



Antitumoral and antioxidant effects of a hydroalcoholic extract of cat's claw (*Uncaria tomentosa*) (Willd. Ex Roem. & Schult) in an *in vivo* carcinosarcoma model

Arturo Alejandro Dreifuss^a, Amanda Leite Bastos-Pereira^b, Thiago Vinicius Ávila^b, Bruna da Silva Soley^b, Armando J. Rivero^c, José Luis Aguilar^a, Alexandra Acco^{b,*}

^a Immunology Laboratory, Cayetano Heredia Peruvian University, Lima, Peru

^b Department of Pharmacology, Federal University of Paraná, Curitiba, Paraná, Brazil

^c Pharmaceutical Technology Department, National University of San Marcos, Lima, Peru

ARTICLE INFO

Article history:

Received 23 January 2010

Received in revised form 5 April 2010

Accepted 20 April 2010

Available online 6 May 2010

Keywords:

Uncaria tomentosa

Cat's claw

Walker-256

ROS

Oxidative stress

Tumor

Urea

ABSTRACT

Aim of the study: The present work intended to study the antitumoral and antioxidant effects of *Uncaria tomentosa* (UT) hydroalcoholic extract in the Walker-256 cancer model.

Methods and materials: Walker-256 cells were subcutaneously inoculated in the pelvic limb of male Wistar rats. Daily gavage with UT extract (10, 50 or 100 mg kg⁻¹, Groups UT) or saline solution (Control, Group C) was subsequently initiated, until 14 days afterwards. For some parameters, a group of healthy rats (Baseline, Group B) was added. At the end of treatment the following parameters were evaluated: (a) tumor volume and mass; (b) plasmatic concentration of urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH); (c) hepatic and tumoral activity of catalase (CAT) and superoxide dismutase (SOD), as well as the rate of lipid peroxidation (LPO) and glutathione (GSH); and (d) hepatic glutathione-S-transferase (GST) activity. The reactivity of UT extract with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed in parallel.

Results: UT hydroalcoholic extract successfully reduced the tumor growth. In addition, treatment with UT reduced the activity of AST, which had been increased as a result of tumor inoculation, thus attempting to return it to normal levels. UT did not reverse the increase of LDH and GGT plasma levels, although all doses were remarkably effective in reducing urea plasma levels. An important *in vitro* free radical-scavenging activity was detected at various concentrations of UT extract (1–300 µg mL⁻¹). Treatment also resulted in increased CAT activity in liver, while decreasing it in tumor tissue. SOD activity was reduced in liver as well as in tumor, compared to Group C. No statistical significance concerning ALT, GST, LPO or GSH were observed.

Conclusions: This data represent an *in vivo* demonstration of both antitumoral and antioxidant effects of UT hydroalcoholic extract. The antineoplastic activity may result, partially at least, from the ability of UT to regulate redox and metabolism homeostasis.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Uncaria tomentosa (UT) is a vine that has been used for centuries by many indigenous tribes of South America for a wide range of affections. Its use has been proposed for a broad array of diseases such as arthritis, gastric ulcers, rheumatism, various infections and

cancer (Cerri et al., 1988; Aquino et al., 1991; Rizzi et al., 1993; Wurm et al., 1998; Lemaire et al., 1999).

The pharmacological properties of UT have been most studied in inflammatory processes. Extracts from the bark and the root of UT have been proved successful in inhibiting nitrite as well as TNF α production (Sandoval et al., 2002). The immunomodulatory properties of UT were also related to its ability of suppress TNF α synthesis (Sandoval et al., 2000). Another study using the carrageenan-induced paw edema model in mice to compare the anti-inflammatory activity of a hydroalcoholic and an aqueous extract of UT concluded that the former has a greater favorable activity (Aguilar et al., 2002). Conversely, an interesting sup-

* Corresponding author at: Federal University of Paraná, Biological Science Sector, Pharmacology Department, Jardim das Américas, Cx. P. 19031, Curitiba – PR Zip Code 81530-990, Brazil. Tel.: +55 41 33611743.

