Antioxidant and Immune-Modulatory Effects of *Aloe vera* Decolorized Leaf Juice *vs.* Inner Leaf Juice

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Introduction

The aloe vera (*Aloe vera*) plant **[fig. 1]** has a long history of traditional therapeutic topical use (Shelton, 1991). Its leaf and inner leaf juices are also ingested in various forms (Vogler, 1999). Aloe vera leaf juice is made from macerated aloe leaves, and when it is intended for human ingestion, it is purified of latex constituents such as anthraquinones via a charcoal filtration process known as decolorization (Ramachandra, 2008).

Several publications have shown immunomodulatory effects (reviewed in Choi, 2003), as well as antibacterial (Habeeb, 2007), antifungal (Olaleye, 2005) and anti-parasitic (Dutta, 2008) properties of aloe vera inner leaf juice, but limited information is available regarding these effects for aloe vera leaf juice after the decolorization process.

Fig. 1: An *Aloe vera* plant (left) and leaf sections showing the mucilaginous inner gel (right)

Objective

The aim of this preliminary study was to compare biologically-relevant antioxidant and immune-modulatory effects of aloe vera inner leaf and decolorized leaf preparations using a variety of *in vitro* techniques.

Materials and Methods

Direct immune-modulatory effects in the absence of an immune stimulant were assessed by treating human peripheral blood mononuclear cells (PBMCs) from three healthy donors with each aloe vera preparation for 24 hours, followed by multi-parameter flow cytometry, which evaluated the activation status (as measured by expression of CD25 and CD69) of several immune cell populations [natural killer (NK) cells (CD3-/CD56+), CD3+/CD56+ immune cells (including NKT cells), and T lymphocytes (CD3+/CD56-)] that were identified by double-staining with monoclonal antibodies specific for CD3 and CD56. To investigate potential immune modulatory effects of each aloe vera preparation in response to inflammatory stimulus, PBMCs were also pre-treated with decolorized leaf (DL) and inner-leaf (In-L) aloe vera preparations and stimulated by either lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (poly I:C) to mimic bacterial and viral insults, respectively.

Antioxidant activity of DL and In-L were assessed for total antioxidant capacity by the Folin-Ciocalteu method (Singleton, 1965).

Figures 2-7: Immune cell activation
Cultures of human peripheral blood mononuclear cells (PBMCs) from three healthy donors were treated with DL and In-L at concentrations from 0.004 to 2 g/L and the activation status of lymphocytes, monocytes, natural killer (NK) cells, CD3+/CD56+ cells (including NK-T cells) and T cells were measured by double staining with fluorescent antibodies for activation markers C25 and C69. Results are shown in mean fluorescence intensity. Asterisk indicates a significant ($p < 0.05$) difference between a test preparation and control.

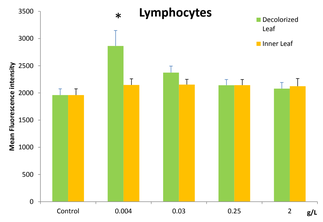

Fig. 2: Lymphocyte activation in the presence of DL and In-L.

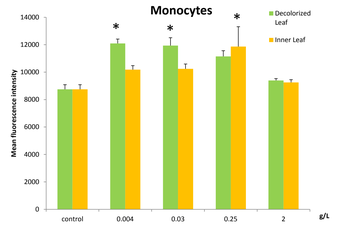

Fig. 3: Monocyte activation in the presence of DL and In-L.

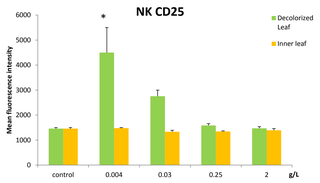

Fig. 4a: Expression of NK activation marker CD25 in the presence of DL and In-L

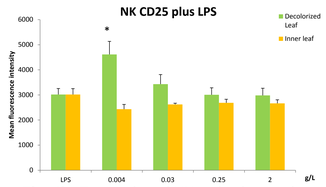

Fig. 4b: Expression of NK activation marker CD25 in the presence of DL and In-L and LPS

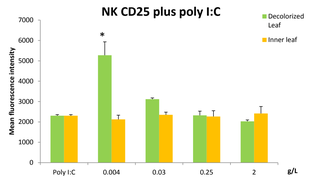

Fig. 4c: Expression of NK activation marker CD25 in the presence of DL and In-L and poly I:C

