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Z. Naturforsch. 2017; 72(5-6)c: 219–226

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Effects of *Hypericum perforatum* extract on oxaliplatin-induced neurotoxicity: in vitro evaluations

DOI 10.1515/znc-2016-0194
Received September 29, 2016; revised December 7, 2016; accepted December 31, 2016

Abstract: *Hypericum perforatum L.* has been used for centuries as a natural remedy for the treatment of many disorders. Neuropathic pain is a common side effect of oxaliplatin-based chemotherapy and often the cause of therapy discontinuation. Thanks to its anti-inflammatory and analgesic effects, the use of *H. perforatum* may be a novel therapeutic strategy for neuropathy. The aim of this paper was to evaluate the effect of *H. perforatum* hydrophilic extract on an in vitro model of oxaliplatin-induced neurotoxicity. The antioxidant potential of extract was first evaluated in cell-free models by the thiobarbituric acid-reactive substances assay and nitro blue tetrazolium oxidation test; the ability of *H. perforatum* extract to reduce oxaliplatin-induced caspase-3 activity in rat astrocytes and its potential interference with the cytotoxic effects of oxaliplatin in a colorectal cancer in vitro model (HT-29 cells) were also evaluated. The extract showed a significant antioxidant effect and was able to reduce caspase-3 activity in rat astrocytes. Of note, the extract alone exerted a cytotoxic effect in HT-29 cells and did not reduce the cytotoxicity of oxaliplatin in HT-29 cells. These data suggest that *H. perforatum* could be used as a novel therapeutic strategy for counteracting chemotherapy-induced neuropathy.

Keywords: astrocyte; HT-29; *Hypericum perforatum L.*; neuropathy; oxaliplatin.

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1 Introduction

Hypericum perforatum L. (St. John's Wort) is a perennial flowering plant that has been used for centuries as a natural remedy for the treatment of a variety of disorders [1, 2]. *Hypericum perforatum* extracts contain several compounds with biological activity such as hypericin, pseudohypericin, flavonoids, oligomeric procyanidins and hyperforin [3]. In traditional medicine, this extract is used in patients with depression disorders [4]; it possesses antiviral and antibacterial activities and was reported to be beneficial in the management of hyperlipidemia and atherosclerosis [3]. *Hypericum perforatum* is well tolerated and does not cause major side effects, and it is considered a safe herbal therapeutic agent [5, 6]. Neuropathic pain severely limits the quality of life and can be caused by a wide variety of injuries to peripheral nerves, including diabetes, traumatic injuries and treatment with chemotherapeutic drugs. Platinum analogues, such as oxaliplatin, are one of the most common antineoplastic drugs successfully employed as first-line treatment for several solid and blood cancers [7]. The most common side effect of these drugs is chemotherapy-induced neuropathy which often leads to reduction or discontinuation of therapy [7]. Oxaliplatin is able to induce neuropathic pain through several mechanisms including the modulation of Na^+ channels [8], alteration in Ca^{2+} homeostasis [7], production of oxygen reactive species [9] and increase in caspase-mediated apoptosis [10]. Among those, oxidative stress is a key pathological feature of neuropathic pain [11–13]. Hyperforin, one of the major compounds found in *H. perforatum*, showed many pharmacological effects in vitro such as anti-inflammatory and antioxidant [14]. Recently, *H. perforatum* extract was able to act on the onset and progression of neuropathic pain by a protective role on Ca^{2+} entry through TRPM2 channels in rat dorsal root ganglia [15]. Galeotti and co-workers found that *H. perforatum* extract was able to relieve neuropathic pain in rat models (chronic constriction injury and oxaliplatin administration). They also demonstrated that hyperforin and hypericin

mediated its antihyperalgesic action through an opioid-dependent pathway and the phosphorylation of PKC [16]. The aim of our paper was to characterize the content and the biological activities of a novel *H. perforatum* extract on a cellular model of oxaliplatin-induced neurotoxicity. First, we evaluated *H. perforatum* effects on oxidative stress and apoptosis in an in vitro model of neuropathic pain. The interaction between *H. perforatum* extract and oxaliplatin was also evaluated in an in vitro model of colorectal cancer.