E-mail address: aleacco@ufpr.br (A. Acco).

pression of the transcription pathway mediated by NF- κ B was also observed with both extracts. Other reports suggest that the anti-inflammatory properties of UT may be related to a synergic combination of compounds (Rizzi et al., 1993; Reinhard, 1997).

Beside the discovery of UT effects in inflammatory and immune scenarios, there is recent interest in its effects in neoplastic processes, although to this point most of the studies have been based on tumor cell lines *in vitro*. Indeed, it has been observed that some aqueous extracts of UT significantly inhibited the proliferation of HL60 human leukemia cells (Sheng et al., 1998). Other study observed enhanced DNA repair activity (Sheng et al., 2001). The first demonstration of the potentially beneficial effects of UT on solid tumors was performed by Riva et al. (2001) using bark extracts and fractions of the plant on the MCF7 breast cancer lineage. Their data suggest that in addition to demonstrating antimutagenic properties, UT had a direct antiproliferative activity against neoplastic cells. More recently, a UT component called mitraphylline demonstrated *in vitro* antitumoral activity against cellular lines of human neuroblastoma and glioma (García Prado et al., 2007).

Despite these results, until now there are only few studies applying UT in *in vivo* tumor models (Caballero et al., 2005; Fazio et al., 2008). Moreover, little attention has been given to the association between the aforementioned antineoplastic effects and its previously observed antioxidant properties (Sandoval et al., 2002; Pilarski et al., 2006). In face of the promising potential of UT for the adjuvant management of neoplastic diseases, this investigation attempted to evaluate the joint antiproliferative and antioxidant activity of an UT hydroalcoholic extract in the Walker-256 (W-256) cancer model, which is a murine carcinosarcoma that has been considered an appropriate model for studies concerned to solid tumors, oncologic pain, metastasis and cancer-related cachexia (De Almeida Salles Perroud et al., 2006; Brigatte et al., 2007).

2. Materials and methods

2.1. Plant material and extraction procedure

All plant material was kindly provided specifically for this study by Peruvian Heritage S.A.C. It consisted on a hydroalcoholic extract of the bark of *Uncaria tomentosa* prepared by decoction using ethanol and water in the proportion of 70:30 for 1 h at 20 °C, and subsequently dried by atomization, resulting in a powder extract. The total alkaloid content was of 5.03%.

To further ascertain the quality of the extract, we determined its oxindole alkaloids content by means of high performance liquid chromatography (HPLC) techniques according to Laus and Keplinger (1994). Detection was at 245 nm, with measurement of the absorbance ratio to that at 230 or 260 nm, peak isolation by semi-preparative chromatography used for quantification. The pentacyclic were found the most significant concentration of alkaloids in the extract.

2.2. Animals

Male Wistar rats (200–300 g) were obtained from the Central Animal House of the Federal University of Paraná (Curitiba, Brazil). Animals were housed at 22 ± 1 °C under a 12-h light–dark cycle, and had free access to standard laboratory food (Purina®) and tap water. No other experiments were conducted in these animals prior to those of the current research. All experimental protocols using animals were performed following the recommendations of Brazilian Law 6638, 05/11/1979 for the scientific management of animals and the “Principles of Laboratory Animal Care” (NIH Publication 85-23, revised in 1985). Both Institutional Animal Ethics Committee of Federal University of Paraná and of Cayetano Heredia Peruvian

University revised and approved all procedures of this study, under certificate numbers 324 and 53973, respectively.

2.3. Tumor cells

Walker-256 tumor cells were kindly donated by Prof. Dr. Luiz Cláudio Fernandes from the Physiology Department of the Federal University of Paraná. The maintenance of Walker-256 cells (W-256) was carried out by weekly passages through intraperitoneal (IP) inoculation according to Vicentino et al. (2002). After 5–7 days of growth in ascitic fluid, the liquid collected was centrifuged for 10 min at 1126 × g at 4 °C. The supernatant was discarded, and the precipitate suspended in 1.0 mL of PBS buffer (16.5 mM phosphate, 137 mM NaCl and 2.7 mM KCl). The viability of tumor cells was assessed by the Trypan blue-exclusion method in a Neubauer chamber. Finally, approximately 10⁷ W-256 cells were injected subcutaneously in the right pelvic limb of each animal.

