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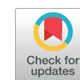
Water and ethanol extracts of *Plantago major* leaves show anti-inflammatory activity on oral epithelial cells



Muhammad Zubair [a, b, *], Cecilia Widén [a, c], Stefan Renvert [c], Kimmo Rumpunen [a]

[a] *Swedish University of Agricultural Sciences, Department of Plant Breeding and Biotechnology Balsgård, Fjälkestadsvägen 459, SE-291 94 Kristianstad, Sweden*
[b] *Department of Bioinformatics and Biotechnology, Government College University (GCU), Faisalabad, Pakistan*
[c] *Kristianstad University, School of Health and Society, SE-291 88 Kristianstad, Sweden*

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ABSTRACT

Context: The leaves of *Plantago major* have been used for the treatment of wounds and inflammation in folk medicine from prehistoric times. However there is no report on the use of *P. major* to treat inflammation in oral epithelial cell lines.
Objective: The present study was undertaken to reveal possible anti-inflammatory effects of *Plantago major* leaf extracts on oral epithelial cells *in-vitro.*
Materials and methods: Water- and ethanol-based extracts of *P. major* leaves were prepared from freeze-dried plant material, and tested *in-vitro* using the oral epithelial cell line H400. The anti-inflammatory activity of *P. major* was tested against *E. coli* lipopolysaccharide (LPS) using the nuclear factor kappa beta (NF-kB) assay.
Results: Both the water- and the ethanol-based extracts, as well as a combination of the two extracts, showed anti-inflammatory activity. A concentration of 0.1 mg/mL (on dry weight basis) yielded the best results for all extracts.
Discussion and conclusion: The results show that synergistic effects of both polyphenols and water-soluble compounds (possibly polysaccharides) are responsible for anti-inflammatory activities of *P. major.*
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1. Introduction

Plantago major leaves have been used for its wound healing and anti-inflammatory effects for centuries. Greek physicians described the traditional use of *P. major* in wound healing and for reducing inflammation already during the first century. There are many compounds in the leaves that may contribute to the anti-inflammatory effects of plant extracts. In *Plantago* species, phenolic compounds have thus been reported to have anti-inflammatory activity.[1,2]

Numerous methods have been used to study the anti-inflammatory activity of plant extracts. Nuclear factor kappa-B (NF-kB) assay is a commonly used *in-vitro* assay to reveal anti-inflammatory activity of both crude extracts and pure compounds.[3,4] The NF-kB assay is based on recognition of microbial products by Toll-like receptors (TLRs), that have been shown to be present in e.g. oral epithelial cells.[5] These receptors activate the NF-kB intracellular pathway that subsequently triggers the inflammatory and immune response processes. This assay investigates the translocation of NF-kB from cytosol to nucleus as a result of inflammation. The difference between the presence of NF-kB in nucleus and cytoplasm describes the degree of inflammation. The higher the nuclear and cytoplasmic differences, the higher is the activation of the NF-kB intracellular pathway, and the higher is also the inflammation. Studies on animal models have confirmed a correlation between NF-kB activation and inflammatory disease,[6,7] and the assay is thus highly relevant for study of anti-inflammatory potential.

The aim of the present study was to evaluate the anti-

* Corresponding author. Department of Bioinformatics and Biotechnology, Government College University (GCU), Faisalabad, Pakistan.
E-mail addresses: muhammad.zubair1751@gmail.com, zoobi_pbg@yahoo.com (M. Zubair).
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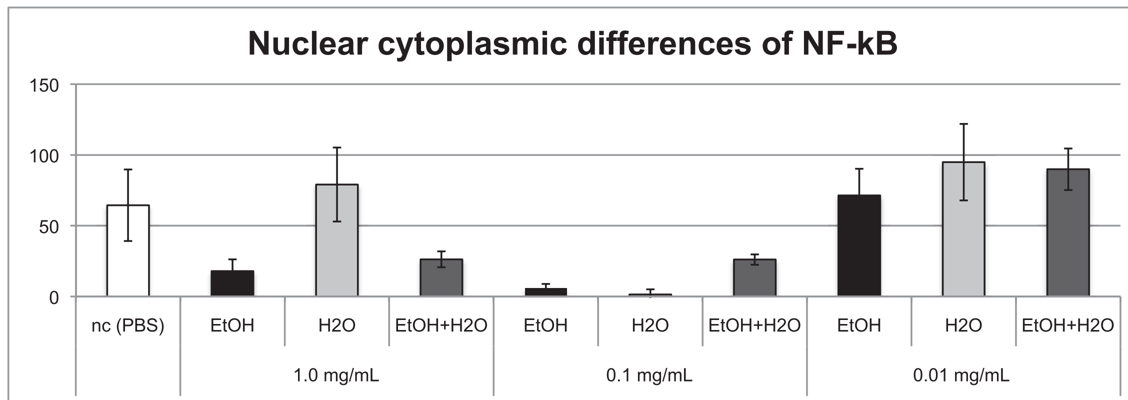

