

**A039 Inhibitory effects of leaf extracts of *Stachytarpheta jamaicensis* (Verbenaceae) on the respiratory burst of rat macrophages**E. Álvarez<sup>a</sup>, J. M. Leiro<sup>b</sup>, M. Rodríguez<sup>c</sup> and Francisco Orallo<sup>a</sup><sup>a</sup> Dpto. de Farmacología, <sup>b</sup> Dpto. de Microbiología y Parasitología. Facultad de Farmacia, Universidad de Santiago de Compostela, 15782, Santiago de Compostela, Spain. <sup>c</sup> Dpto. de Farmacología, Facultad de Medicina, Universidad de Panamá, Campus Universitario, Panamá.

The antioxidant effects of ethyl acetate (EAc) and *n*-hexane (HE) fraction obtained from an alcoholic extract of *Stachytarpheta jamaicensis* Vahl. (Verbenaceae) on the generation of reactive oxygen species (ROS) during the respiratory burst of rat peritoneal macrophages were investigated. The lyophilized ethanol extract of the leaves and stems was fractionated between water and chloroform. EAc was obtained from the aqueous phase and the HE from the methanol solution of the dried chloroform fraction. Nitrite concentration was assayed by the Griess reaction (1) in cultures of *in vivo* pre-stimulated macrophages in the presence of 10 U/ml of gamma interferon and 100 ng/ml of lipopolysaccharide. The production of ROS by macrophages stimulated with 10 µg/ml of phorbol 12-myristate 13-acetate (PMA) was measured by fluorescent probes (2). Superoxide anions (O<sub>2</sub><sup>-</sup>) were generated in a hypoxanthine/xanthine oxidase (HX/XO) system and quantified spectrophotometrically (3). In the same system, the effect on XO activity was studied measuring the uric acid formation. Only EAc (0.4 - 40 µg/ml) inhibited, like L-(+)-ascorbic (100 µM), the extracellular release of ROS by resident peritoneal macrophages stimulated with PMA. In addition, EAc, like superoxide dismutase (1 U/ml), showed a potent O<sub>2</sub><sup>-</sup> scavenging activity in the HX/XO system. At concentrations of about 40 µg/ml, EAc inhibited XO activity and the production of nitric oxide in macrophages, like allopurinol (10 µM) and N-methyl-L-arginine acetate (250 µM), respectively. These results suggest that the EAc extract of *S. jamaicensis* and not HE fraction, contains some drugs that may have a potential pharmaceutical value in the treatment of immunopathological diseases where an over production of ROS is implicated, as inflammation, atherosclerosis or even carcinogenesis and neurodegenerative disorders.

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**References:** 1. Green, LC. et al. (1982) Anal. Biochem. 126: 131-138. 2. Leiro, J. et al. (2001) Exp. Parasitol. 98: 1-9. 3. Huguet, Al. et al. (1990) Z. Naturforsch. 45c: 19-24.

**A040 Effect of (-)-epigallocatechin-3-gallate on respiratory burst of rat macrophages**E. Álvarez<sup>a</sup>, J. Leiro<sup>b</sup> and F. Orallo<sup>a</sup><sup>a</sup> Departamento de Farmacología, <sup>b</sup> Departamento de Microbiología y Parasitología, Laboratorio de Parasitología. Facultad de Farmacia, Universidad de Santiago de Compostela, E-15782, Santiago de Compostela, Spain.

The toxic effects derived from over production of oxygen radicals (ROS) by immune cells can be partially abolished by the antioxidant activities of plant polyphenols. In the present study we investigated the potential effects of (-)-epigallocatechin-3-gallate (EGCG) on the respiratory-burst responses of rat peritoneal macrophages. *In vivo* pre-stimulated macrophages were incubated in the presence of 10 U/ml of gamma interferon and 100 ng/ml of bacterial lipopolysaccharide, and nitrite concentration in the cultures was evaluated by the Griess reaction (1). The production of ROS by macrophages was investigated in cells stimulated with 10 µg/ml of phorbol 12-myristate 13-acetate (PMA) using fluorescent probes (2). Superoxide anions (O<sub>2</sub><sup>-</sup>) were generated in a phenazine methosulphate (PMS)-NADH system and quantified by the spectrophotometric measurement of the product of the reduction of nitro blue tetrazolium (NBT) (3). EGCG (50-200 µM) blocked the production of nitric oxide (NO) by macrophages, being the concentration of 200 µM as active as N-methyl-L-arginine 1 mM. EGCG (1-100 µM), like L-(+)-ascorbic acid (100 µM, positive control), also inhibited the extracellular liberation of ROS by resident peritoneal macrophages stimulated with PMA. At low concentrations (1-5 µM), EGCG increased the reduction of NBT by the O<sub>2</sub><sup>-</sup> generated in the PMS/NADH system acting as a pro-oxidant agent, while at concentrations of about 10 µM EGCG exhibited, like superoxide dismutase (10 U/ml), a potent O<sub>2</sub><sup>-</sup> scavenger activity. These results show that EGCG is capable of modulating ROS production during respiratory burst of rat peritoneal macrophages by O<sub>2</sub><sup>-</sup> scavenging. Therefore, these antioxidant properties of EGCG may be useful in the prevention of both, the tissue injury caused by inflammatory process like atherosclerosis, asthma or arthritis, and some human diseases as Alzheimer and cancer.

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