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Haematological, immunomodulatory and cancer chemopreventive activities of the mature leaf concentrate of *Carica papaya* L Sri Lankan wild type cultivar

A thesis submitted for the Degree of Doctor of Philosophy

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Abstract

Herbal medicines have been invaluable as therapeutic agents since history of mankind, with current wide public acceptance of these as a safe mode of therapy. Thus prudent use of traditional medicinal knowledge may support the search for effective plant based drug leads. The leaf concentrate of *Carica papaya* is a traditionally acclaimed phytotherapy against haematological and immunological disorders, and cancers. Nonetheless, comprehensive scientific validation of these claims remains obscure. A comprehensive study was thus carried out to corroborate the haematological, immunomodulatory and cancer chemopreventive activities of the mature leaf concentrate of *C. papaya* Sri Lankan wild type cultivar (MLCC) using *in vitro, ex vivo* and *in vivo* murine models, accompanied by toxicological evaluation and chemical characterization of the MLCC.

Oral gavage of the MLCC to thrombocytopenicWistar rats at three doses ([low dose (LD)-0.18, human equivalent dose (HED)-0.36 and high dose (HD)-0.72 ml/100g BW] manifested significant platelet increasing activity ($p<0.05$). *In vitro* up regulated synthesis of interlukin-6 (IL-6) in bone marrow cultures and *in vivo* regulation of serum thrombopoetin (TPO) and platelet activiating factor (PAF) levels may have revealed the potential mechanism of platelet increasing activity. Moreover, pre incubation of platelets with the MLCC mounted significant protection against platelet cytotoxicity ($p<0.05$). In parallel, the MLCC exhibited haemostatic activity through modulating both coagulation and fibronolytic pathways of rat blood, *in vitro*. Counts of rat platelet, total and differential leukocytes, bone marrow cells (BMCs) and of phagocytic activity of rat peritoneal macrophages were significantly augmented ($p<0.05$) with significant decrease in plasma concentrations of IL-6 and TNFα following oral gavage of the MLCC (HD) ($p<0.05$). At low concentrations the MLCC elicited *ex vivo* proliferation of rat BMC and phagocytic activity of peritoneal macrophages while high concentrations manifested cytotoxicity of both these cell types. Oral gavage of the MLCC, markedly inhibited leukocyte migration, prostaglandin E2 (PGE2) and TNFα levels ($p<0.05$) of the carrageenan induced rat peritonitis model. Membrane stabilization potential of the MLCC reiterated antiinflammatory activity of the MLCC.

The MLCC explicated strong anti-proliferative activity against HEp-2 cells (human laryngeal carcinoma cells; IC_{50}:56 $\mu g m L^{-1}$). Though, the anti-proliferative activity of the MLCC was lower than of the reference drug cyclophosphamide, it imparted cytoprotection on normal rat BMCs. The MLCC enhanced antitumor immunity by up-regulation of IFNγ of both cancer and normal cell types. Quenching of ABTS, DPPH, NO, SO and H_2O_2 and enhancement of endogenous antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GR) in oxidative stressed rats reiterated the antioxidant activity of the MLCC ($p<0.05$). *In vivo* sub-acute toxicological evaluation of the MLCC demonstrated that it was devoid of general toxicity, plus hepato, nephro and endocrine toxicities. The GC-MS/LC-MS analyses of the MLCC explicated the presence of several immunomodulatory and cytotoxic compounds. When the MLCC was fractionated using the Kupchan method, the high and mid polar fractions showed higher cytotoxicity while *in vitro* phagocytic activity was lower than of the crude MLCC. This may reflect the synergistic activity of the bioactive compounds of the MLCC that may well contribute to the immunomodulatory activity of the MLCC.

In the light of the above findings, the present study provided ample affirmation of the traditional claims of the MLCC as a hematological, an immunomodulatory and a cancer chemopreventive remedy; Extensive examination of the MLCC is therefore strongly warranted both as a safe, inexpensive and readily available multi-component formulation, and as starting material to explore for novel drug lead(s).