2.4. Experimental design

The treatment began 1 day after tumor cell inoculation, and continued for 14 days. The hydroalcoholic dried extract of *Uncaria tomentosa* was dissolved daily in distilled water and administered by gavage in doses of 10, 50 or 100 mg kg⁻¹ (Group UT, n=9). Animals of the control group (Group C, n=9) received a similar volume of distilled water. For some parameters, another group called baseline (Group B, n=5) was added, which was composed of individuals not inoculated with tumor, but treated with saline solution. After 14 days of treatment, all animals were anesthetized with ketamine (Dopalen®, Vetbrands, Paulínia), in a dose of 60 mg kg⁻¹, and xylazine (Anasedan®, Vetbrands, Paulínia), in a dose of 7.5 mg kg⁻¹; and blood samples from the inferior cava vein were obtained for biochemical assays. Subsequently, animals underwent euthanasia by diaphragm puncture. All tumors were removed and weighted in an analytical balance. The tumor volume was calculated by means of the measure of its diameters, according to Mizuno et al. (1999). After these determinations, tumor and liver samples of each animal were stored at –70 °C for further analyses.

2.5. Blood biochemical assays

Serum samples were collected after blood centrifugation at 3000 × g for 10 min. These samples were used for determination of plasmatic urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH) by means of commercial kits (Labtest Diagnostica, Lagoa Santa, Brazil).

2.6. Oxidative stress parameters

2.6.1. *In vitro* free radical-scavenging activity

The reactivity of the UT hydroalcoholic extract (concentrations of 1–300 μg mL⁻¹) with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed through an adaptation in the method of Chen et al. (1999). The system consisted of 750 μL of test solution (UT extract) and 250 μL of a methanolic solution of DPPH (1 mg 25 mL⁻¹). After 5 min, the decrease in absorbance was measured. A solution of the reducing agent ascorbic acid (50 μg mL⁻¹) was used as positive control and distilled water was used as negative control.

2.6.2. Determination of catalase, superoxide dismutase and glutathione-S-transferase activities

For the biochemical analyses of these enzymes, liver and tumor samples were homogenized in phosphate buffer pH 6.5. Catalase

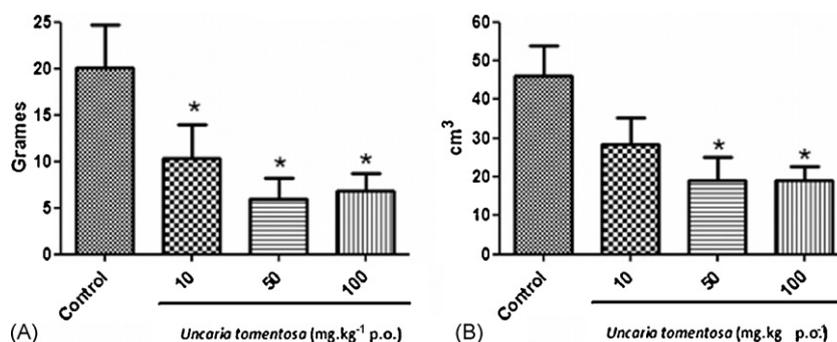


Fig. 1. Tumor mass (A) and volume (B) after 14 days of treatment with *Uncaria tomentosa* (10, 50 or 100 mg kg⁻¹) or distilled water (control) to rats bearing Walker-256 carcinosarcoma. The animals were treated as described in Section 2.4. Data are expressed as mean ± standard error of mean and were analyzed by one-way ANOVA and *post hoc* Neumann–Keuls. Abbreviation: p.o. = *per os*. **p* < 0.05 compared with control group.

