614/E2

Optimization of alkaline comet assay (*in vitro*) and preliminary studies to evaluate genotoxicity of *Flueggea leucopyrus* Willd (Katupila)

H N Thirimanne, C D Wijayarathna*, R C Deshani, N M D T M Jayathilaka and N V Chandrasekharan

Department of Chemistry, Faculty of Science, University of Colombo, Colombo 03

The comet assay is a simple, rapid, sensitive and cost effective technique used to detect DNA damage in single cells. Briefly, in this technique cells are exposed to the suspected genotoxic agent, suspended in Low Melting Agarose (LMA) and placed on a microscopic slide precoated with normal melting point agarose. Cells are then lysed and DNA allowed to unwind and electrophoresed. Damaged DNA moves towards the anode forming a comet like structure. The length of the tail of the comet is proportional to the extent of the damage. In this study, assay conditions were optimized and used to evaluate the genotoxic potential of *Flueggea leucopyrus* Willd. It is a plant commonly used in traditional medicine to treat many diseases including cancer.

Several assay parameters were optimized including duration of lysis (90 min), unwinding (30 min) and electrophoresis (30 min). Electrophoresis was performed at ~1.3 V/cm and 300 mA. The slides were stained with ethidium bromide and observed under a fluorescence microscope. The parameter used for scoring comets was the ratio of the length of the comet tail to the diameter of the head. The score is directly proportional to the extent of DNA damage.

Aqueous extracts of leaves (from two different regions of the country) were prepared by boiling in water and then freeze-dried. Three concentrations of the plant extract (0.005 mg/ml, 0.01 mg/ml and 0.02 mg/ml) were tested using 20 µl of whole blood. Included in each assay was a negative control using Hank's Balanced Salt Solution and a positive control using a H_2O_2 (200 µM) instead of the extract.

The mean score for the first test sample (0.005 mg/ml, 0.01 mg/ml, and 0.02 mg/ml) and negative and positive controls were 1.48 $\pm$ 0.31, 1.54 $\pm$ 0.50, 1.72 $\pm$ 0.43, 1.28 $\pm$ 0.25 and 3.87 $\pm$ 1.43 respectively. Similar results were obtained with the duplicated trial. Based on the above analysis the leaf extracts of *Flueggea leucopyrus* Willd do not appear to be genotoxic at lower concentrations (0.005 mg/ml, 0.01 mg/ml) ($p < 0.05$), though at the highest concentration (approximately three times the amount estimated to be generally consumed) they appear to be marginally toxic. As these studies are preliminary in nature, further trials would need to be carried to confirm these findings.

dilruksh@chem.cmb.ac.lk Tel: 0112503367