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# Flavonoids as protective agents against oxidative stress induced by gentamicin in systemic circulation. Potent protective activity and microbial synergism of luteolin

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ABSTRACT

The flavonoids effect on gentamicin (GEN)-induced oxidative stress (OS) in systemic circulation was evaluated in terms of reactive oxygen species (ROS) production, enzymatic antioxidant defenses superoxide dismutase (SOD) and catalase (CAT), and lipid peroxidation (LP) *in vitro* on human leukocytes and *in vivo* on rat whole blood. The inhibitory activity of ROS was ATS < QTS < isovitexin < vitexin < luteolin. Luteolin, the most active, showed more inhibition in ROS production than vitamin C (reference inhibitor) in mononuclear cells and a slightly lower protective behavior compared to this inhibitor in polymorphonuclear cells. In both cellular systems, luteolin tends to level SOD and CAT activities modified by GEN, reaching basal values and preventing LP. In Wistar rats, GEN plus luteolin can suppress ROS generation, collaborate with SOD and CAT and diminish LP produced by GEN at therapeutic doses. Finally, luteolin and antibiotic association was evaluated on the antimicrobial activity in *S. aureus* and *E. coli* showing a synergism between GEN and luteolin on *S. aureus* ATCC and an additive effect on *E. coli* ATCC. Therefore, simultaneous administration of luteolin and GEN could represent a potential therapeutic option capable of protecting the host against OS induced by GEN in the systemic circulation while enhancing the antibacterial activity of GEN.

## 1. Introduction

Gentamicin (GEN) is an aminoglycoside antibiotic used in clinical practice for the treatment of Gram-negative bacterial infections that has now regained popularity due to widespread resistance of these bacteria to other antibiotic classes (Adil et al., 2016; Denamur et al., 2011). However, its clinical use has been restricted due to important adverse effects, which would be related to oxidative stress induction (Adil et al., 2016; Noorani et al., 2011; Sweetman, 2009; Veljković et al., 2016).

In previous studies, it was demonstrated that GEN is able to produce leukotoxicity, in systemic circulation, related to an excessive production of ROS, alterations in antioxidant defense mechanisms, and an increased lipid peroxidation in human leukocytes and whole blood from rats treated with therapeutic doses of this antibiotic (Bustos et al., 2016). Leukocytes play an important role in host defense against infectious agents by producing ROS, but an exacerbated ROS production can damage the host organism and the cell that produces them, being necessary the oxidant/antioxidant balance maintenance in these cells (Mytar et al., 1999; Paiva and Bozza, 2014).

In recent years, attention has been focused on the ability of natural antioxidants to protect against the toxic effects associated with increased oxidative stress, caused by GEN (Moreira et al., 2014; Noorani et al., 2011; Veljković et al., 2016). Within the natural antioxidants, one of the chemical groups of great interest are the flavonoids, polyphenolic

*Abbreviations:* ATS, quercetin- 3-acetyl-7, 3′,4′-trisulphate; CAT, catalase; DMSO, dimethyl sulfoxide; GEN, gentamicin; HBSS, Hank's balanced salt solution; $H_2$-DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; IV, isovitexin; LT, luteolin; MDA, malondialdehyde; MIC, minimal inhibitory concentration; MN, mononuclear leukocytes; NBT, nitroblue tetrazolium; PMN, polymorphonuclear leukocytes; QTS, quercetin-3,7,3′,4′-tetrasulphate; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; V, vitexin

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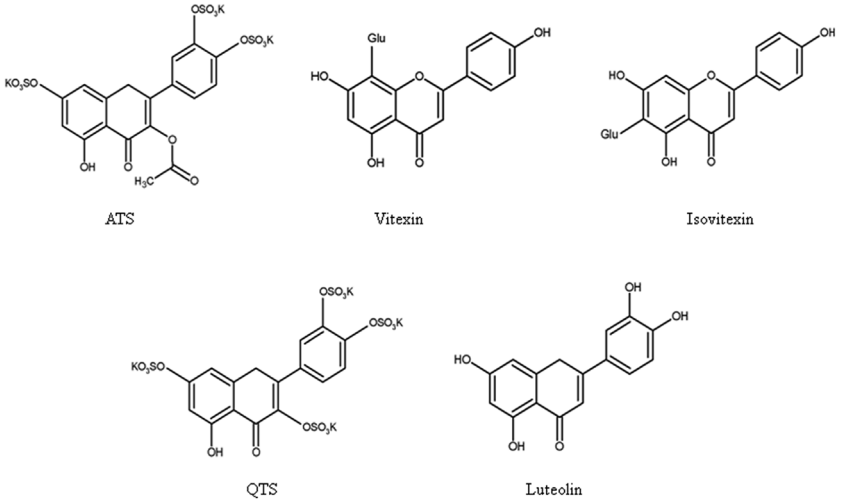


**Fig. 1.** Chemical structure of flavonoids isolated.

compounds found in the plant kingdom, due to its antioxidant power, i.e. its ability to reduce free radicals and chelate metals blocking their catalytic power (Mira et al., 2002; Rice-Evans et al., 1996). Recently, it was evaluated the effect of quercetin (Q), a flavonoid with strong antioxidant capacity obtained from *Flaveria bidentis*, demonstrating an important Q-protective effect on GEN-induced oxidative stress in human leukocytes *in vitro*, and in rat whole blood and plasma *in vivo* without important modification of GEN antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* strains (Bustos et al., 2016). However, due to the need to achieve therapeutic agents with potential in clinical application, it is necessary to search natural products that, in addition to avoiding GEN-induced toxic effects in systemic circulation, can enhance the antimicrobial activity of this antibiotic.

