

Effect of *Aristolelia chilensis* (maqui) on inflammation response

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SUMMARY

In this study the effects of *Aristolelia chilensis* (maqui) on COX-2 expression, NF- κ B activation, PI3K/Akt and ERK1/2 phosphorylation, and cellular viability in the human cell line of colon cancer Caco-2 are shown. On the other hand, an *in vivo* study about the effect of *Aristolelia chilensis* in carrageenan-induced rat paw inflammation is shown.

RESULTS

COX-2 expression is strongly up-regulated in inflammation and colon cancer. In this sense, we analyzed the COX-2 expression in Caco-2 cells; the cells were incubated with *Aristolelia chilensis* (5 μ g/ml) for different times (0, 24, 48 and 72 hr), and the Cox-2 expression was analyzed by western blotting. *Aristolelia chilensis* reduced the basal level of COX-2 expression at 24 hr of treatment (Fig 1).

The NF- κ B pathway was studied through I κ B α analyze and p65 NF- κ B localization by immunocytochemistry. The cells were treated with *Aristolelia chilensis* (5 μ g/ml) between 0 – 120 min, then probed with antibodies against I κ B α and p65 NF- κ B, and analyzed by fluorescence microscopy. The cells showed a cytoplasm positive stain of I κ B α at time 0, however an increase in intensity of signal was observed at 30 and 60 min of treatment (Fig 2). The results of p65 NF- κ B did not show differences between the different times of treatment with *Aristolelia chilensis*. On the other hand, HL-60 cells were transfected with a reporter vector NF- κ B-luciferase and the effect of *A. chilensis* was evaluated. *A. chilensis* (50 μ g/ml) significantly reduced the NF- κ B activity.

COX-2 and NF- κ B pathway activation are controlled by signalling pathways such as PI3K/Akt and ERK1/2 MAPK.

The ERK1/2 and PI3K/Akt pathways was studied at 0 and 24 hr of *A. chilensis* treatment by western blot (Fig. 3). An increase of ERK1/2 phosphorylation at 2 and 4 hr was detected. The PKB phosphorylation increased at 30 min of *A. chilensis* treatment was observed. The cellular viability measured by MTT and FACS showed that *A. chilensis*, did not induce cytotoxicity at the concentrations used in whole experiments.

Because, *A. chilensis* reduced the COX-2 expression we tested the effect in the carrageenan-induced rat paw inflammation (Fig. 4). It was observed that 100 mg/kg of *A. chilensis* i.p., significantly reduced the inflammation measured as area under curve between 0-6 hrs, effect was similar to 2 mg/kg of diclofenac an unspecific COX inhibitor. Moreover only at 100 mg/kg of *A. chilensis* a reduction of COX2 in the rat paw, measure by immunohistochemistry was observed.

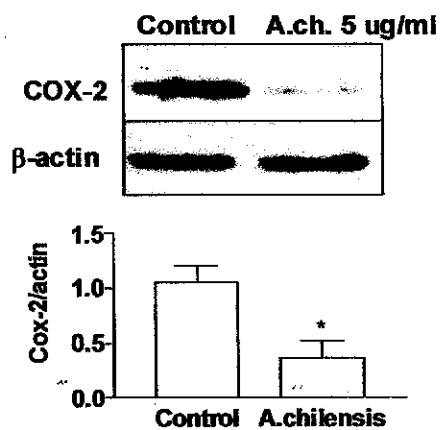
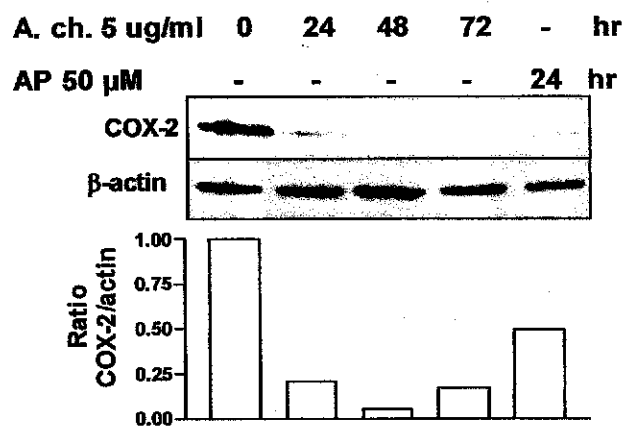


Figure 1. Effect of *A. chilensis* on COX-2 expression in Caco-2 cells. *A. chilensis* (5 ug/ml) reduced the COX-2 expression at 24 hrs of incubation.

