# A technique to screen plant extracts for anti-inflammatory activity on ovine neutrophils

Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

# A technique to screen plant extracts for anti-inflammatory activity on ovine neutrophils

M. Farinacci, M. Colitti, S. Sgorlon & B. Stefanon

**To cite this article:** M. Farinacci, M. Colitti, S. Sgorlon & B. Stefanon (2007) A technique to screen plant extracts for anti-inflammatory activity on ovine neutrophils, Italian Journal of Animal Science, 6:sup1, 548-550

**To link to this article:** https://doi.org/10.4081/ijas.2007.1s.548

# A technique to screen plant extracts for anti-inflammatory activity on ovine neutrophils

M. Farinacci, M. Colitti, S. Sgorlon, B. Stefanon

Dipartimento di Scienze Animali. Università di Udine, Italy

*Corresponding author:* Bruno Stefanon. Dipartimento di Scienze Animali. Facoltà di Medicina veterinaria, Università di Udine. Via delle Scienze 208, 33100 Udine, Italy - Tel. +39 0432 558581 - Fax: +39 0432 558585 - Email: bruno.stefanon@uniud.it

**ABSTRACT:** Neutrophils play an essential role in host defense and inflammation. In recent years, several studies in men and dairy animals have shown that administration of natural compounds can influence the immune system. In the present study the extracts of six patented protected plants, named C1, C2, C3, C4, C5, C6, have been screened for their effect on ovine neutrophils. Three, clinically healthy, non lactating, non pregnant sheep were used. Freshly isolated neutrophils were incubated with extracts at increasing doses and tested for viability, adhesion and superoxide production induced by PMA. The residues of C1, C2, C3 strongly inhibited adhesion and superoxide production, demonstrating anti-inflammatory activity. Further *in vivo* experiments are required to validate their use for productive animals.

**Key words:** Neutrophils, Natural extracts, Adhesion, Superoxide production.

**INTRODUCTION** – The historic use of herbal remedies to treat and prevent infectious diseases has been supplanted with the emergence of specific synthetic medicines and antimicrobial agents. However, selected herbs, known to possess natural antioxidant, immunomodulatory activity and other characteristics, can profitably be used in animal feed additives. This is also in line with recent EU directives and the concern for the use of chemical drugs in animal production. Adverse environmental conditions increase the occurrence of infectious diseases in animals. In ruminant intensive husbandry, hot or cold environments, early weaning, crowding and transport, induce a metabolic stress that causes immunosuppression and increase the risk of infectious diseases (Matteri *et al*., 2001). It has been seen that neutrophils adhesion, migration and phagocytosis-induced respiratory burst activities become depressed in parturient cows. Calving-associated immunodepression has been considered one of the main causes related to the appearance of different pathologies and affections in dairy cows, such as retained placenta or environmental mastitis (Drackley, 1999). On the other hand, under pathological conditions, uncontrolled activation of neutrophils can contribute to propagation and maintenance of acute and chronic inflammation by several mechanisms.

Considering the essential role of neutrophils in both host defence and inflammation, we investigated whether and to what extent six plant extracts could modulate *in vitro* neutrophil activity.

**MATERIAL AND METHODS** - All reagents were purchased from Sigma (Si.Louis, MO) and the extracts were supplied from Indena S.p.a. (Settala, Milano, Italy). The extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50mg/ml and stored at -20°C until use. Populations of ovine neutrophils were obtained from healthy, non pregnant and non lactating sheep, fed a hay and concentrate diet. Neutrophils were isolated from the buffy coats of sheep according to the procedure of Carlson and Kaneko (1973). After isolation, cells were incubated with increasing doses of extracts (6.7, 20, 60,180 µg/ml) for 60 minutes, at 37° C and 5% $CO_2$. Two kinds of control cells were used: cells incubated with Hanks' balanced salt solution (HBSS) medium alone and cells incubated with medium plus DMSO. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Pruett and Loftis, 1990). The adherence ability (ADH) of PMA-activated neutrophils was assayed by a colorimetric assay of acid phosphatase activity of adherent cells and superoxide production (SO) by measuring superoxide dismutase-inhibitable reduction of ferricytochrome C, as previously described

(Paltrinieri *et al*., 2002). The MTT, ADH, SO tests were performed on neutrophils isolated from 3 sheep in 5 replicates for each dose. The mean value of replicates were calculated and the individual data are expressed as a percentage of the absorbance of treated cells in comparison to DMSO control cells. For concentration-response studies the dependent variables (MTT, ADH, SO) were regressed against the natural logarithm of extract concentrations with Prism GraphPad 4.03 software (GraphPad Software Inc.). This program analyzed the data for slope, IC50 values, 95% confidence intervals, and regression significance.