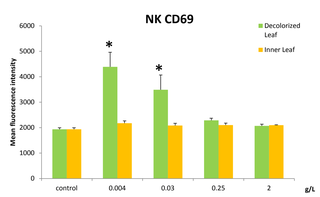

Fig. 5a: Expression of NK activation marker CD69 in the presence of DL and In-L

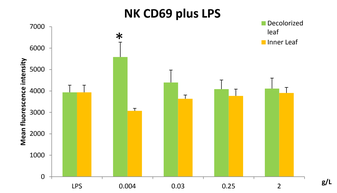

Fig. 5b: Expression of NK activation marker CD69 in the presence of DL and In-L and LPS

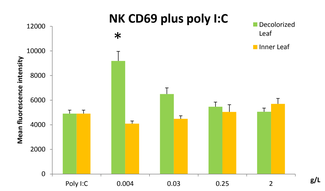

Fig. 5c: Expression of NK activation marker CD69 in the presence of DL and In-L and poly I:C

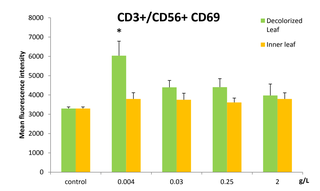

Fig. 6a: Expression of CD3+/CD56+ activation marker CD69 in the presence of DL and In-L

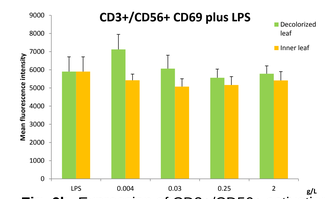

Fig. 6b: Expression of CD3+/CD56+ activation marker CD69 in the presence of DL and In-L and LPS

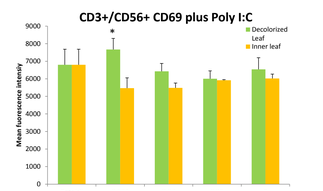

Fig. 6c: Expression of CD3+/CD56+ activation marker CD69 in the presence of DL and In-L and poly I:C

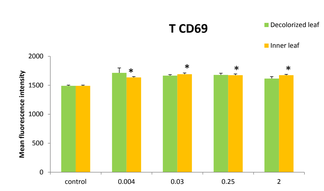

Fig. 7a: Expression of T cell activation marker CD69 in the presence of DL and In-L

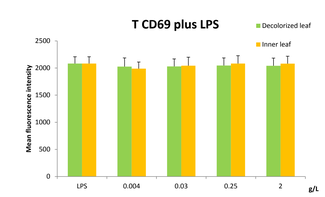

Fig. 7b: Expression of T cell activation marker CD69 in the presence of DL and In-L and LPS

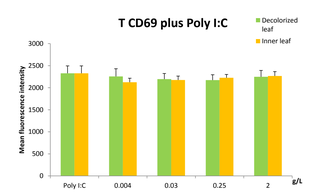

Fig. 7c: Expression of T cell activation marker CD69 in the presence of DL and In-L and poly I:C

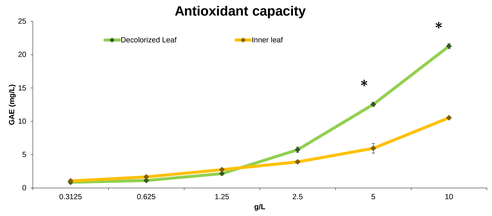

Fig. 8: Total antioxidant capacity of DL and In-L at concentrations from 0.3125 to 10 g/L, expressed in gallic acid equivalents (GAE) in mg/L. Asterisk indicates significance ($p < 0.05$)

Results and Discussion

DL showed a significant increase in the activation of T lymphocytes **[fig. 2]** and monocytes ($p<0.01$) **[fig. 3]**. DL also increased the expression of the activation markers CD25 **[fig. 4a]** and CD69 **[fig. 5a]** of NK cells ($p<0.05$). This increased expression was also observed when the cells were stimulated by poly I:C ($p<0.01$) **[figs. 4c** and **5c]**. The CD3+/CD56+ cell population also increased its expression of CD69 when pre-treated with DL alone **[fig. 6a]** and also when the pre-treated PBMCs were stimulated by poly I:C ($p<0.01$) **[fig. 6c]**.

In contrast, In-L material showed a relatively limited immune modulatory activity, increasing the expression of CD69 only in T cells ($p<0.01$) **[fig. 7a]**. No difference was seen in the presence of LPS or poly I:C **[figs. 7b and 7c].**

DL showed higher total antioxidant capacity than In-L, at 5 and 10 mg/ml concentrations ($p<0.05$) **[fig. 8]**.

Conclusion

The decolorized aloe vera leaf preparation showed higher antioxidant and immune-modulatory activity than the inner-leaf preparation. The increase in immune cell activation markers representing activation status of the immune cells suggests that DL supports innate immunity, particularly through the sustained activation of NK cells, both in terms of direct effects, and in the context of a viral-mimetic insult.

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