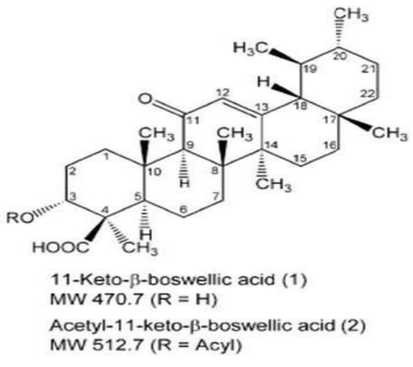
CALU-3 CELLS AS *IN VITRO* MODEL OF BRONCHIAL EPITHELIUM FOR PHARMACOLOGICAL STUDIES OF NATURAL COMPOUNDS

A. Meneghel[1], D. Catanzaro[1], V. Cocetta[1], S. Dall'Acqua[1], G. Pasut[1], E. Ragazzi[1], M. Montopoli[1]
[1] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Largo E. Meneghetti 2, 35131 Padova, Italy.

INTRODUCTION

Inflammation is a normal component of host defence, but elevated unresolved chronic inflammation can leads to a wide range of chronic conditions. Therefore, focus on the resolution of the inflammatory situation is crucial for the remission of certain diseases. Currently there is considerable interest for the potential antioxidant role that some plant extracts may have. The identification of new natural compounds capable of modulating, at least in part, dysfunctions caused by inflammation, assumes a significant importance due mainly to their low toxicity. A natural compound being studied is *Boswellia Serrata*. The extract of *Boswellia serrata* gum-resin (Salai Guggal, Indian olibanum) has been traditionally used in Ayurvedic medicine for the treatment of inflammatory diseases such as arthritis or asthma [Gupta I *et al.*, 1998]. The early pharmacological studies of Singh and Atal [Singh GB & Atal CK, 1986] have shown an anti-inflammatory activity of this extract. Its action leads to 5-lipoxygenase selective inhibition, in concentration-dependent manner, blocking the production of characteristic inflammatory mediators, resulting in decreased inflammatory response, typical of immunological and allergic reactions, which would normally cause bronchoconstriction, chemotaxis, and increased vascular permeability [Ammon *et al.*, 1991; Altmann A. *et al.*, *2004*]. The boswellic acids with their anti-inflammatory activity do not interfere with the synthesis of prostaglandins and therefore do not cause peptic ulcer, gastrointestinal bleeding and hepatic disease. We previously demonstrated an anti-inflammatory and antioxidant capacity of natural compounds derived from *Boswellia serrata* in an *in vitro* model of intestinal epithelium. In this study, we evaluated if this activity was maintained in one other epithelium model, the bronchial epithelium, that it's extremely important under the profile of inflammation and immune response. Calu-3 is an epithelial cell line derived from human lung adenocarcinoma. It represents a suitable *in vitro* model of bronchial epithelium because it presents an intrinsic ability to differentiate leading to the formation of occluding junctions. According to the literature, we used experimental approaches to verify the effects of inflammatory stimuli like H_2O_2. We tested *Boswellia serrata,* in the form of extract of gum-resin (BS-EGR) (0.1 µg/ml-10 µg/ml) and as acetyl-11-keto-β-boswellic acid (AKBA) (0.027 µg/ml equal to 2.7% of 1 µg/ml of BS-EGR), *to* evaluate the effect on inflammation. The results obtained have shown that *Boswellia serrata* is able to modulate the induced stress damage in bronchial epithelium.



Boswella's resin contains both pentacyclic and tetracyclic triterpenes and among the first there are some boswellic acids, such as acetyl-11-keto-β-boswellic acid (AKBA), that are responsible for most of the pharmacological effects.