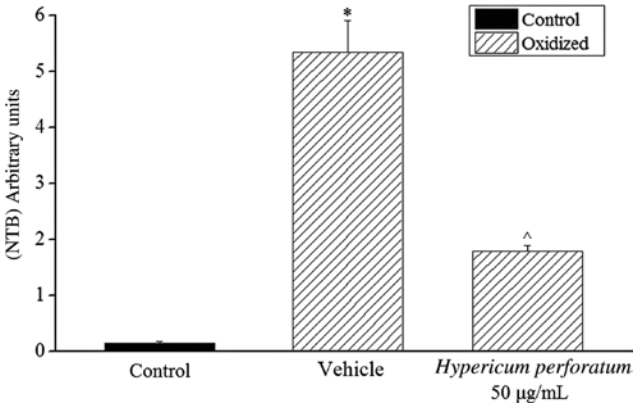

Figure 1: *Hypericum perforatum* extract's antioxidant properties evaluated by biochemical assay in a cell-free model. NBT (nitro blue tetrazolium) levels oxidized by superoxide anion generated from the hypoxanthine-xanthine oxidase reaction. NBT levels were expressed as fluorescence arbitrary units. *$p < 0.01$ vs. control; ^$p < 0.01$ vs. oxidized control.

2 Materials and methods

2.1 *Hypericum perforatum* hydrophilic fraction production

The production process is characterized by extracting the flowering tops of *H. perforatum* (cultivated and harvested by Aboca S.p.A., Sansepolcro, Italy). The dried top flowered herb undergoes extraction with ethanol 75% (v/v) (D/S 1 : 12). After 6–8 h, at 50 °C, the hydroalcoholic herb's mixture was dropped for 1 h and filtered to remove the exhausted herb. The corresponding extract was concentrated under vacuum, till the alcohol concentration is reduced to 1%–3%. The concentration step provided a mixture where water insoluble compounds resulted as an amorphous precipitate. The solid was removed by means of a Decanter centrifuge and the resulting hydrophilic fraction underwent to freeze-drying for 72 h. The freeze-dried fraction so obtained was stored until use, away from light and moisture.

2.2 Hyperforin and hypericin analysis

2.2.1 Sample preparation

The HPLC analysis in *H. perforatum* extract was performed according to the following method. Methanol (25 mL) was added to a sample of freeze-dried extract (0.5 g) and the resulting mixture was extracted protected from the light in an ultrasound bath at 35 °C for 30 min and then filtered in a volumetric flask. The filter was rinsed with further 25 mL of methanol and extracted again in the same conditions. After filtration the collected solutions were brought to 50 mL. The resulting solution was filtered on a cellulose acetate filter (0.45 µm) and used to perform HPLC analysis (Figure 1).

2.2.2 Hyperforin assay instrumental conditions

The measures were carried out by an Agilent 1100 Series LC/UV system consisting of a vacuum degasser, a quaternary pump, a Peltier autosampler thermostated at 20 °C, a Peltier column compartment thermostated at 20 °C and a UV detector. The column used was from Waters (Milford, MA; Spherisorb ODS2, 250 mm × 4.6 mm, 5 µm) and the elution was performed with H_2O 0.2% phosphoric acid (solvent A), CH_3CN (solvent B) and CH_3OH (solvent C). The gradient program used was 0 min 85% A, 15% B, 0% C; 10 min 70% A, 20% B, 10% C; 20 min 15% A, 75% B, 10% C in isocratic conditions till 45 min. The flow rate was 1.2 mL/min. The detector UV/Vis was set at 270 nm. Rutin anhydrous (Extrasynthese), dissolved in methanol/water 90/10, was used as a standard, and a response factor of 1.75 was calculated against hyperforin. The linearity of the method was found between 0.0127 and 0.1 mg/mL. The working solutions were 0.1, 0.05, 0.025, 0.0127 mg/mL. The correlation found was $r = 0.999998$. Each solution was injected in triple; the CV was inferior to 1%.