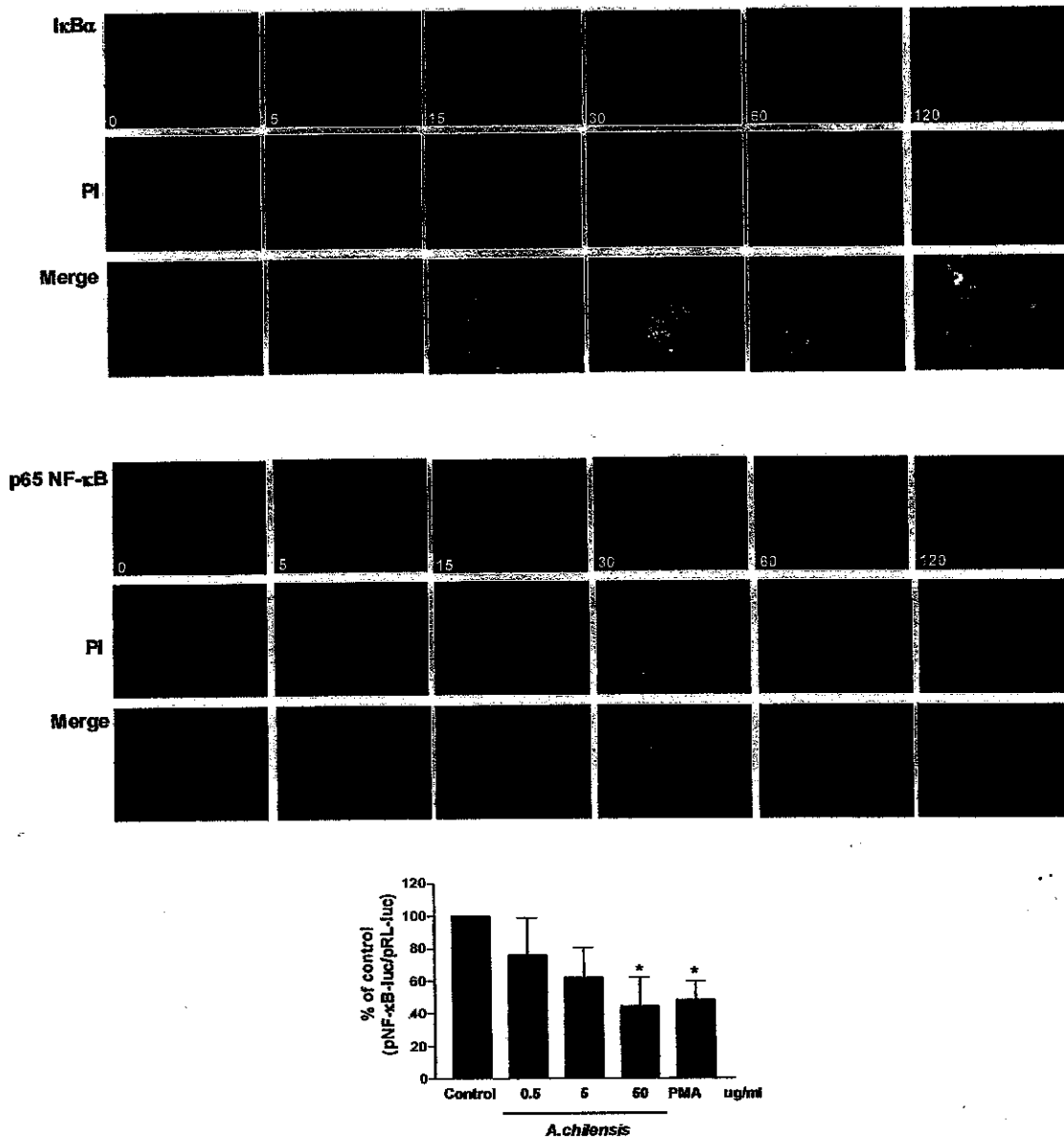


Figure 2. **Effect of *A. chilensis* on NF-κB activation in Caco-2 cells.** *A. chilensis* (5 ug/ml) increased the stain of IkBα between 30-60 min of treatment (arrow), but did not changes in the p65 NF-κB levels were observed. PI: propidium iodide (nuclear staining), merge: overlay of IkBα or p65 NF-κB with PI.