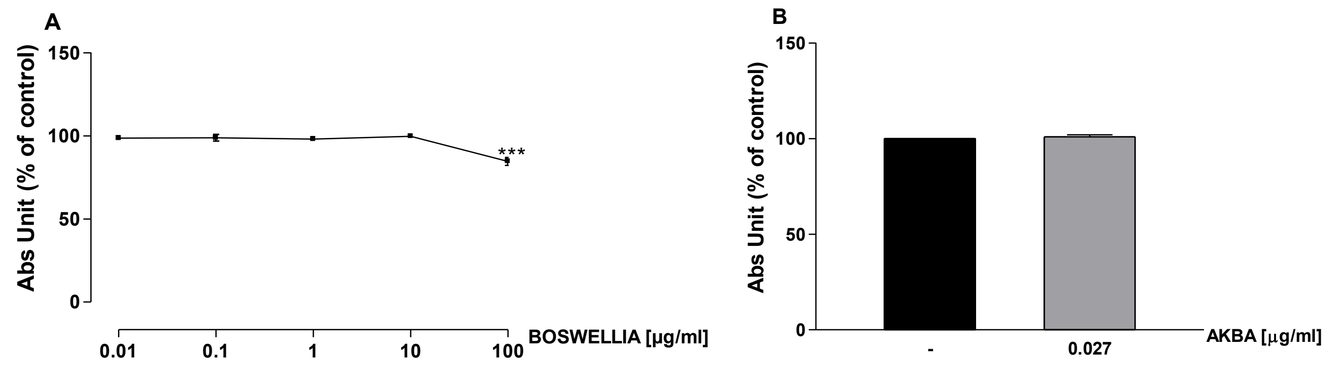


Fig. 1: Effect on cell viability of Boswellia serrata (**A**) (0,01 µg/ml - 100 µg/ml) and AKBA (**B**) (0.027 µg/ml) in Calu-3 cells after 24h of treatment, evaluated by MTT assay. Values are expressed as mean ± SEM percentage of Abs compared to control.
*** P <0.001 treated vs control.

MATERIALS AND METHODS

Cell lines and reagents: epithelial cell line derived from human lung adenocarcinoma grown in E-MEM supplemented with 10% fetal bovine serum (FBS), 1% glutamine and 1% 100 U/ml penicillin and 100 µg/ml streptomycin , in humidified condition at 5% CO2 and 37° C. All reagents used were from Cambrex-Lonza (New York, USA).

Viability assay: Cell viability, following a treatment, can be estimated by using a yellow soluble tetrazolium salt [3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide] (MTT, Sigma-Aldrich) that is reduced to formazan salt, an insoluble crystal in purple color water, by the mitochondrial respiratory enzyme succinate-tetrazolium reductase. The MTT is internalized by the cells via endocytosis, while the formazan crystals are transported to the cell surface by exocytosis, and that such transport mechanisms occur only in living cells, therefore this test is considered a useful indicator of cell viability. The intensity of the purple color is therefore directly proportional to the concentration of formazan.

Ros: Ros level are quantified using the 2′,7′-Dichlorofluorescin diacetate probe (H2DCF-DA, Sigma-Aldrich); this fluorophore penetrates easily through the cell membrane into the cell and is hydrolyzed to H2DCF by cytoplasmic esterase. H2DCF is not fluorescent, but in the cell can be oxidized by ROS DCF, fluorescent compound which, when excited at 488 nm, emits in green (525 nm) and this fluorescence is measured by VICTOR ™ X3 PerkinElmer.

Statistical analysis: Before any statistical comparison, data were checked if normally distributed, and thereafter ANOVA and Student's t test for unpaired data were used to verify the statistical significance of the results.

RESULTS

Cell viability: The cytotoxic activity was evaluated by MTT assay.
Boswellia serrata (0.01-100 µg/ml) and AKBA (0.027 µg/ml of 2.7% to 1 µg/ml of *Boswellia serrata*) (Fig. 1), don't show cytotoxic activity in Calu-3 cell line in the concentrations used; it's only showed a reduction of 15% of cell viability with *Boswellia serrata* to the concentration 100 µg/ml (Fig. 1A).
ROS: Fig. 2-3 present the total ROS production under baseline condition and after oxidative stress and treated with *Boswellia serrata* (0.01-10 µg/ml) or AKBA (0.027 µg/ml).
The concentration 1 µg/ml of *Boswellia serrata* under baseline condition decreases the production of ROS (Fig. 2A). After induction of oxidative stress (Fig. 2B) is interesting to observe that *Boswellia serrata* is capable of decreasing significantly the generation of ROS to different concentrations 0.01-0.1-1 µg/ml, particularly of 15% to 0.1 µg/ml and about 35% to 1 µg/ml; concentration 10 µg/ml detect an increased production of radical species. As reported in Fig. 3 also with AKBA (0.027 mg/ml) occurs a reduction of ROS production, although not statistically significant. After induction of stress (Fig. 3B), it shows significant reduction of the radical species generation of about 15%.