2.2.3 Hypericin assay instrumental conditions

The measures were carried out by an Agilent 1100 Series LC/UV system consisting of a vacuum degasser, a quaternary pump, a Peltier autosampler thermostated at 20 °C, a Peltier column compartment thermostated at 20 °C, and a UV detector. The column used was from Waters (Milford, MA; Spherisorb ODS2, 250 mm × 4.6 mm, 5 µm) and the elution was performed with sodium dodecyl

sulfate (SDS) 10 mM solution, pH 2.5 controlled with phosphoric acid (solvent A) and CH_3CN (solvent B). The gradient program used was 0 min 25% A, 75% B; 15 min 5% A 95% B. The flow rate was 1.2 mL/min. The detector UV/Vis was set at 590 nm. Hypericin (Sigma-Aldrich, Milan, Italy) was dissolved in methanol/pyridine 18/2. The linearity of the method was found between 0.06 and 0.3 mg/mL. The working solutions were 0.061, 0.122, 0.244 mg/mL. The correlation found was $r = 0.999966$. Each solution was injected three times; the CV was inferior to 1%.

2.3 UPLC-MS/QToF fingerprint method

2.3.1 Sample preparation

MeOH 50% v/v (100 mL) was added to the sample (0.25 g). The resulting mixture was extracted in an ultrasonic bath at 35 °C for 10 min and then centrifuged for 10 min at 4000 rpm. The pellet was suspended again in MeOH 50% v/v (100 mL) and extracted in the same conditions. After centrifugation the second extract was combined and the solution so obtained was transferred into a 200 mL flask. The sample was filtered on a 0.22 µm cellulose acetate syringe filter and then employed for the acquisition of the ESI-MS chromatographic profile (negative and positive ion mode).

2.3.2 Instrumental conditions

The measurements were carried out by an MS/Q-ToF (Xevo G2XS, Waters) system equipped with an ESI source (LockSpray, Waters). The system was coupled to an UPLC (H Class Aquity, Waters), equipped with a quaternary pump, an thermostated autosampler at 10 °C and a column compartment thermostated at 50 °C.

The parameters used were the following:

- [ESI(−)]: polarity ES−, analyzer resolution mode, capillary (kV) 2.5, sampling cone 40, source temperature (°C) 150, source offset 80, desolvation temperature (°C) 500, cone gas flow (L/h) 50, desolvation gas flow (L/h) 1000.
- [ESI(+)]: polarity ES+, analyzer resolution mode, capillary (kV) 2.5, sampling cone 40, source temperature (°C) 150, source offset 80, desolvation temperature (°C) 500, cone gas flow (L/h) 50, desolvation gas flow (L/h) 1000.

The column used was an RP-C18 (Cortecs C18 Column, 2.1 mm × 100 mm × 1.6 µm coupled to a VanGuard Pre-Column 2.1 mm × 5 mm, Waters).

The elution was performed with H_2O/HCOOH 125 mM (solvent A), MeOH (solvent B), H_2O (solvent C) and MeOH/HCOOH 125 mM (solvent D). The gradient program used was 0 min 20% A, 5% B, 75% C, 0% D; 1 min 20% A, 5% B, 75% C, 0% D; 4 min 20% A, 45% B, 35% C, 0% D; 13 min 20% A, 80% B, 0% C, 0% D; 14 min 20% A, 80% B, 0% C, 0% D; 14.10 min 5% A, 80% B, 0% C, 15% D; 16 min 5% A, 80% B, 0% C, 15% D; 16.10 min 20% A, 5% B, 80% C, 0% D; 18 min 20% A, 5% B, 75% C, 0% D. The flow rate was 0.5 mL/min. The injection volume was 3 µL.