Figure 1. Immunocytochemical analysis (nuclear cytoplasmic differences) of NF-kB activity in OEC H400 following 47 hours treatment with different *Plantago major* extracts of dried leaves (water and ethanol-based extracts and a combination of equal volumes of the two extracts) each in three different concentrations (1.0, 0.1 and 0.01 mg/mL) and 1 hour of exposure to *E. coli* LPS 0.02 μg/mL. PBS was used as a negative control (nc).

inflammatory activity of different extracts of *P. major* leaves on oral epithelial cells *in-vitro*.

2. Results and discussion

We found that the water- and the ethanol-based extracts as well as the combination of the two extracts, had a significant anti-inflammatory activity as revealed by the NF-kB assay (Fig. 1). NFκB is normally present in the cytoplasm, and translocation from the cytoplasm to the nucleus is a definitive measure of NFκB activation. In this assay, translocation of the activated transcription factor from the cytoplasm to the nucleus is automatically detected and quantified for each cell. The ratio of intensity between the cytoplasm and nucleus is shown in Fig. 1. This confirms that this is a translocation event as it indicates a drop in cytoplasmic intensity along with an increase in nuclear intensity.

The concentration of 0.1 mg/mL (with a content of 1.24, 0.19 and 0.71 μg plantamajoside and a content of total phenols of 1.67, 0.22 and 0.94 μg GAE per mL for ethanol-based, water-based and the combination of both extracts, respectively) had the highest anti-inflammatory activity on the oral epithelial cells (OEC H400). The anti-inflammatory response was reduced or absent both at the lower and higher concentrations tested.

The fact that the content of plantamajoside and total phenols in the ethanol-based extract at a concentration of 0.01 mg/mL (0.12 μg plantamajoside and a content of total phenols of 0.17 μg GAE per mL) is comparable to the contents of plantamajoside and total phenols in the 0.1 mg/mL water-based extracts but there is significant difference between the in-vitro anti-inflammatory response of both extracts show that not only plantamajoside and total phenols but also other water-soluble compounds (possibly polysaccharides) are responsible for the anti-inflammatory activities and ultimately wound healing activities of *P. major.* However at a concentration of 1.0 mg/mL both the ethanol-based and the combination of the water- and ethanol-based extracts showed anti-inflammatory activity.

Our results revealing anti-inflammatory activity of *P. major* leaf extracts are in accordance with[8] albeit with another kind of cells and another type of anti-inflammatory reaction. They have verified anti-inflammatory activities of methanol extracts of *P. major* and *P. lanceolata* leaves by the COX-1 (cyclooxygenase, expressed constitutively) and 12-LOX (lipooxygenase), enzyme assay and human platelets.

We have in a previous study found that plantamajoside is the major polyphenol in ethanol extracts of *P. major* leaves.[9,10] It is therefore possible that this compound is contributing to the anti-inflammatory effect noticed in the present study. Anti-inflammatory activity can be one of the ways in which extracts of *P. major* contribute to wound healing as proposed by.[11] Extracts of *P. major* leaves have previously shown effects on the formation of granulation tissue by stimulating the cell proliferation and migration process.[12]

The results of the present study, in combination with results from our previous studies, corroborate the wound healing effects of *P. major* as expected from its traditional use.

3. Experimental

The experimental section is available in supplementary material.

4. Conclusion

We report for the first time that extracts of *P. major* leaves can protect against inflammatory mediators on human oral epithelial cell lines H400 *in-vitro*. Both ethanol-based and water extracts of leaves protected against inflammatory mediators. This makes *P. major* an interesting source of bioactive substances with anti-inflammatory activity. The authors are aware of that additional cell-lines should be tested to corroborate the conclusions. The results justify further studies to elucidate the role of specific compounds responsible for this anti-inflammatory activity. A careful optimization of the concentration of water and ethanol-based extracts and the combination of both these extracts, is also needed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jtcme.2017.09.002.

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