**RESULTS AND CONCLUSIONS -** We used an *in vitro* assay system to evaluate whether the extracts could modulate PMA-induced neutrophil adhesion and superoxide production. PMA is a good activator of ovine neutrophils, (Paltrinieri *et al*., 2002) and involves the receptor-independent activation and translocation of the protein kinase C (PKC). Pretreatment of neutrophils with C1, C2, C3, C4, C5 showed a significant concentration-dependent inhibition of adhesion and superoxide production (Figures 1 and 2). By comparing the IC50 (Table 1), C1, C2 and C3 were the most effective extracts. However, all doses of these extracts reduced neutrophils viability to values less than 95%, as determined by MTT test. The MTT assay measures cell metabolic status based on the activity of mitochondrial dehydrogenases, therefore it does not distinguish between apoptosis and necrosis. We hypothesize that the effect of extracts on MTT reduction and functional status of neutrophils could be due to apoptosis induction. Infact, neutrophils apoptosis is accompanied by down regulation of cellular functions, such as chemokinesis, chemotaxis, phagocytosis, oxidative burst and degranulation (Maianski *et al*., 2004).
C4 and C5 also inhibited adhesion and superoxide production induced by PMA in a dose-dependent manner, but without affecting cell viability. As PMA activate neutrophils bypassing the receptor, a PKC-dependent mechanism might be interfered by these extracts. C6 extract slightly influenced neutrophils viability in a dose-dependent way; at concentration 6.7 and 20 µg/ml, MTT reduction was 83.07% and 90.48% respectively, and at 60 and 180 µg/ml the MTT reduction resulted 97.06% and 104.07%. Superoxide production resulted significantly enhanced by C6; the maximal positive effect on PMA stimulation was of 130.19% at a concentration of 180 µg/ml.
In conclusion, the *in vitro* model indicated a potential use of extracts C1, C2, C3, C4, C5 as anti-inflammatory agents, and of C6 as an immunostimulant agent. Further studies are required to elucidate the molecular mechanism involved and *in vivo* trials will be used to validate these results.

Figure 1. Effect of extracts on PMA-induced neutrophils adhesion (A) and superoxide production (B). The original data were converted to percentage values relative to the DMSO controls. Values are mean and vertical lines sd. * and ** regression are significant at P<0.01 and 0.001 respectively.

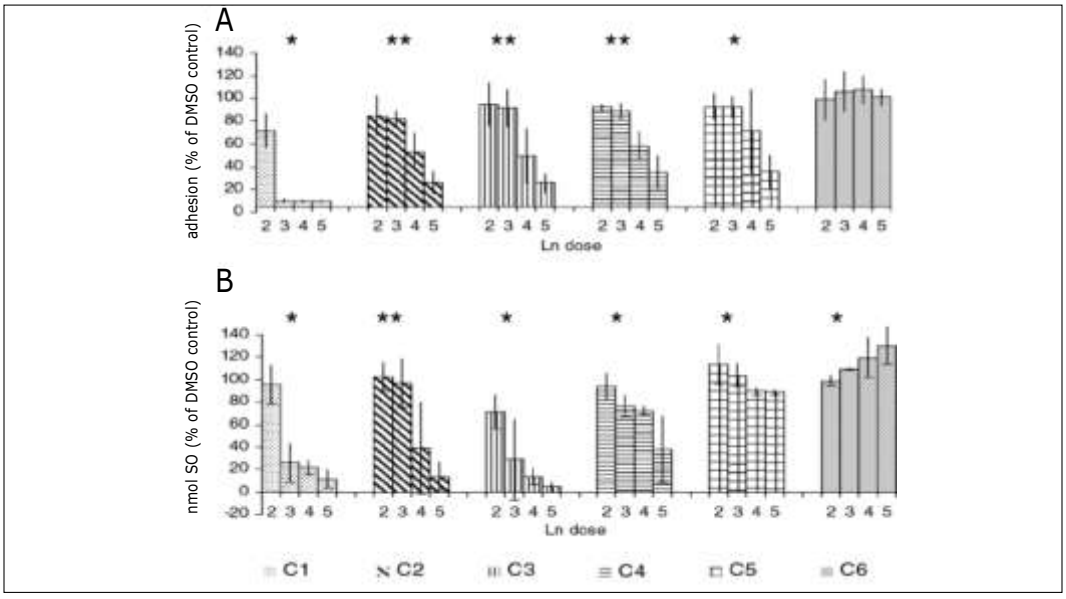

Table 1. IC50 (μg/ml) values and 95% confidence intervals (CI) of the six patented protected plants.

| Compound | ADH | | | SO | | |
|---|---|---|---|---|---|---|
| | IC50 | 95% CI | | IC50 | 95% CI | |
| | | min | max | | min | max |
| C1 | 7.92 | 0.38 | 28.93 | 21.35 | 5.94 | 57.74 |
| C2 | 62.12 | 27.06 | 177.15 | 49.50 | 20.25 | 148.12 |
| C3 | 66.89 | 26.66 | 229.06 | 12.85 | 2.18 | 35.98 |
| C4 | 92.02 | 46.57 | 227.92 | 107.13 | 36.71 | 878.31 |
| C5 | 126.98 | 33.78 | 2620.18 | 7793.15 | 743.23 | 168+E6 |
| C6 | ns* | ns | ns | nd** | nd | nd |

** not significant, ** not determinable.*

The research was supported by PRIN 2005-2007, U.O. Bruno Stefanon.

**REFERENCES** - **Carlson**, G.P., Kaneko, J.J., 1973. Isolation of leukocytes from bovine peripheral blood. Proc. Soc. Exp. Biol. Med., 142:853-856. **Drackley**, J. K., 1999. Biology of dairy cows during the transition period: The final frontier? J. Dairy Sci., 82:2259–2273. **Maianski**, N.A., Maianski A.N., Kuijpers, T.W., Roos, D., 2004. Apoptosis of neutrophils. Acta Haematol-Basel, 111:56-66. **Matteri**, R.L., Carroll, J.A., Dyer, C.J., 2001. Neuroendocrine response to stress. In "*The Biology of Animal Stress. Basic principles and implications for animal welfare*" (G.P. Moberg and J.A. Mench eds) chapter 3, pp. 43-76. CABI Publishing New York, USA. **Paltrinieri**, S., Panelli, S., Comazzi, S., Sartorelli, P., 2002. Effect of 1-24 ACTH administration on sheep blood granulocyte functions. Vet. Res., 33:71-82. **Pruett**, S.B., Loftis, A.Y., 1990. Characteristics of MTT as an indicator of viability and respiratory burst of human neutrophils. Int. Arch. Aller. a Imm., 92(2):189-92.