2.4 Lipid peroxidation (thiobarbituric acid-reactive substances assay)

Thiobarbituric acid-reactive substances (TBARS) assay was assessed as an index of lipid peroxidation. The TBARS determination was carried out in rat brain homogenate obtained from brain tissue homogenized in PBS at the final concentration of 10% w/v. Then were added $FeCl_3$ (20 µM, Sigma-Aldrich, St. Louis, MO, USA) and ascorbic acid (100 µM, Sigma-Aldrich) to obtain the Fenton reaction both in the presence and in the absence of *H. perforatum* extract (50 µg/mL). At the end of incubation, the mixture was added to 4 mL reaction mixture consisting of 36 mM thiobarbituric acid (Sigma-Aldrich) solubilized in 10% CH_3COOH, 0.2% SDS, and pH was adjusted to 4.0 with NaOH. The mixture was heated for 60 min at 100 °C and the reaction was stopped by placing the vials in an ice bath for 10 min. After centrifugation (at 1600 *g* at 4 °C for 10 min) the absorbance of the supernatant was measured at 532 nm (PerkinElmer spectrometer).

2.5 Nitro blue tetrazolium oxidation test

The assay evaluated the nitro blue tetrazolium (NBT) superoxide anion-mediated oxidation and was performed as described by Ciuffi et al. [17]. Briefly, the superoxide anion generated by the reaction between hypoxanthine (600 mM, Sigma-Aldrich) and xanthine oxidase (10 mU/mL, Sigma-Aldrich) caused the NBT (10 mM, Sigma-Aldrich) oxidation that was evaluated both in the presence and in the absence of *H. perforatum* extract (50 µg/mL). The reaction was appraised measuring the absorbance at 560 nm (PerkinElmer spectrometer).

2.6 Cell cultures

Rat astrocytes were extracted as described by McCarthy and de Vellis previously [18], and human colorectal

adenocarcinoma cell HT-29 was a kind gift from Professor Enrico Mini (University of Florence).

2.7 Caspase-3 activity

Astrocyte and HT-29 cells were seeded in six-well plates (5×105/well) and grown until confluent. After 4 and 8 h of incubation with treatments (oxaliplatin 100 μM and *H. perforatum* extract 5, 50, 250 μg/mL) the cells were scraped in 100 μL lysis buffer (200 mM Tris–HCl buffer, pH 7.5, containing 2 M NaCl, 20 mM EDTA and 0.2% Triton X-100). Fifty microliters of the supernatant was incubated with 25 μM fluorogenic peptide caspase substrate rhodamine 110 bis-(N-CBZ-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid amide) (molecular probes) at 25 °C for 30 min. The amount of cleaved substrate in each sample was measured in a 96-well plate fluorescence spectrometer (PerkinElmer; excitation at 496 nm and emission at 520 nm).

3 Results

3.1 *Hypericum perforatum* hydrophilic fraction characterization

The production process of the fraction used for this study is characterized by the extraction of the dried flowered aerial part of *H. perforatum* by means of ethanol 75%, a solvent known to extract most of the metabolite produced from medicinal plants, comprising some terpenes and free fatty acids. During the concentration step under vacuum, required to prepare the concentrate to be subject to freeze-drying, a precipitate was formed. This feature indicated that the starting hydroalcoholic extract 75% contained a mixture of compounds with different solubility characteristics, molecular species present being more polar, soluble in water, and less polar, soluble in ethanol 75% but insoluble in water. So, before freeze-drying, the concentrate was treated by centrifugation affording two different fractions: the first one, the surnatant, is composed of compounds completely soluble in water (hydrophilic fraction), and a second "less polar" fraction, the pellet, characterized by compounds soluble in ethanol 75% but insoluble in water. To perform a rational characterization of the "hydrophilic fraction", the presence of the most specific markers of HP, hyperforins and hypericins, was analyzed. The hydrophilic fraction obtained as reported before has a hyperforin content of 0.248% (as sum of hyperforin and adhyperforin, calculated as hyperforin) and 0.154% of total hypericins (as sum of hypericin and pseudohypericin, calculated as hypericin). This result must be correlated with the results of the other fraction separated during the process. The percentage of hypericins was similar, but hyperforins reach values from 50- to 100-fold higher, confirming that the production process used is convenient to obtain an original fraction of HP depleted in hyperforin content. Comparing this result with a classical HP freeze-dried extract, we can observe again a difference on the hyperforin content, as generally the hydrophilic fraction has one fifth of hyperforin content. The hydrophilic fraction was further characterized acquiring its chromatographic fingerprint by means of an LC-MS/Q-ToF. Several other not quantified compounds were identified in *H. perforatum* extract, as reported in Table 1. The hydrophilic fraction contains hyperforins 0.248% (sum of hyperforin and adhyperforin, calculated as hyperforin) and 0.154% of total hypericins (sum of hypericin and pseudohypericin, calculated as hypericin). The fraction was further characterized. A chromatographic fingerprint analysis by means of an LC-MS/Q-ToF was performed and the characteristic compounds of *H. perforatum*, reported in Table 1, were detected. A chromatographic profile of *H. perforatum* hydrophilic fraction is reported in supplementary materials.