(CAT) activity was measured according to Aebi (1984). The reaction was monitored at 240 nm in spectrophotometer for 60 and 90 s for liver and tumor samples, respectively. Superoxide dismutase (SOD) activity was measured in both tissues by the ability of this enzyme to inhibit pyrogallol auto-oxidation, in microplate at 440 nm (Gao et al., 1998). The amount of enzyme that inhibited the reaction by 50% (IC₅₀) was defined as one unit of SOD, and the enzyme activity was expressed in units of SOD per milligram of total protein (USOD mg protein⁻¹). The activity of glutathione-S-transferase (GST) in liver was measured following the method of Habig et al. (1974) using a microplate reader, which assessed the linear increase in molar extinction at 340 nm.

2.6.3. Determination of reduced glutathione levels

Reduced glutathione (GSH) levels were measured by the method described by Sedlak and Lindsay (1968). Tumor and liver tissue were diluted in phosphate buffer 0.1 M (pH 6.5) in the proportion of 1:10. Subsequently, 250 μL of the homogenate were mixed with trichloroacetic acid (200 μL of 12.5% purity) and kept in ice for 30 min thus allowing protein precipitation. The supernatant was separated by centrifugation at 13,750 × *g* for 10 min at 4 °C. Then, 30 μL of the clear supernatant was mixed with 270 μL of phosphate buffer 0.1 M (pH 8.5) and 5 μL of 5,5'-dithiobis-(2-nitrobenzoic acid) in methanol. The absorbance of the reaction solution was measured at 415 nm in a microplate reader, using reduced glutathione as external standard.

2.6.4. Determination of lipid peroxidation rate

Lipid peroxidation (LPO) rate was measured by the FOX-2 method (Jiang et al., 1991). This technique determines lipid hydroperoxide synthesis during peroxidation. Tumor and liver samples were homogenized in methanol (1:5 ratios) at 25,000 rpm min⁻¹, and then centrifuged at 5000 × *g* for 5 min at 4 °C. The absorbance of the supernatant was measured at 560 nm in a spectrophotometer model Ultrospec 4300 pro. The results were expressed in nmol mg protein⁻¹.

2.6.5. Quantification of proteins

The quantification of proteins in liver and tumor samples was performed according to the Bradford method (1976). The reaction was accomplished at 595 nm in a microplate reader, using bovine serum albumin (BSA) as protein standard.

2.7. Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) with *post hoc* Neumann–Keuls multiple range testing in the Graph Pad Prism program version 4.0. Differences were considered significant when *p* ≤ 0.05.

3. Results

3.1. Tumor mass and volume

Fourteen days after the inoculation of W-256 cells, the tumor mass of the control group was 20 ± 4.70 g. Treatment with all doses of UT extract notably reduced the tumor mass, as shown in Fig. 1A. Considering that tissue mass can vary because of the water contents, the volume of tumors were as well evaluated. The tumor volume of the control group was 46 ± 8 cm³. Compared to control group, the tumor volume reduced 46%, 58% and 64% at doses of 10, 50 and 100 mg kg⁻¹ of UT extract, respectively (Fig. 1B), thus showing a dose-dependent effect. Animals had their body weight monitored during the entire treatment period. However, no statistical significance between the groups was observed.

3.2. Plasmatic levels of urea and hepatic markers

Considering the liver as the main organ responsible for the metabolism of xenobiotics, such as UT extract, and for the oxidative stress homeostasis, hepatic function was evaluated accessing plasmatic levels of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH). Also plasma urea concentration was measured as a marker for kidneys function and ureagenesis, once this metabolic process takes place in hepatocytes. Basal plasma levels for these parameters were considered as those obtained in the baseline group (Group B) and were compared with distilled water and UT-treated groups. These results are shown in Table 1.

Animals of the control group presented enhanced levels of all plasma enzymes when compared to baseline condition. These increases were represented by about 50% for LDH, 533% for GGT, 62% for ALT and 163% for AST. These data indicate that some of the hepatic functions are modified by the presence of W-256 tumor. Regarding these parameters in treated rats, the most prominent effect of UT was in AST. All three tested doses of UT reduced by about 14% the plasma levels of AST. However, these levels still remained 127% higher than those found in baseline group. In contrast, ALT levels did not show significant changes between control and UT groups. Despite lacking statistical significance, it should be stressed that LDH levels from the group treated with 100 mg kg⁻¹ of UT reduced about 30% when compared to control group. Nevertheless, these levels were 15% higher than Group B. Interestingly, GGT was the enzyme that presented the highest elevation in all tumor-bearing rats. GGT levels in UT-treated rats were higher than those presented by control group, reaching ~600% of increasing compared to basal levels.

