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Modulatory Effect of Kenaf Extracts on Macrophage Activation

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Objectives

To investigate the modulatory effect of *Hibiscus cannabinus* (Kenaf) on macrophage activation, the production of nitric oxide, a representative cytotoxic molecule secreted from macrophages was examined using lipopolysaccharide (LPS)-activated macrophage-like cells (RAW264.7 cells).

Material and Methods

Materials

The Kenaf extracts were prepared with various organic solvents. 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazlium bromide (MTT), Griess reagents, and LPS were purchased from Sigma Chem. (St. Louis, MO, USA). RAW264.7 cells were obtained from ATCC (Rockville, MD, USA). Fetal bovine serum, penicillin and streptomycin were obtained from GIBCO (Grand Island, NY, USA)

Methods

Cell protective and NO modulatory effects of *Hibiscus cannabinus* extracts were evaluated by the methods previously reported (Cho et al., 2000). Briefly, *Hibiscus cannabinus* extracts (50 or 100 μg/ml) were added to each well and cultured for 24 h. Cell viability and NO metabolites in culture supernatants were determined by measuring OD value at 570 nm (OD_{570}).

Results and Discussion

As shown in Table I, Kenaf extracts showed different modulatory effect on NO production in LPS-activated RAW264.7 cells. Of tested fractions, thus, hexane fraction prepared with 80% EtOH only suppressed NO production in LPS-activated RAW264.7 cells up to 30% at 50 μg/ml. However, subfractions (BuOH, Hexane and ethyl acetate) prepared with 80% EtOH significantly protected LPS-induced cell death up to 30 to 45%. Therefore, our data suggest that Kenaf may have a beneficial protective effect in various macrophage-mediated immunopathological diseases, in addition to its industrial merits as a raw material in manufacturing papers and filters. Taken together, a possibility as an immunomodulatory bioproduct will be further carefully evaluated using other experimental approaches such as the atopic and allergic diseases conditions.

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Table I. Effects of Kenaf extracts on NO production and cell protection in LPS-treated RAW264.7 cells.

Fraction	Concentration tested (μg/ml)	% of control (mean ± SD)	
		NO production	Cell protection
Vehicle		100	0
80% methanol extract	100	93.4 ± 2.1	27.5 ± 7.2
50% methanol extract	100	98.7 ± 0.3	17.5 ± 5.3
30% methanol extract	100	100.4 ± 0.4	19.3 ± 6.6
80% ethanol extract	100	100.1 ± 1.8	20.9 ± 1.8
Butanol	50	105.2 ± 2.7	30.1 ± 6.2
Hexane	50	69.1 ± 2.6	34.3 ± 7.4
Ethyl acetate	50	102.2 ± 3.0	43.5 ± 14.3
Water	50	97.7 ± 1.9	04.8 ± 1.9
50% ethanol extract	100	101.1 ± 3.2	15.8 ± 0.5
30% ethanol extract	100	105.9 ± 1.5	13.5 ± 4.4
water	100	99.8 ± 2.7	19.7 ± 4.8