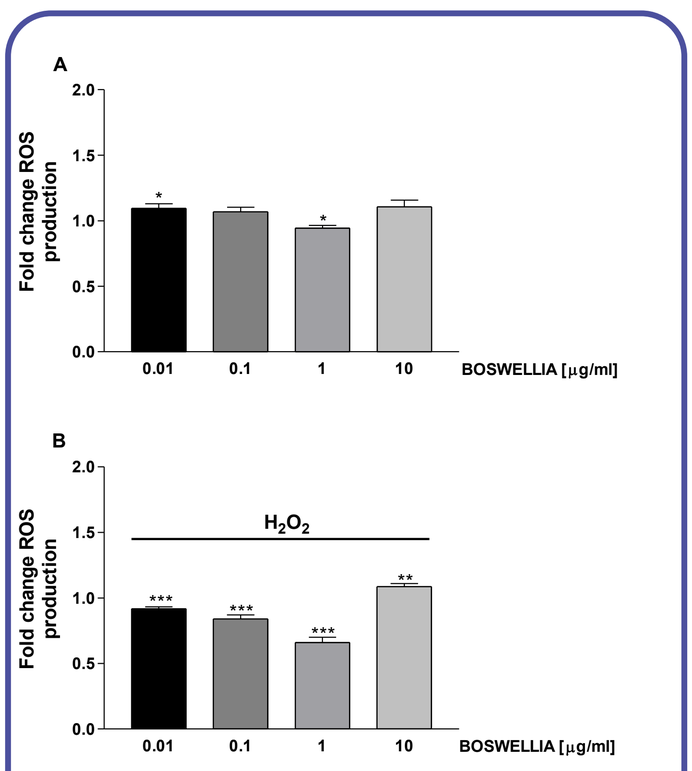


Fig. 2: Effect of *Boswellia serrata* (0.1 µg/ml-10 µg/ml) on ROS production in Calu-3 cells after 24h, in absence of H2O2 (**A**) and after its addition (**B**), through the use of H2DCFA-DA 50µM probe. Values are expressed as ratio of treated compared to control ± SEM. *** P <0.001, ** p <0.01, * p <0.05.

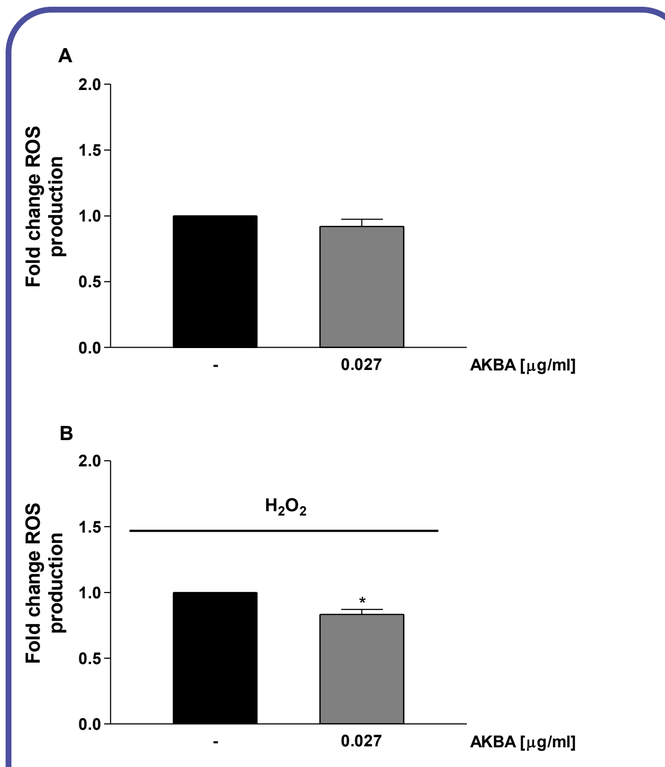


Fig. 3: Effect of AKBA (0.027 µg/ml) on ROS production in Calu-3 cells after 24h, in absence of H2O2 (**A**) and after its addition (**B**), through the use of H2DCFA-DA 50µM probe.
Values are expressed as ratio of treated compared to control ± SEM. * P <0.05.

CONCLUSIONS

The extract of *Boswellia serrata*, among its active compounds, contains antioxidants with anti-inflammatory action. Despite the anti-inflammatory activity of *Boswellia serrata* needs to be further deepened, the data obtained in this study seem to be very promising. Indeed there's a proven antioxidant effect in Calu-3 cells (bronchial epithelium model) comparable to the one previously identified in Caco-2 cells (model of intestinal epithelium). The results obtained so far have shown that *Boswellia serrata* is able to modulate the ROS production level in bronchial epithelium; this presupposes we need more study aimed to investigate the molecular mechanism behind this anti-inflammatory compound.

REFERENCES

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