Table 1
Plasmatic levels of hepatic markers and urea after 14 days of treatment with *Uncaria tomentosa* extract to rats bearing the tumor Walker-256, compared with control and baseline groups.

Parameter	Groups					Units
	Baseline	Control	<i>Uncaria tomentosa</i>			
			10 mg kg ⁻¹	50 mg kg ⁻¹	100 mg kg ⁻¹	
LDH	175 ± 35.2	262.9 ± 49.5	214.8 ± 22.5	202.6 ± 25.0	201.3 ± 30.8	UL ⁻¹
GGT	1.5 ± 0.7	9.5 ± 0.9 [#]	10.9 ± 2.3 [#]	10.6 ± 1.9 [#]	11.3 ± 1.6 [#]	UL ⁻¹
ALT	55.5 ± 1.7	90.4 ± 4.3	79.4 ± 2.8	80.4 ± 2.6	78.5 ± 3.8	UL ⁻¹
AST	69.5 ± 1.6 [*]	183.3 ± 4.6	157.6 ± 7.5 ^{*,#}	157.6 ± 4.9 ^{*,#}	158.2 ± 5.9 ^{*,#}	UL ⁻¹
Urea	40.7 ± 12.3 ^{***}	208.9 ± 47.9	23.1 ± 3.3 ^{***}	37.5 ± 6.9 ^{***}	60.8 ± 4.7 ^{***}	mg dL ⁻¹

Animals were inoculated with W-256 cells and treated as described in Sections 2.3 and 2.4. Data are expressed as mean ± standard error of mean and were analyzed by one-way ANOVA and *post hoc* Neumann–Keuls. Abbreviations: LDH = lactate dehydrogenase; GGT = gamma-glutamyltransferase; ALT = alanine aminotransferase; ALT = aspartate aminotransferase.

^{*} $p < 0.05$ comparison with control group.

^{***} $p < 0.001$ comparison with control group.

[#] $p < 0.05$ comparison with baseline group.

Treatment with *Uncaria tomentosa* showed a remarkable reduction in urea plasmatic levels in order of 89%, 82% and 70% for the groups treated with 10, 50 and 100 mg kg⁻¹ of UT extract, respectively (Table 1). Interestingly, the influence of UT in reducing plasma urea concentration demonstrated an inverse dose-dependent effect.

3.3. Assessment of the ability of *Uncaria tomentosa* to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Due to previous studies have demonstrated the antioxidant properties of *Uncaria tomentosa*, we decided to evaluate the *in vitro* scavenging activity of DPPH by several concentrations of our extract. A statistically significant reduction of the absorbance was obtained compared to the negative control (water) as well as the positive control (ascorbic acid). The scavenging activity was detected since the lowest UT concentration (1.0 µg mL⁻¹), and the reaction reached the steady state at concentration of 100 µg mL⁻¹, as shown in Fig. 2.

3.4. Evaluation of oxidative stress in liver and tumor tissue

Considering the observation of an expressive *in vitro* antioxidant action of UT extract (Section 3.3), we deemed appropriate to measure *in vivo* oxidative stress parameters. Catalase (CAT), super-

oxide dismutase (SOD) and glutathione-S-transferase (GST) were measured in liver and/or tumor tissues. Additionally, the rate of lipid peroxidation (LPO) and reduced glutathione (GSH) were as well assessed on both tissues.

3.4.1. Oxidative stress parameters in liver tissue

The presence of W-256 tumor induced significant changes in all of hepatic enzymes related to oxidative stress. In the control group the mean activity of CAT was reduced by 79%, SOD increased by 252% and GST decreased by 59% when compared to baseline group, as shown in Table 2. On the other hand the treatment with UT successfully normalized the activities of these enzymes. Regarding to CAT and GST, all tested doses of UT extract significantly increased its activities, drawing both enzymes to similar levels of those found in baseline condition. It should be stressed, however, that the activity of hepatic SOD was normalized only by the highest dose of UT extract (100 mg kg⁻¹), while the lower (10 mg kg⁻¹) and intermediary (50 mg kg⁻¹) doses did not succeed in achieving any statistically significant reduction in the activity of this enzyme.

3.4.2. Oxidative stress parameters in tumor tissue

The assays of CAT demonstrated that this enzyme has lower activity in tumor than in liver. The obtained values of CAT activity for the group treated with 10 mg kg⁻¹ UT reached 291 ± 17.8 µmol min mg protein⁻¹ in liver, but only 59.9 ± 5.2 µmol min mg protein⁻¹ in tumor. Thus, in UT-treated rats, CAT activity was found to be 4.3-fold elevated in liver when compared with tumor tissue. Another important observation concerning CAT activity is that UT reduced its activity in tumor while remarkably enhancing it in liver, as compared to control group. Like so, activity of tumor SOD was significantly reduced in all groups treated with UT extract. In contrast, treatment with UT did not lead to any significant differences in reduced glutathione levels (GSH) and lipid peroxidation (LPO) rates on the tumor tissue. These results are shown in Table 3.

4. Discussion

Previous work on the antiproliferative potential of *Uncaria tomentosa* had been centered on *in vitro* testing of various extracts or even isolated compounds of the plant on neoplastic lineages, predominantly mammary, hematological and neurological (Sheng et al., 1998, 2001; Riva et al., 2001; Bacher et al., 2006; García Prado et al., 2007; Pilarski et al., 2006; García Giménez et al., 2010). The antitumoral action of UT extract shown by reduction of volume and mass of W-256 tumor in all tested doses (10, 50 and 100 mg kg⁻¹) constitutes the first observation of such effect on W-256, an *in vivo* solid rat tumor. These results corroborate pre-

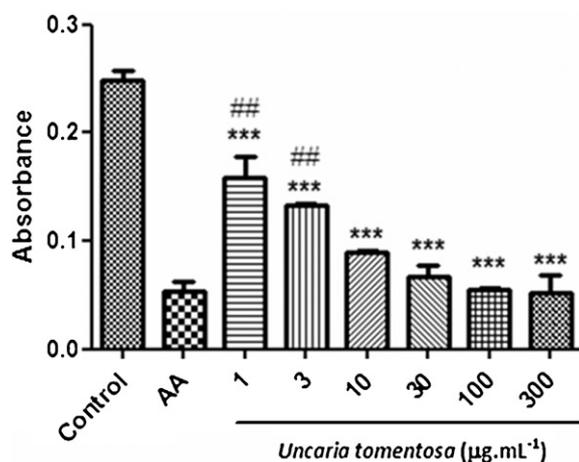


Fig. 2. Evaluation of scavenging activity of DPPH (2,2-diphenyl-1-picrylhydrazyl) by different concentrations of *Uncaria tomentosa* hydroalcoholic extract. Negative control was water; positive control was ascorbic acid (AA) 50 µg mL⁻¹. Data are expressed as mean ± standard error of mean and were analyzed by one-way ANOVA and *post hoc* Neumann–Keuls. ^{***} $p < 0.001$ compared to control; [#] $p < 0.01$ compared to AA.

Table 2

Oxidative stress parameters evaluated in the liver homogenate after 14 days of treatment in baseline, control, and *Uncaria tomentosa* (10, 50 and 100 mg kg⁻¹) groups of rats bearing the Walker-256 tumor.

Parameter	Groups					Units
	Baseline	Control	<i>Uncaria tomentosa</i>			
			10 mg kg ⁻¹	50 mg kg ⁻¹	100 mg kg ⁻¹	
CAT	392.7 ± 50.1*	82.5 ± 22.9	291 ± 17.8*	249.1 ± 17.3 [†]	213.4 ± 14.5 [†]	μmol min mg protein ⁻¹
SOD	1.9 ± 0.2*	6.7 ± 0.9	6.7 ± 1.3	5.2 ± 0.5	3.1 ± 0.3*	UnSOD mg protein ⁻¹
GST	607.5 ± 29.5*	248.9 ± 54.4	732 ± 119.2 [†]	602.9 ± 83.3 [†]	508.8 ± 85.9 [†]	nmol min mg protein ⁻¹
GSH	589.3 ± 76.1	397.3 ± 65.3	495.1 ± 44.1	510.5 ± 66.5	597 ± 100.6	nmol mg protein ⁻¹
LPO	1.5 ± 0.7	5.6 ± 0.6 [#]	6.9 ± 0.9 [#]	4.2 ± 0.6 [#]	5.2 ± 0.3 [#]	nmol mg protein ⁻¹

Animals were inoculated with W-256 cells and treated as described in Sections 2.3 and 2.4. Data are expressed as mean ± standard error of mean and were analyzed by one-way ANOVA and *post hoc* Neumann–Keuls. Abbreviations: CAT = catalase; SOD = superoxide dismutase; GST = glutathione-S-transferase; GSH = reduced glutathione; LPO = lipoperoxidation.

* $p < 0.05$ comparing with control group.

$p < 0.05$ comparing with baseline group.

vious observations of UT effect upon subcutaneous tumors in mice (Caballero et al., 2005; Fazio et al., 2008). Moreover, it has been stated that both healthy and malignant cells achieve their termination via apoptosis or necrosis, and that the balance of ROS is quite crucial in determining which of these two pathways will be unfolded (Skulachev, 2000; Pelicano et al., 2004; El-Najjar et al., 2009). In the present work we observed an important correlation between the antitumoral properties of UT and its potential as a free radical scavenger and as a provider of reactive oxygen species (ROS) homeostasis, corroborating previous reports (Wirth and Wagner, 1997; Choi et al., 2002; Pilarski et al., 2006; Cheng et al., 2007).

More than an antioxidant effect *per se* as demonstrated by DPPH assay, our results show that UT extract is able to reduce hepatic SOD activity in the highest dose (100 mg kg⁻¹). Previous experiences had demonstrated that different UT extracts showed different degrees of SOD-like activity, ultimately concluding that UT exerts an expressive antioxidant activity, especially when using alcohol-based extracts such as the one employed in the present work (Pilarski et al., 2006). Indeed, we observed an important reduction of SOD activity in liver and in tumor when compared to control values, even attempting to revert it to baseline values. This result may suggest a reduction in generation of anion superoxide (O₂^{•-}) as a result of treatment with UT. This observation is important due to the fact that rats bearing W-256 can generate great amounts of anion O₂^{•-} through the respiratory chain (Bastos-Pereira et al., 2009), thus fomenting an oxidant cascade that leads to necrosis in an untreated scenarios (Portugal et al., 2009; Calabrese et al., 2010).

The lessened capacity of tumors to detoxify hydrogen peroxide (H₂O₂) is related to lower CAT levels (Valko et al., 2006). In this case, the animals treated with all doses of UT presented elevation in hepatic activity of CAT enzyme. This data confirms the potential

of the liver as an antioxidant organ in systemic oxidative states like that induced by the presence of W-256 tumor. Moreover, we report a significant diminished CAT activity in tumor tissue as a result of UT treatment at the dose of 100 mg kg⁻¹. Tumor cells that exhibit high CAT activity are resistant against ROS-induced apoptosis, and it has been observed that the inhibition of this enzyme resensitizes such malignant cells (Sandstrom and Buttke, 1993; Tome et al., 2001; Von Eynatten and Bauer, 2001; Moran et al., 2002; Vandenabeele et al., 2006; Fang et al., 2007; Bechtel and Bauer, 2009). Therefore, the CAT inhibition in tumor cells that we observed is indeed a highly favorable result, which indicate that W-256 cells are sensitive to ROS-induced apoptosis.