3.2 *Hypericum perforatum* extract's antioxidant properties

Hypericum perforatum whole extract showed antioxidant activity both in cellular and cell-free models. In a cell-free model, *Hypericum* extract was able to reduce the oxidized NBT (mediated by superoxide anion from the hypoxanthine-xanthine oxidase reaction) (Figure 1), and in a tissue-derived model, the TBARS content (indicator of lipid injury mediated by oxygen reactive species generated by the Fenton reaction) was reduced by the treatment with *Hypericum* extract (Figure 2). In a primary culture of rat astrocytes, the treatment with oxaliplatin (100 μM for 4 h) induced $O_2^{-}\cdot$ production which was significantly reduced by the co-administration of *Hypericum* extract (Figure 3).

3.3 Effects of *H. perforatum* extract on oxaliplatin-induced apoptosis in primary rat astrocytes

In primary rat astrocytes, oxaliplatin induced a marked increase of caspase-3 activity both after 4 and 8 h of

Table 1: Compound identified on *Hypericum perforatum* hydrophilic fraction.

Compound	RT	Ion polarity	Adduct ion	Experimental mass (scan base peak mass), m/z	MS^E ions	Theoretical neutral mass, m/z	Delta, ppm
Vanillic acid	2.15	Negative	$[M-H]^-$	167.0322	108.0189	168.0422	16.5
Neochlorogenic acid	2.40	Negative	$[M-H]^-$	353.0857	191.0581	354.0951	6.3
Chlorogenic acid	3.18	Negative	$[M-H]^-$	353.0857	191.0553	354.0951	6.3
Cryptochlorogenic acid	3.32	Negative	$[M-H]^-$	353.0857	173.0462 191.0553	354.0951	6.3
Luteolin-7-O-glucuronide	4.51	Negative	$[M-H]^-$	461.0739	285.0409	462.0798	2.8
Hyperoside	4.60	Negative	$[M-H]^-$	463.0938	300.0290	464.0954	12.0
Quercetin 3-O-glucoside-6″-acetate	4.71	Negative	$[M-H]^-$	505.0986	300.0290	506.1060	0.4
Orientin/homoorientin	4.81	Negative	$[M-H]^-$	447.0932	327.0497	448.1006	0.5
Rutin	4.87	Negative	$[M-H]^-$	609.1471	300.0254	610.1534	1.5
Quercitrin	4.92	Negative	$[M-H]^-$	447.0975	151.0036	448.1006	9.2
Quercetin	5.44	Negative	$[M-H]^-$	301.0368	151.0036	302.0426	4.5
Eupatorin-5-methyl ether	7.15	Negative	$[M-H]^-$	357.0976	342.0714	358.1052	1.0
I3,II8-Biapigenin/amentoflavone	8.08	Negative	$[M-H]^-$	537.0821	375.0508	538.0900	1.3

