

A sensitive *in vitro* method for evaluation of immunostimulatory properties of natural products

John Kalns, Ph.D., Jakob Kirchner, Ph.D., Samuel Ebong, Ph.D., Cherie Oubre, Ph.D.

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ABSTRACT

Background- Some natural products may enhance the performance of the human immune system. However, source and method of preparation can have a significant impact on efficacy. Products that are of similar chemical composition may differ significantly in terms of biological activity. In order to ensure that products have the desired property, methods for evaluating bioactivity are required. The human monocytic cell line, THP-1, responds *in vitro*, in a manner similar to that of primary human dendritic macrophages and may predict activity of natural products *in vivo*. *Objective-* Determine if Aloe Vera gel obtained from different sources differ in their abilities to induce production of Tumor Necrosis Factor (TNF) in THP-1 cells. *Methods-* Aloe Vera gel from five different sources was tested. Aloe Vera gel, was suspended in phosphate buffered saline with 0.05% Tween 20 added to facilitate solubilization. In some instances the suspensions were filtered sequentially through a 0.22 mm syringe filter followed by a 100,000 MW cut-off filter. THP-1 cell were induced to a macrophage-like phenotype by addition of phorbol myristyl acetate (PMA) 50ng/ml. Aloe Vera gel suspensions were then added (10% v/v) and 24 hours later TNF levels determined using a microbead ELISA assay (Luminex), and cell viability measured by mitochondrial function (reduction of tetrazolium, MTT). Lipopolysaccharide (LPS) from *E. Coli* O157 was used as a positive control. *Results-* Two of five Aloe Vera gel preparations at 100 µg/ml induced TNF at a level similar to that of high dose LPS, 500 ng/ml. Active preparations demonstrated TNF induction at Aloe Vera concentrations as low as 1 µg/ml. In contrast, three did not induce TNF. Of the two active compounds, filtration through a 0.22 mm filter reduced TNF induction by 80% and subsequent filtration through a 100,000 MW cut-off filter reduced induction 100%. Heating to 100°C for 30 minutes prior to exposure had no effect on TNF expression. Level of TNF expression was inversely related to cell viability measured by MTT. *Conclusions-* The results demonstrate that chemically similar compositions differ significantly with regard to ability to elicit an immune response. Filtration results suggest that Aloe Vera gel particulates are responsible for observed activity, a property that cannot

be detected using conventional analytical methods. The method is useful for discovery of new, immunologically active natural products as well as for assessment of the activity of materials from different sources or vendors.

METHODS

Ingredients Tested

The ingredients were tested in a blinded fashion. No information was provided prior to testing except that all ingredients are Aloe Vera gel. The ingredients were provided to Hyperion Biotechnology, Inc. by Mannatech, Inc. of Coppell, Texas.

Preparation of Ingredients

Thirty to forty milligrams of each ingredient were placed in to a plastic 50ml centrifuge tube and then suspended in either cell culture media or distilled, deionized water, or water with 0.05% Tween 20 to solubilize and wet the Aloe preparations. The suspensions were then vortexed at high speed for 5 minutes and then sonicated for 30 minutes and then vortexed for another 5 minutes just prior to addition to cells.

TNF Measurement

After 24 hour incubation with Aloe preparations 100 µl of media was collected and assayed for TNF using microbead-based ELISA (TNF Bioplex Kit, Biorad) measured on a Luminex fluidic device.

Evaluation of cell viability using MTT

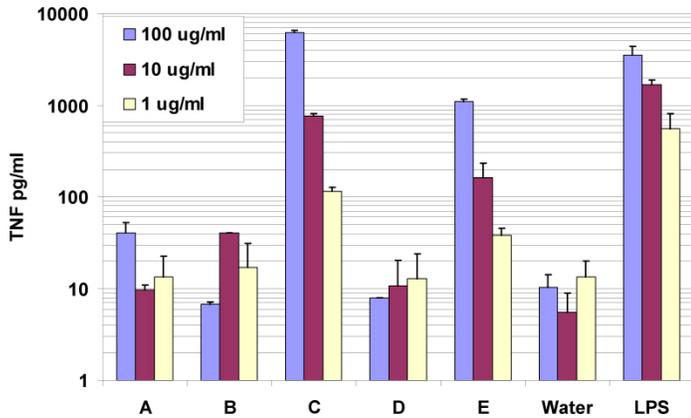
After collection of supernatant media containing tetrazolium salt, 20 µl, was added and cells returned to the incubator for 4h. After 4 hours, 80 µl of lysis solution was added tetrazolium crystals and absorbance read at 620 nm. Higher absorbance values indicate more mitochondrial activity and thus, indirectly, the number of living cells present. Cultures that are completely non-viable have an observed absorbance near 0.

Turbidity of Aloe Preparations

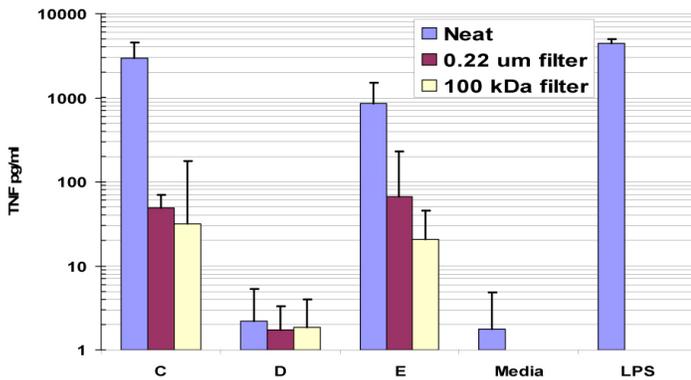
Turbidity was measured by determination of absorbance at 620 nm in a 96-well plate.

RESULTS

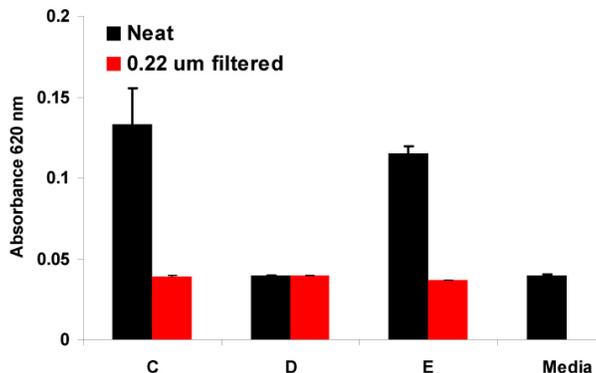
The figure shows that two Aloe preparations are highly active (C and E) whereas three (A, B and D) are not. Significant activity to 1 µg/ml is observed. LPS 500, 50 and 5 ng/ml. N=6, error bars SEM.



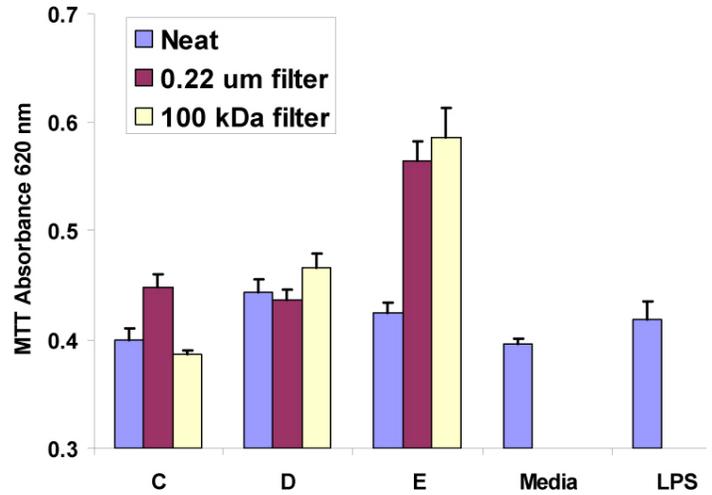
The figure below shows that filtration removes most activity of suggesting that particulates are responsible for most of the observed immunological activity. Heating to 1000C does not reduce activity of active Aloe (data not shown). N=6, error bars SEM.



The figure below shows that preparations that are active are also turbid supporting the hypothesis that particulates are responsible for activity. Filtration is sufficient to remove all turbidity and also most activity. N=6, error bars SEM.



The figure below shows that THP-1 viability is also linked to formulation suggesting a complex behavior. N=6, error bars SEM.



CONCLUSIONS

- Chemical composition and source do not always predict biological activity.
- Particulates can be important in eliciting immune effects. More work is required to determine if particulates are present in the gastrointestinal tract following ingestion of Aloe.
- The approach used here may be useful in predicting biological activity *in vivo*.

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For more information: send inquiries by mail to: Hyperion Biotechnology, Inc., 13302 Langtry, San Antonio, Texas, 78248; or email: johnkalns@hyperionbiotechnology.com