In the bottom graph, HL-60 cells were transfected with a reporter vector NF-κB-luciferase and the effect of *A. chilensis* was evaluated. *A. chilensis* (50 ug/ml) reduced the NF-κB activity.

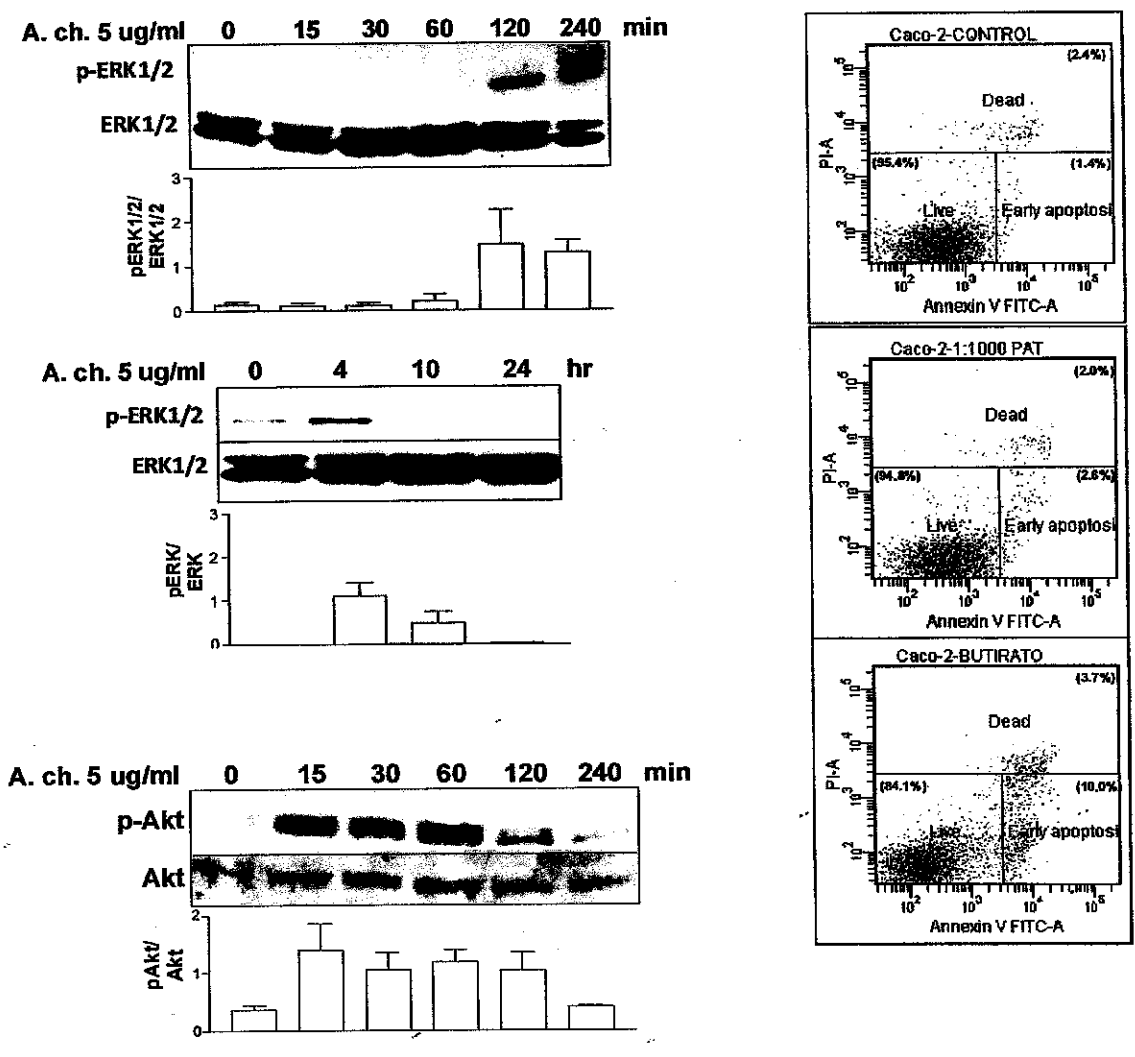


Figure 3. Effect of *A. chilensis* on ERK1/2 and Akt phosphorylation in Caco-2 cells. Western blot analysis of Caco-2 cells treated at different times with *A. chilensis* (5 $\mu\text{g/ml}$).

In the right panel the cellular viability and apoptosis in cells incubated for 24 hr with *A. chilensis* (5 $\mu\text{g/ml}$), stained with iodide propidium and Annexin-V, and measured using flow cytometry, as control of apoptosis butyric acid was used.

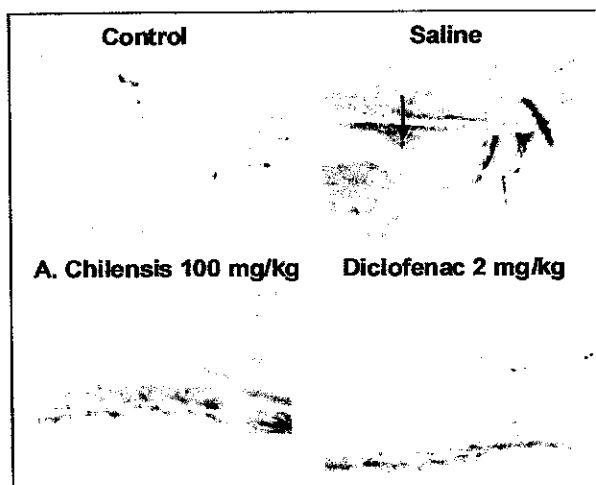
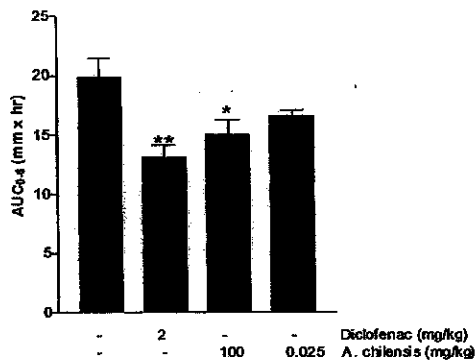
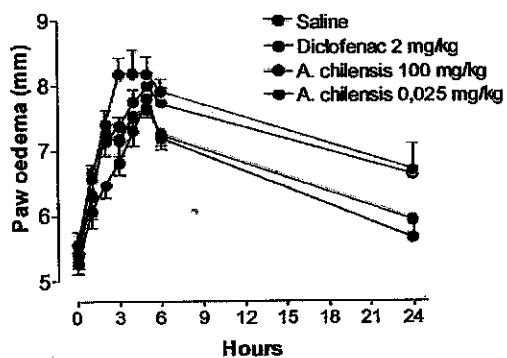


Figure 4. Effect of *A. chilensis* in the carrageenan-induced rat paw inflammation model. *A. chilensis* i.p. (100 mg/kg), significantly reduced the inflammation measured as area under curve between 0-6 hrs. In the bottom panel, immunohistochemistry of COX2 in tissue of rat paw. The COX-2 stain (brown) is shown by arrow.

CONCLUSIONS AND PERSPECTIVES

- *A. chilensis* reduces the expression of COX-2 in vitro en Caco-2 cells
- The reduction of COX-2 expression could be explained by an interference in the NF- κ B pathway
- The potential anti-inflammatory effect of *A. chilensis* was demonstrated using the carrageenan-induced rat paw inflammation model. In the inflammatory paw a reduction of COX-2 expression was also observed
- The evidence in this work strongly suggests that *A. chilensis* could be a potent nutraceutical in the control of chronic inflammatory process.

ACKNOWLEDGEMENTS

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