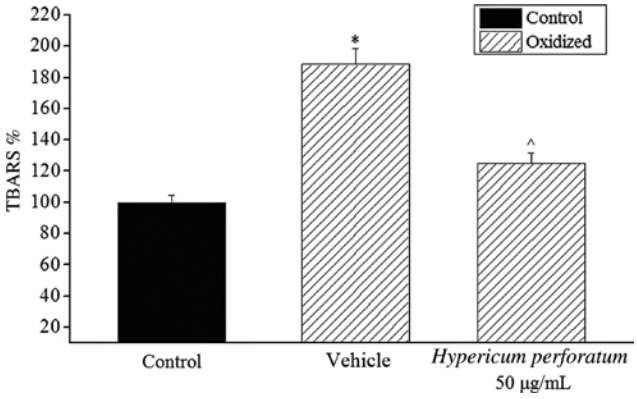

Figure 2: *Hypericum perforatum* extract's antioxidant properties in a tissue-derived model. TBARS (thiobarbituric acid reactive substances) levels obtained by the Fenton reaction. Values were expressed as percentage with respect to control. *$p < 0.01$ vs. control; ^$p < 0.01$ vs. oxidized control.

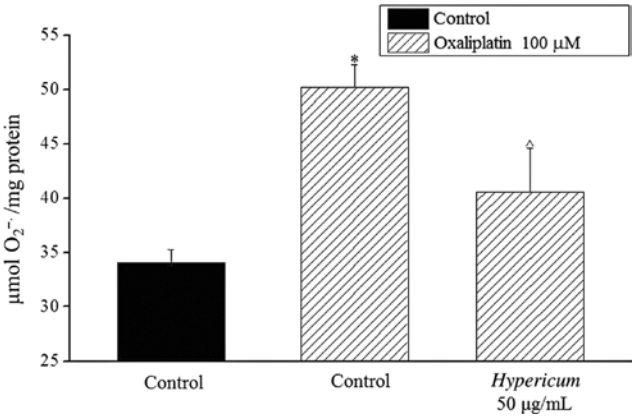

Figure 3: Antioxidant properties of *Hypericum perforatum* extract in a primary culture of rat astrocytes. The production of $O_2^{\cdot-}$ was evaluated after 4 h of 100 µM oxaliplatin treatment both in the absence and in the presence of 50 µg/mL *Hypericum perforatum* extract. Values are expressed as mean ± SEM of three independent experiments performed on three different biological samples for each treatment. *$p < 0.01$ vs. untreated control and ^$p < 0.01$ vs. oxaliplatin treatment.

treatment. *Hypericum perforatum* extract, at all concentrations tested (5, 50, 250 µg/mL), was able to reduce the increased caspase-3 activity only after 4 h, whereas after 8 h, only the highest concentration (250 µg/mL) had a significant effect (Figure 4).

3.4 Effects of *H. perforatum* extract alone or in combination with oxaliplatin on apoptosis in HT-29 colon cancer cells

In oxaliplatin-treated HCT-29 colon cancer cells, *H. perforatum* extract did not interfere with the cytotoxic activity of oxaliplatin at both 4 and 8 h of treatment. Of note, the extract alone was able to exert an unusual effect inducing a proapoptotic response similar to that produced by oxaliplatin after 4 h of treatment. After 8 h this effect was lost (Figure 5).

4 Discussion

Neuropathic pain is one of the most common side effects of platinum-based chemotherapy, significantly affects

