

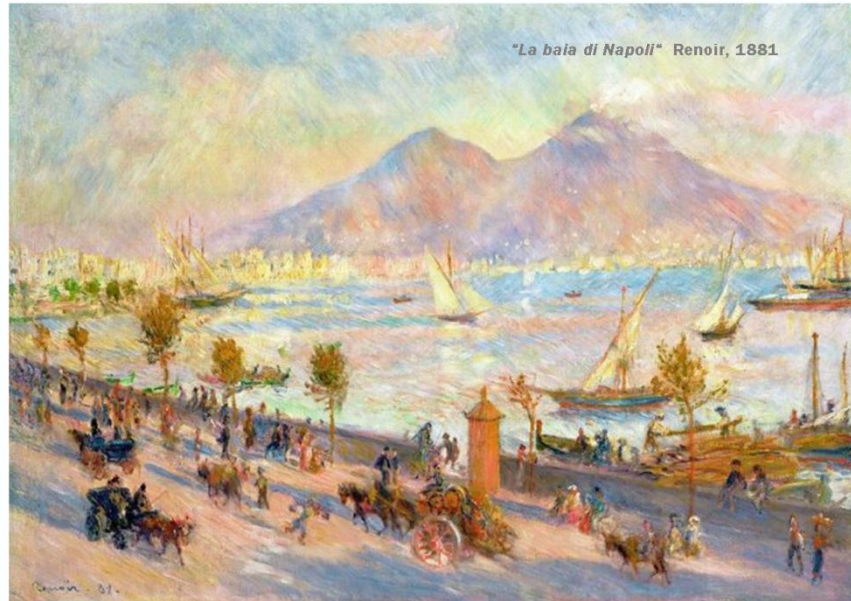
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EVALUATION OF ANTIBACTERIAL PROPERTIES OF NON TRANSFUSIONAL HEMO-COMPONENTS: AN IN VITRO STUDY IN VETERINARY MEDICINE

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The decline in the antibiotic pipeline and the continuous increase in resistance have become a serious global threat in human and veterinary medicine [1]. In recent years, more and more interest was addressed in discovering new natural antibacterial molecules. Activated non transfusional hemo-components (NTHC) have been demonstrated to promote tissue regeneration, to enhance the action of the natural physiological responses, and to inhibit bacterial growth [2]. The aims of the study were to evaluate: -the antibacterial activity of NTHC obtained from canine blood; -the role of leucocytes and platelets in antibacterial effect; -the properties of NTHC in relation to the different Gram's stain affinity to antibiotic susceptibility profile. Platelet lysate, fibrin glue, thrombin, pure- and leucocyte-platelet-poor plasma (P-PPP, L-PPP), and pure- and leucocyte-platelet-rich plasma (P-PRP, L-PRP) with and without calcium gluconate or thrombin, pure- and leucocyte-platelet gel (P-PG, L-PG) at different quantities (10, 20, 40, 180 μ l) were tested. Different strains of *Staphylococcus aureus*, *Staphylococcus cohnii*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, previously isolated from canine skin infections and classified as sensitive, multi-resistant and resistant to a whole panel of ten commercial antibiotics, were used. The assessment was carried out at 4, 18, 24 hours both by Kirby Bauer method and inhibition in broth into microtiter plate with a spectrophotometer reading (OD_{540nm}). In comparison to each bacterial control growth (OD_{540nm}), platelet lysate, fibrin glue, thrombin, PPP and PG, with and without leucocytes, showed greater inhibitory effect against Gram negative bacteria ($P < 0.05$). Antibacterial activity of P-PG and L-PG was documented already after 4 hours of incubation and confirmed after 18 and 24 hours (Kirby-Bauer). The maximum bacteriostatic effect was highlighted at 18 hours (microdilution in broth). The antibacterial action was directly proportional to the amount of PG, when tested against Gram negative (L-PG 180 μ l: mean OD=0.90 vs. control OD=1.60, $P=0.001$; P-PG 180 μ l: mean OD=0.70 vs. control OD=1.60, $P < 10^{-4}$), multi-resistant (L-PG 180 μ l $P=0.031$; P-PG 180 μ l $P=0.019$) and resistant to all panel strains (P-PG 180 μ l $P=0.021$). The similar bacteriostatic action of PPP and PRP ($P > 0.05$) demonstrated to be linked to plasma components rather than to platelets. The presence of leucocytes in NTHC did not result in a significant reduction of bacterial growth ($P > 0.05$). The study allowed demonstrating the antibacterial efficacy of NTHC against different microorganisms, in particular resistant strains. For the first time, the activity of platelet lysate was evaluated in veterinary medicine. The results contributed to the enrichment of knowledge on this field, in order to provide answers to relevant questions recently raised by different Authors in human medicine. Further studies are in progress to evaluate the mechanism of action underlying the antimicrobial activity of NTHC.

[1] Cloeckaert et al. Antimicrobial resistance in animals and the environment. *Veterinary Microbiology*, 194:1-4, 2016.

[2] Del Fabbro et al. Antimicrobial properties of platelets rich preparations. A systematic review of the current preclinical evidence. *Platelets*, Early on line:1-10, 2016.