Different species were incorporated in this study in order to obtain several flavonoids: *Prosopis strombulifera* (Lam.) Benth var. *strombulifera* a rhizomatous shrub popularly known as “retortuño” or “mastuerzo”, *Prosopis nigra* (Gris.) Hieron. var. *nigra* a spiny tree known by the common names of “algarrobo negro” or “algarrobo dulce”, both South American species grow at Central and Northern Argentina (Barboza et al., 2006), and *Flaveria bidentis* (L.) Kuntze, known as “fique” or “contrayerba”, a native species distributed in America that grows in Córdoba, Argentina (Guglielmone et al., 2002; Ortega et al., 2010). From fruits of *P. strombulifera* the flavonoid luteolin (LT) has been isolated, which has a strong, anti-inflammatory, antioxidant, antifungal and antibacterial activity (An et al., 2016; López-Lázaro, 2009). From leaves of *P. nigra,* two flavonoids isomers, vitexin (V) and isovitexin (IV), were obtained, both with wide range of pharmacological effects including antioxidant activity, anticancer, antiviral, anti-inflammatory, among others (Xiao et al., 2016). Finally, from leaves of *F. bidentis* two quercetin sulfated derivatives have been isolated: quercetin-3-acetyl-7,3′,4′-trisulphate (ATS) and quercetin-3,7,3′,4′-tetrasulphate (QTS), which although they have anticoagulant and antiplatelet effects, there are no direct studies regarding their antioxidant activity (Guglielmone et al., 2002).

Thus, the aim of this study was to search flavonoids that can counteract toxic effects of GEN related to ROS production at the systemic level evaluating ROS generation, endogenous antioxidant defenses, and lipid peroxidation in human leukocytes *in vitro* and in rat whole blood and plasma, *in vivo*. It was also evaluated the antibacterial effect of flavonoid plus GEN combination on *S. aureus* and *E. coli,* in order to determine if flavonoid can enhance the antibacterial activity of GEN.

## 2. Materials and methods

### 2.1. Plant material

Plant material was collected in Argentina. Fruits of *Prosopis strombulifera* (CORD 1285) were collected in Mendoza, leaves of *Prosopis nigra* (ACORD AMP 1285) were collected in Traslasierra and leaves of *Flaveria bidentis* (CORD 2813) were collected in Santa Rosa de Río Primero. *P. strombulifera* and *F. bidentis* were identified by experts from Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET) and voucher specimens were deposited at the CORD (UNC Botanical Museum) as reference material. *P. nigra* was identified by experts from ACORD (Facultad de Ciencias Agropecuarias, UNC) and a voucher specimen was deposited as reference material. The plant material was dried at room temperature and powdered.

### 2.2. Extraction and purification of flavonoids

Fruits or leaves from *Prosopis* powdered (500 g) were extracted with ethanol (EtOH) by soxhlet and the solvent evaporated under reduced pressure to obtain the crude EtOH extract which was suspended in boiling water and, after cooling at room temperature, it was defatted with hexane and then extracted with ethyl ether (EtOEt) and ethyl acetate (AcOEt). For the *P. strombulifera* species, the EtOEt extract was selected (455 mg), this was chromatographed on preparative paper Whatman 3 MM with acetic acid 15% and the band with Rf of 0.44 was cut out and eluted with EtOH. The eluate was concentrated under reduced pressure, and carried out a column chromatography with Sephadex LH-20 and eluted with EtOH to obtain four fractions. From fraction 2 was obtained a pure compound (13 mg) identified as luteolin. For *P. nigra* species, work continued with the AcOEt extract (1.47 g), which was deposited in a column of microcrystalline cellulose and eluted with $H_2O$ to obtain three fractions. From fraction 1 was obtained isovitexin (150 mg) and from fraction 2 was obtained vitexin (260 mg). Finally, quercetin- 3-acetyl-7, 3′,4′-trisulphate (ATS) and quercetin-3,7,3′,4′-tetrasulphate (QTS) were isolated from the leaves of *F. bidentis* as previously described (Guglielmone et al., 2002) (Fig. 1).

Flavonoids identity (Fig. 1) was confirmed by spectrophotometric UV–Vis data (Mabry et al., 1970), and chromatographic HPLC analysis against authentic sample. The HPLC analysis was performed in a Varian Pro Star chromatograph (model 210, series 4171), equipped with a reversed-phase column (Phenomenex Hipersil C18, 4.6 × 30 mm), UV–Vis detector and the conditions were as follow: wavelength of the