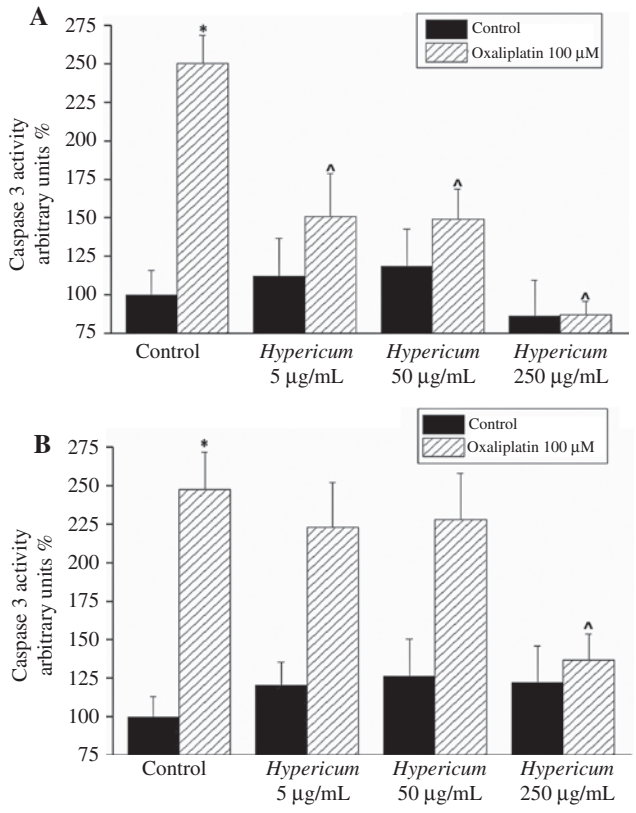

Figure 4: Caspase activity in astrocytes culture. Cells were treated with oxaliplatin 100 µM for 4 h (panel A) and 8 h (panel B) both in the absence and presence of 5, 50, 250 µg/mL *Hypericum perforatum* extract. Values, expressed as mean ± SEM from four independent experiments, were reported as percentage compared with control. *$p < 0.01$ vs. control. ^$p < 0.01$ vs. control treated with oxaliplatin.

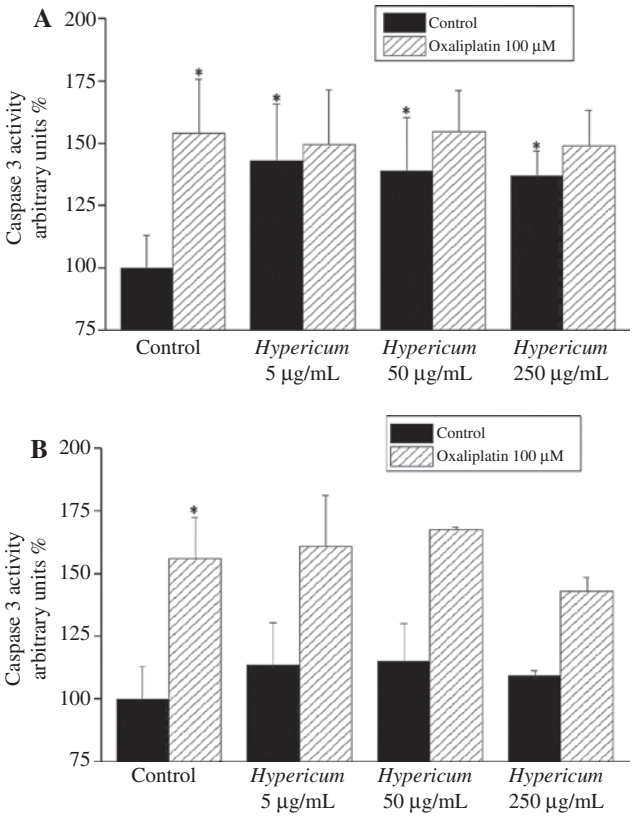

Figure 5: Caspase-3 activity evaluated on HT-29 cells. The figure shows the effect of oxaliplatin administration on caspase-3 activity evaluated both in the presence and in the absence of *Hypericum perforatum* extract after 4 h (panel A) and 8 h (panel B). The values are expressed in percentage compared with control and reported as the mean ± SEM of four independent experiments, performed on three different biological samples for each treatment. *$p < 0.01$ vs. control. ^$p < 0.01$ vs. control treated with oxaliplatin.

quality of life and is often the cause of therapy discontinuation. The identification of novel supplementary therapeutic strategies able to counteract the onset and the progression of chemotherapy-induced neuropathy is therefore of paramount importance. The mechanisms behind the onset of chemotherapy-induced neuropathy are far to be fully clarified, but a key role of oxidative stress has been suggested. Di Cesare Mannelli and co-workers described the pivotal role of oxidative stress in a rat model of painful oxaliplatin-induced neuropathy. In the plasma of oxaliplatin-treated rats, the increases in carbonylated protein and thiobarbituric acid reactive substances were the index of the resultant protein oxidation and lipoperoxidation, respectively. The same pattern of oxidation was also found in the sciatic nerve and in the spinal cord [11]. The characterization of our *H. perforatum* extract revealed the presence of several compounds with antioxidant properties such as phenolic acids, and its ability to reduce oxidative stress was demonstrated in two cell-free models. Our extract reduced both the presence of oxygen reactive species and the oxidative stress-induced damage to biological molecules. Several lines of evidence suggest a relationship between oxaliplatin-induced neurotoxicity and oxidative stress [9]. Other data supported this observation, but the relationship between the increased generation of ROS after platinum treatment and other mechanisms of oxaliplatin neurotoxicity remains poorly understood [7]. Data from Joseph et al. suggest that oxaliplatin-induced neuropathy is mediated by IB4 nociceptors and induces oxidative stress [19]. Zheng and collaborators reported that oxaliplatin is able to produce deleterious effects on axonal mitochondria leading to electron transport chain disruption and cellular energy failure in DRG neurons. The mitochondrial damage could be the starting point for increased ROS generation [20]. Thanks to its antioxidant properties, *H. perforatum* extract seems to be a promising candidate as a therapeutic agent against oxidative

stress-induced neuropathy. *Hypericum perforatum* extract showed protective effects against H_2O_2-induced apoptosis in PC12 cells [21] and exerted local and systemic protective effects in ischemia reperfusion injury in the rat liver [22].

Ta and co-workers demonstrated the involvement of caspases in oxaliplatin-induced neuropathy [10], whereas prolonged exposure to oxaliplatin induced the activation of MAP-kinase proteins p38 and ERK1/2, which in turn mediate apoptosis in rat dorsal root ganglia [23]. Conversely, oxaliplatin treatment was able to downregulate protective JNK/Sapk [24]. Our data highlight that *H. perforatum* extract exerts a protective effect on apoptosis induced by oxaliplatin in rat astrocytes (model of glial cells as the target of chemotherapy-induced damage), and this effect was evident at all concentrations tested after 4 h, whereas only the higher one maintained its efficacy after 8 h. We also evaluated the possible interference of *H. perforatum* extract on the cytotoxicity of oxaliplatin. To evaluate this aspect, the antiapoptotic effects of *H. perforatum* extract were assessed also on an in vitro model of colorectal cancer (HT-29 cells). In this model *H. perforatum* showed no ability to reduce caspase-3 activity induced by oxaliplatin treatment both after 4 and 8 h, suggesting that *H. perforatum* does not interfere with the cytotoxic activity of oxaliplatin against cancer cells and therefore it may be used as adjuvant therapy to counteract chemotherapy-induced neuropathy. We are also among the first to report that *H. perforatum* extract exerts a proapoptotic effects similar to that exerted by oxaliplatin in HT-29 cells after 4 h of treatment. These peculiar results are in line with those reported in previous studies showing that the oxaliplatin apoptosis-inducing mechanism is preferentially mediated in astrocytes by the intrinsic pathway and by the extrinsic pathway in the colon cancer cell HT-29 [25]. The intrinsic pathway is promoted by a mitochondrial alteration characterized by membrane potential changes, release of cytochrome C and redox unbalance [25]. Several studies showed that oxaliplatin promotes the release of cytochrome C from mitochondria to the cytosol in normal glial cells and increases of swollen and vacuolated mitochondria in peripheral nerve axons of neuropathic rats [20, 26]. Caspase-8 is one of the principal initiators of the extrinsic process and directly activates the effector caspase-3. Zanardelli and co-workers confirmed the activation of the extrinsic pathway in HT-29 cells by oxaliplatin treatment and demonstrated a lesser sensitivity of the mitochondria functionality against oxaliplatin toxicity in HT-29 in comparison to astrocytes [25]. *Hypericum perforatum* might also act differently in two different apoptotic pathways causing an apoptotic impulse in HT-29 cells and a protective effect in astrocytes. In conclusion, our data suggest the use of *H. perforatum* extract as a novel strategy for chemotherapy-induced neuropathy.

Acknowledgments: This research was funded by Aboca S.p.A. Società Agricola. CG received a grant from Aboca S.p.A. AM and LM are employees of Aboca S.p.A. The other authors declare no conflict of interest.

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Supplemental Material: The online version of this article (DOI: 10.1515/znc-2016-0194) offers supplementary material, available to authorized users.