

## Procoagulant Factors Inhibition Potential of Ingredient of PHYTOCEE®: *Emblica officinalis*

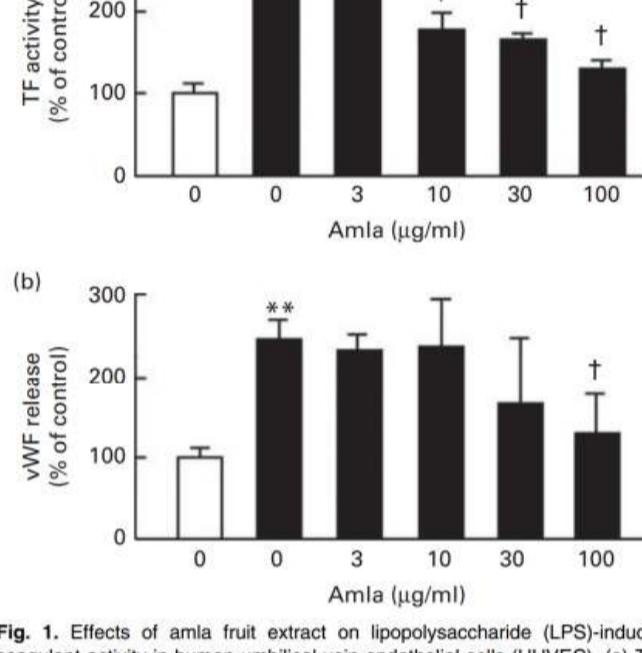
### OBJECTIVE

To evaluate the anticoagulant properties of amla (*Emblica officinalis*) fruit extract.

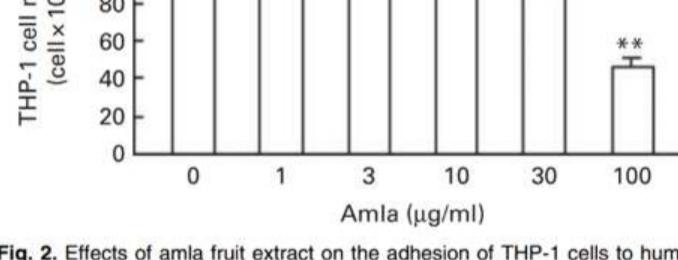
### MATERIALS AND METHODS

Primary HUVEC and Human monocytic cell line THP-1 cells were used in this study. The test material was a commercial amla fruit extract. The tissue factor (TF) activity was measured as a factor of X activation by factor VIIa and TF complex in HUVEC after stimulation with TNF- $\alpha$  using standardized assay protocol. After the stimulation of the HUVEC with lipopolysaccharide (LPS) at various concentrations (0, 3, 10, 30 and 100mg/ml) of the amla fruit extract, the concentration of the von Willebrand factor (vWF) antigen in the supernatant was determined with ELISA method. The cell adhesion assay in HUVEC cells stimulated with 1mg/ml of stimulant LPS for 2 h along with different concentrations (0, 1, 3, 10, 30 and 100mg/ml) of the amla fruit extract was carried out by measuring labelled THP-1 cells in a standardized microplate assay method.

### RESULTS



**Fig. 1.** Effects of amla fruit extract on lipopolysaccharide (LPS)-induced coagulant activity in human umbilical vein endothelial cells (HUVEC). (a) Tissue factor (TF) activity of HUVEC stimulated with 1  $\mu$ g/ml LPS (■) or PBS (□) in the presence of 0–100  $\mu$ g/ml of amla fruit extract was determined. (b) von Willebrand factor (vWF) release from HUVEC into the supernatant was determined by ELISA. Values are means of three independent experiments, with standard deviations represented by vertical bars. \*\*Mean value was significantly different from that of the PBS control ( $P < 0.01$ ; Dunnett's test). †Mean value was significantly different from that of the LPS-stimulated HUVEC without amla fruit extract ( $P < 0.05$ ; Dunnett's test).



**Fig. 2.** Effects of amla fruit extract on the adhesion of THP-1 cells to human umbilical vein endothelial cells (HUVEC). HUVEC were stimulated with lipopolysaccharide in the presence of 0–100  $\mu$ g/ml of amla fruit extract. The number of THP-1 cells adhered to the HUVEC was determined. Values are means of three independent experiments, with standard deviations represented by vertical bars. Mean values were significantly different compared with those of the group stimulated in the absence of amla fruit extract: \*  $P < 0.05$ ; \*\*  $P < 0.01$  (Dunnett's test).

### CONCLUSIONS

- Amla fruit extract potentially and significantly reduced lipopolysaccharide (LPS)-induced tissue factor expression and von Willebrand factor release in human umbilical vein endothelial cells (HUVEC) at clinically relevant concentrations (1–100 mg/ml).
- Furthermore, in a leucocyte adhesion model of inflammation, Amla at clinically relevant concentrations (1–100 mg/ml) also significantly decreased LPS-induced adhesion of human monocytic cells (THP-1) to the HUVEC.

### OUTCOME

These results suggest that amla fruit extract may be an effective anticoagulant agent.

#### Reference:

Rao TP, Okamoto T, Akita N et al. Amla (*Emblica officinalis* Gaertn.) extract inhibits lipopolysaccharide-induced procoagulant and pro-inflammatory factors in cultured vascular endothelial cells. Br J Nutr. 2013;110(12):2201-6.