A (7).

The mechanism of activation of lymphocytes by low doses of mangiferin vis-à-vis the decrease in blastogenesis at high doses could not be entirely elucidated. However, the fact that the structural requirements for such stimulations are not at all rigid, since a wide variety of chemical agents ranging from metal ions to macromolecules (lectins, antibodies), with varied specificity for the cell surface receptors, can stimulate lymphocytes (7), suggests a common final pathway for triggering blastogenesis. PHA and Con A induce blastogenesis via changes in the level of intracellular nucleotide (increase in cGMP) and Ca^{2+} ion. Conversely, cytokinin-like activity of mangiferin was observed (3) in higher plants. Zn^{2+} ion has also been implicated in lymphoblast transformation and is associated with the activities of thymidine kinase and polymerase involved in DNA synthesis. In lymphocytes, specific receptors for Zn-transferin were recognized and enhanced uptake of transferin-bound Zn ion by PHA-stimulated lymphocytes was reported (8). In view of the pronounced metal ion (Cu^{2+}, Zn^{2+}, Fe^{2+}) chelating property of mangiferin (9), it would seem likely that this compound activates lymphocytes by transferring one or more of these/similar metal ions, from the *in situ* mangiferin–metal ion complex to the cell, and thereby promotes the DNA-synthesizing enzymes. These possibilities are currently being investigated to elucidate the mechanism of the immunostimulatory activity of mangiferin.

The LD_{50} of mangiferin in albino mice, determined as before (10), was 402 mg/kg (344–453 mg/kg at 95% fiducial limits).

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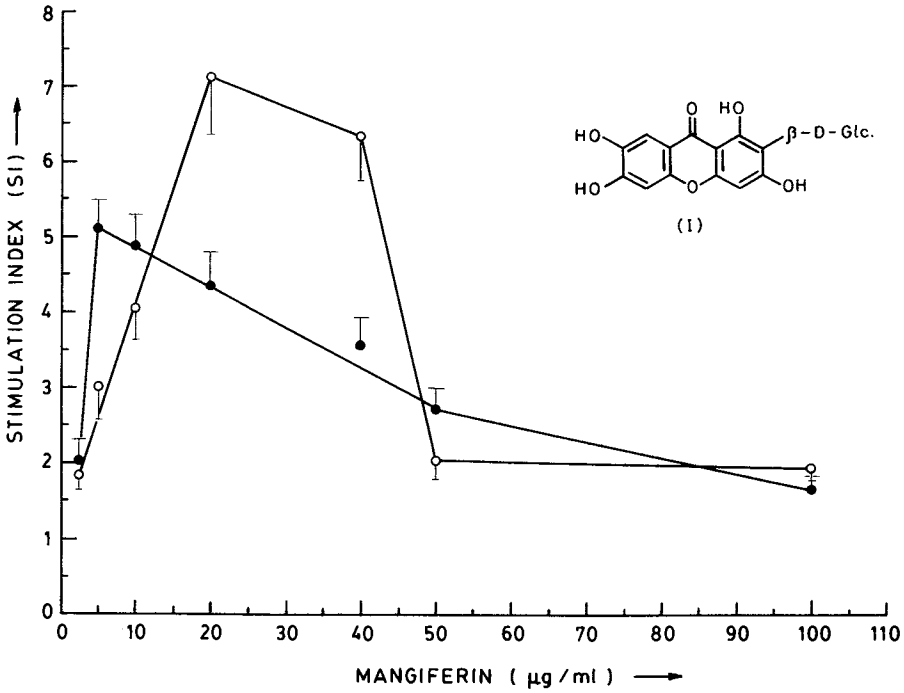

Fig. 1. Dose–response [SI (mean ± SD); mean of five to nine replicates was taken] of mangiferin (I) on activation of thymocytes (●) and splenic lymphocytes (○) of mice. SI of PHA (thymocytes, 3.70 ± 0.30; lymphocytes, 3.81 ± 0.41); SI of Con A (thymocytes, 4.05 ± 0.23; lymphocytes, 6.08 ± 0.61).

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