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*Investigation of the immunostimulatory effects of  
some New Zealand honeys and characterization of an  
active component*

A Thesis submitted in partial fulfilment of the requirements for the degree of

Master of Technology  
in  
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## *Abstract*

Medicinal use of honey has re-emerged recently indicating that honey accelerates wound healing activity. Honey has been shown to stimulate TNF- $\alpha$  production from monocytes and macrophages which is apparently correlated with a high molecular weight fraction, and not lipopolysaccharide (LPS, an immunostimulatory endotoxin) levels. Cytokine production by honey has been attributed to the endotoxin content. The aim of this study was to investigate the ability of Comvita sourced honeys to elicit a TNF- $\alpha$  cytokine response from acute monocytic leukemia (THP-1) cells as well as identify the responsible component.

Five honey samples were used together with sugar and methylglyoxal controls. The samples were incubated with THP-1 cells, with and without LPS. After incubation, the cell culture supernatants were collected and TNF- $\alpha$  was measured by the enzyme-linked immune sorbent assay (ELISA). The most active honey samples were further heat-treated to remove enzyme/protein/peptide-like stimulation; the samples were treated with polymixin B (PmB) to remove LPS-like stimulation and not protein fraction. The samples were then filtered by molecular weight centrifugal filters to separate constituents according to their size and the fractions were re-analysed.

All five honey samples in the absence of LPS stimulated TNF- $\alpha$  release from THP-1 cells, whereas untreated, sugar- and methylglyoxal-treated cells did not. The cytokine production was partially inhibited by heating, but mostly by PmB. In the filtered honey samples, the activity was observed in the >30 kDa fraction. These results suggest that the activity may be associated with one or more components which are partially heat-labile, LPS-like stimulated with a high molecular weight.

Further, honey samples were analyzed for the concentration of LPS present. The tests revealed that the cytokine stimulation was higher than would be expected from the concentration of LPS present in the honey. The possibility that this component was a plant-derived  $\beta$ -glucan, which is known to have LPS-like activity and can interfere with detection of LPS in the LAL assay, was investigated. Subsequent analyses confirmed the presence of arabinogalactan, a large complex carbohydrate.

The data presented in this study suggests that arabinogalactans in honey may stimulate inflammatory responses and the release of cytokines that are crucial in regulating wound-healing. This heralds a significant advancement in the usage and understanding of medicinal honey.

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*To my Husband Subrahmanyeswarlu, and daughters, Yashaswini and Harika  
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With all my love*

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## *List of Abbreviations*

NH <sub>4</sub> OH	Ammonium hydroxide
ATCC	American Type Culture Collection
ANOVA	Analysis of variance
AG	Arabinogalactan
AGP	Arabinogalactan-protein
AH	Artificial honey
BSA	Bovine serum albumin
(CH <sub>3</sub> ) <sub>2</sub> SO	Camptothecin
CO <sub>2</sub>	Carbon dioxide
°C	Degrees Celsius
DMSO	Dimethylsulfoxide
KH <sub>2</sub> HPO <sub>4</sub>	Dipotassium hydrogen orthophosphate
Na <sub>2</sub> HPO <sub>4</sub>	Disodium hydrogen orthophosphate anhydrous
ECM	Extra cellular matrix
ESR	Environmental Science Research
ELISA	Enzyme-linked immune sorbent assay
FBS	Fetal bovine serum
GC-MS	Gas chromatography-mass spectrometry
<i>g</i>	Gram or Acceleration due to gravity
>	Greater than
HPAEC	High-performance anion-exchange chromatography
h	Hour
THP-1	Human monocytic leukemia cells
HMF	Hydroxymethylfurfural
HRP	Horse radish peroxidase
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
IRL	Industrial Research Limited
IU	International Unit
IL-1	Interleukin-1

IL-6	Interleukin-6
Kg	Kilogram
KGy	kilo Grays
KDa	Kilo Daltons
<	Less than
L	Litre
LPS	Lipopolysaccharide
Ltd	Limited
LiNO <sub>3</sub>	Lithium nitrate
pH	-Log [H <sup>+</sup> ]
MS	Mass spectroscopy
MHz	Megahertz
MGO	Methylglyoxide
CH <sub>3</sub> I	Methyl iodide
MeOH	Methanol
ML	Millilitre
μL	Microliter
mg	Milligram
μm	Micrometer
μM	Micromolar
mM	Millimolar
mm	Millimetre
min	Minutes
M	Molar
ng	Nanogram
nm	Nanomolar
N <sub>2</sub>	Nitrogen
NMR	Nuclear magnetic resonance
ppm	Part per million
%	Percent
% (w/v)	Percent by weight per volume
PMA	Phorbol 12-myristate 13-acetate
PBS	Phosphate Buffered Saline
PBS-T	Phosphate Buffered Saline -Tween 20

PmB	Polymixin B
rpm	Revolution per minute
RPMI	Roswell park memorial institute medium
Complete RPMI	RPMI medium supplemented with 10 % FBS, penicillin at 50 IU units/mL and streptomycin at 5 µg/mL
SEC	Size-exclusion chromatography
NaN <sub>3</sub>	Sodium azide
NaBD <sub>4</sub>	Sodium borodeuteride
NaH <sub>2</sub> PO <sub>4</sub>	Sodium dihydrogen phosphate monohydrate
TMB	Tetra methyl benzidine
TFA	Tri fluoro acetic acid
TNF- $\alpha$	Tumor necrosis factor
USP	United States Pharmacopeia
v/v	Volume per volume
WST-1 reagent	4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzenedisulfonate