Immuno-modulatory effect of Turmeric (Curcuma longa) and Aloe Vera on cultured Polymorphonuclear cells (PMN) and adherent mononuclear cells

Peer review status:
No

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Article ID: WMC005113
Article Type: Research articles
Submitted on: 31-May-2016, 10:34:11 AM GMT   Published on: 02-Jun-2016, 02:00:40 PM GMT
Article URL: http://www.webmedcentral.com/article_view/5113
Subject Categories: ALTERNATIVE MEDICINE
Keywords: Polymorphonuclear cells, Turmeric, Aloe Vera, Cell proliferation, Alternative Medicine, adherent cells

How to cite the article: Gandhi M, Shree R. Immuno-modulatory effect of Turmeric (Curcuma longa) and Aloe Vera on cultured Polymorphonuclear cells (PMN) and adherent mononuclear cells. WebmedCentral ALTERNATIVE MEDICINE 2016;7(6):WMC005113

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Source(s) of Funding:
Departmental funds for B.Tech Research Projects

Competing Interests:
No competing interests

Additional Files:
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Abstract

Complementary and alternative therapeutic measures off-late have gained significance because they have been shown to be clinically efficient with minimal side effects compared to mainstream treatments. Plant medicines are far and away safer, gentler and better for human health than synthetic drugs and with much less side effects. Aloe vera and Curcuma longa are two of such plants, which have been proven to have medicinal properties by several groups of researchers. However, the exact effect of these extracts on cultured human adherent and non-adherent cells is still controversial. Very few studies have been done in this regard. Adherent and non-adherent cells in humans form a very important component of immune system. Studying the effect of Aloe vera and Curcuma longa on these cells, can go a long way in understanding the immuno-modulatory effects of these plants. In this study, we tested the immuno-modulatory effects of mannan from Aloe Vera and Curcumin from turmeric rhizome using human cells (PBMC). We used cell culture and Bradford's protein estimation methods and determined that mannan inhibits the proliferative response in human PBMCs, whereas Curcumin has a proliferative response towards human PBMCs.

Introduction

Complementary and alternative therapeutic measures off-late have gained significance because they have been shown to be clinically efficient with minimal side effects compared to mainstream treatments. In India, several candidate plants of indigenous origin have been shown to have various immuno-modulatory, anti-inflammatory and tonic properties (Sharma 1981, Chopra et al. 1996). Some of them have been shown to have profound impact on the T-cell activity and to some extent also on the Th1/Th2 cytokine profile. Plant medicines are far and away safer, gentler and better for human health than synthetic drugs and with much less side effects. This is so because human beings have co-evolved with plants over the past few million years. Some compounds perform the same functions in plants and in the body. Natural antioxidant phenols in plants, for example, protect plant cells from oxidation, and often perform the same function in the human body. Our bodies recognize the substances that occur in plants, and possess sophisticated mechanisms for metabolizing plant materials.

Another advantage to herbal medicine is cost. Herbs cost much less than prescription medications. Research, testing, and marketing add considerably to the cost of prescription medicines. Herbs tend to be inexpensive compared to drugs.

Genus Aloe vera, family Liliaceae (Active compound-carboxypeptidase and salicylate) extract has been shown to enhance production of IL-1 cytokine (Peng et al, 1991), which in turn can further promote Th1/Th2 response. The antimicrobial activity of aloe extracts was postulated as early as 1939. Clinical studies have revealed several immuno-modulatory properties. Stronger leukocyte infiltration was seen in injured areas where aloe extracts were used in treatment, and surface wound recovery was stimulated by polyuronic acids. Acemannan, the major fraction of aloe polysaccharides, has been extensively studied for immuno-modulatory effects. It has also been found that Acemannan can stimulate TH2 cells (Talmadge et al, 1996; Vanitha et al, 2004). A number of studies indicated immuno-modulating activities of the polysaccharides in A. Vera gel, and suggested that these effects occur via activation of macrophage cells to generate nitric oxide, secrete cytokines (e.g. tumor necrosis factor-alpha or TNF-α, interleukin-1 or IL-1, interleukin-6 or IL-6 and interferon-γ or INF-γ) and present cell surface markers (Tizard et al., 1996).

Genus Curcuma longa, family Zingiberaceae (Active compound- curcumin i.e. diferuloyl methane) has long been known as a spice, remedy, and dye (Aggarwal et al, 2008).Curcuma contains 3-5% curcuminoids (50-60% curcumin) and up to 5% essential oils and resins. (Chaudhary et al, 2010; Aggarwal et al, 2010). The curcuminoid content in turmeric can vary between 2 and 9%, depending on the geographical conditions
The anti-inflammatory effects of curcumin have been postulated on the basis of a number of in vitro and in vivo studies (Basnet et al., 2011; Strimpakos, 2008). It has been shown to be a potent immuno-modulatory agent that can modulate the activation of T cells. In a study carried out in 1999, Kang et al showed that in vivo administration of curcumin resulted in the inhibition of IL-12 production by macrophages stimulated in vitro with either LPS or HKL, leading to the inhibition of Th1 cytokine profile (decreased IFN-γ and increased IL-4 production) in CD4+ T cells. The unusually high biological activity profile of curcumin arises from its unique combination of antioxidant and anti-inflammatory properties (Priyadarsini et al 2003; Ak et al, 2008; Wang et al, 2010). These two activities are complementary, and an effective antioxidant should also be an anti-inflammatory because oxidative stress elicits an inflammatory response.

The effect of turmeric and aloe vera extracts on cultured human adherent and non-adherent cells is still controversial. Very few studies have been done to study the effect of plant extract on cells of the immune system. (Talmadge et al, 1996). Lot remains to be investigated, on the exact effect of these two extracts on cultured human blood cells. We therefore thought it pertinent, to design a study to analyze the effect of these two extracts on the cultured polymorphonuclear cells and monocytes from human blood. we studied, the primary action of turmeric and aloe Vera extracts on peripheral blood mononuclear cells, by checking cells morphologies and cells density in each well under inverted light microscope and by estimating the protein content in each well by Brad ford's protein estimation method.

Materials and Methods

Preparation of turmeric and aloe vera extracts: Turmeric extract was prepared from dried turmeric rhizome by adding hexane and ethanol. The curcuminoid layer was precipitated by petroleum ether, air-dried and dissolved in Dimethyl sulfoxide (DMSO) at a concentration of 10mM and stored in dark-colored bottle. For aloe vera, the leaf pulp was taken and mixed with chloroform and methane in a colored bottle and stored in dark at room temperature.

Isolation of PBMCs from peripheral human blood: 30 ml of venous blood was collected and centrifuged at 1000 rpm for 10 min at 4°C. Buffy coat was separated and washed in PBS. The cells were then suspended in complete DMEM-G medium, viability counted by trypan blue exclusion method and plated at a density of 3X10^6 cells/ml.

Treatment of cultured cells with turmeric extract and aloe vera extract: The cells were plated in 24 well culture plates and treated with the turmeric extract and aloe vera extract. Concavalin A was used as a positive control to observe the proliferation of cells. Cells cultured in media alone were used as media control. Cells were also treated with DMSO and Chloroform/ Methanol solvents alone to study the effect of solvent on the rate of proliferation and cell morphology. Experimental wells were also set up, where cells were treated with undiluted turmeric/ aloe vera extracts dissolved in their respective solvents or with 1:10 diluted extracts (Table-1). Cells were incubated at 37°C in 5% CO₂ for 20 hours. Non-adherent cells were taken in 5 ml storage vials. The adherent cells were scraped and collected in storage vials. The cells were then washed and lysed. The cell lysate was stored at -80°C till further use.

Protein estimation: The protein content in the cells incubated under different conditions of culture was measured by Bradford protein estimation method and BSA was used as the standard.

Results

Cell viability by trypan blue exclusion method: The cells were counted in haemocytometer and the viability of cells was found to range from 95% to 99% under different set of experimental conditions.

Cell morphology: Cells were observed under inverted microscope after 20 hours of incubation. Morphological features of cells and the rate of proliferation were found to vary under different set of conditions. The results for different set of experimental conditions are discussed in Table-2.

Levels of protein: BSA was used as the standard and the protein content measured by Bradford method. The protein concentration of cell lysate under different culture conditions was found to vary. The results are depicted graphically in Figure-1.

Discussion

Concavalin A was found to cause extensive and rapid proliferation of cells under culture conditions and the cell morphology was found to be changed. However, Con A resulted in more extensive proliferation of adherent cells compared to the non-adherent cells, thereby resulting in a higher extent of protein levels in adherent cells. DMSO treated cells were found to be
alive after treatment but the rate of proliferation was found to be reduced, as evidenced by the reduction in protein levels. Morphological features of the DMSO treated cells remained unchanged. **DMSO was therefore found to have no killing effect on the cells.** In Aloe vera treated cells, both adherent cells and non-adherent cells showed extensive cell death and hence the protein content was found to be increased compared to the DMSO control. This observation indicated that **Aloe vera led to killing of adherent cells.** **Chloroform-methanol treated cells showed a very high extent of cell death and clumping.** Non-adherent cells showed little extent of cell death. Adherent cells showed extensive cell death and the protein content was found to be high for adherent cells. In the turmeric extract treated cells, cells were alive and proliferated at a very high rate. The rate of proliferation and the protein content both were quiet high in non-adherent cells compared to the adherent cells. **Turmeric extract was therefore found to have a very good proliferative effect.**

**Conclusions**

Thus, we conclude that Aloe vera extract is inhibitory in nature and tends to inhibit cell proliferation by possibly increasing rate of cell death. The effect is much more pronounced on the adherent cells compared to the non-adherent cells. Turmeric extract has a very high proliferative effect as it causes significant increase in cell density and also in the protein content compared to solvent alone control. The effect is more pronounced on the non-adherent cells. Turmeric and Aloe Vera therefore could be promising candidates for alternative/ complementary medicines for the treatment of a variety of immunological and inflammatory diseases. Depending upon the type of pathological condition and the need for increasing or decreasing the extent of proliferation for immune cells such as PBMCs or monocytes, strategies can be designed by making use of Aloe Vera or Turmeric extracts. The exact cellular mechanisms by which these plant extracts affect the rate of cell proliferation, however is yet to be elucidated.

**References**

Illustrations

Illustration 1

Comparative protein levels in adherent and non-adherent cells

![Illustration 1: Comparative protein levels in non-adherent and adherent cells](image)

Illustration 2

Conditions of treatment in culture

<table>
<thead>
<tr>
<th>WELLS</th>
<th>TREATMENT ON CELLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PURE CONTROL 1 ml media contains cellsx2x(0000000) + 1 ml media</td>
</tr>
<tr>
<td>2</td>
<td>CONTROL + CONCAVALIN A 1 ml media contains cellsx2x(0000000) + 1 ml conc A (5ug/ml)</td>
</tr>
<tr>
<td>3</td>
<td>Neat solvent- DMSO treatment 1 ml media contains cellsx2x(0000000) + 1 ml DMSO</td>
</tr>
<tr>
<td>4</td>
<td>Aloe Vera treatment 1 ml media contains cellsx2x(0000000) + 1 ml aloe Vera extract</td>
</tr>
<tr>
<td>5</td>
<td>1:10 Aloe Vera treatment 1 ml media contains cellsx2x(0000000) + 0.2ml aloe Vera extract + 0.8 ml media</td>
</tr>
<tr>
<td>6</td>
<td>Neat solvent- dichloroform+ methanol (1:1) treatment 1 ml media contains cellsx2x(0000000) + 1 ml dichloroform+ methanol (1:1)</td>
</tr>
<tr>
<td>7</td>
<td>Turmeric extract treatment 1 ml media contains cellsx2x(0000000) + 1 ml turmeric extract</td>
</tr>
<tr>
<td>8</td>
<td>1:10 Turmeric extract treatment 1 ml media contains cellsx2x(0000000) + 0.2ml turmeric extract + 0.8 ml media</td>
</tr>
</tbody>
</table>

Table 1: Conditions of treatment in culture
Illustration 3

Morphological results for different set of experimental conditions

<table>
<thead>
<tr>
<th>WELLS</th>
<th>TREATMENT ON CELLS</th>
</tr>
</thead>
</table>
| 1     | 1. Cells were alive  
       | 3. Cells proliferation occurred but at a low rate. |
| 2     | 1. Cells were alive  
       | 2. Morphology of cells changed a little, size of cells was large.  
       | 3. Cells proliferation rate was high or cells were in high density. |
| 3     | 1. Cells were alive  
       | 2. Some cells were adherent and some in solution.  
       | 3. Low cells proliferation rate.  
       | 4. Less morphological changes occurred. |
| 4     | 1. Cells were dead and clumping of cells occurred. |
| 5     | 1. Very few cells were alive. |
| 6     | 1. Cells were dead and clumping of cells occurred. |
| 7     | 1. Cells were alive  
       | 2. Cells were proliferating in very high density.  
       | 3. Cells were very high in number. |
| 5     | 1. Cells were alive  
       | 2. Cells were high in number.  
       | 3. Change in morphology of cells occurred.  
       | 4. Most of the cells were in solution. |

Table-1: Morphological results for different set of experimental conditions