As well as CAT, the activity of hepatic GST is reduced by W-256 but is induced by UT treatment. Interestingly, despite the fact that this enzyme has been involved in xenobiotics and carcinogens metabolism through glutathione (GSH) conjugation reaction (DeWeese et al., 2001), the tumor and hepatic levels of GSH did not suffer any significant alteration with the UT treatment. In this sense, GSH and LPO, both parameters that were not modified by treatment, may not be targets for the action of UT in W-256. One more data that reinforces that observation is the absence of differences between control and UT-treated groups regarding to GGT plasmatic level. GGT is intimately concerned in the synthesis and metabolism of GSH through the gamma-glutamyl cycle (Goldberg, 1980).

All of the above mentioned parameters play important roles in the redox homeostasis, and the maintenance of their regular level can be useful in various diseases, such as cancer. Despite the ineffectiveness of UT extract in regulating GSH and LPO level, it may be suggested that UT extract is interesting as an adjuvant in the treatment of solid tumors. The fundament of this statement occurs, partially at least, because of its action upon enzymes that regulate oxidative stress. It is well known that the interference in some

Table 3

Oxidative stress parameters evaluated in the tumor homogenate after 14 days of treatment with saline solution (control) or *Uncaria tomentosa* (10, 50 or 100 mg kg⁻¹) extract in rats bearing the Walker-256 tumor.

Parameter	Groups				Units
	Control	<i>Uncaria tomentosa</i>			
		10 mg kg ⁻¹	50 mg kg ⁻¹	100 mg kg ⁻¹	
CAT	74.6 ± 12.4	59.9 ± 5.2	55.7 ± 5.3	55.7 ± 3.4 [†]	μmol min mg protein ⁻¹
SOD	6.3 ± 0.4	4.5 ± 0.7*	4.0 ± 0.4*	2.8 ± 0.3 [†]	UnSOD mg protein ⁻¹
GSH	314 ± 50.3	297 ± 11.4	304.5 ± 18.4	208.7 ± 17.4	nmol mg protein ⁻¹
LPO	9.8 ± 0.8	8.3 ± 1.3	6.9 ± 0.6	8.9 ± 1.1	nmol mg protein ⁻¹

Animals were inoculated with W-256 cells and treated as described in Sections 2.3 and 2.4. Data are expressed as mean ± standard error of mean, one-way ANOVA, *post hoc* Neumann–Keuls. Abbreviations: CAT = catalase; SOD = superoxide dismutase; GSH = reduced glutathione; LPO = lipoperoxidation.

* $p < 0.05$ comparing with control group.

redox processes and ROS metabolism are a possible way of achieving apoptosis in neoplastic cells (De Martino et al., 2006; Cheng et al., 2007).

The presence of W-256 tumor increased plasmatic level of hepatic enzymes in comparison with baseline group. The values of enzymes found in baseline condition are in concordance with the reference ones for rats (Kaneko, 1989; Petterino and Argentino-Storino, 2006). Elevated plasmatic levels of GGT, ALT and AST in control rats indicate that hepatic functions are modified by W-256 tumor. Increased plasma levels of both ALT and AST are generally regarded as important indicators of cellular hepatic lesions (Peichoto et al., 2006), once alterations in hepatocyte function or membrane permeability can result in enzyme extravasation to plasma. However, UT treatment was able to reduce significantly AST plasmatic level. Considering that AST has cytosolic location in hepatocytes (Jagadeesan and Kavitha, 2006), our data indicate that UT extract protected at least partially the hepatocytes, resulting in reduced plasmatic AST. Similar results regarding plasmatic AST and ALT in rats bearing W-256 were recently reported after treatment with extracts of *Agaricus brasiliensis* mushroom, which also has an antioxidant activity (Jumes et al., 2010). It is worthy of mention that when administered to human patients with osteoarthritis, cat's claw had no deleterious effects on liver function parameters (Piscoya et al., 2001).

Plasmatic levels of GGT were found extremely high in control rats. These findings are not surprising in view of several publications reporting that tumor progression and appearance of more aggressive phenotypes are related with GGT role or deregulated expression of this transferase (Hanigan et al., 1999; Maellaro et al., 2000; Pompella et al., 2006; Corti et al., 2009). Prospective studies showed that elevated GGT is associated with mortality from liver disease, cancer or diabetes (Ruhl and Everhart, 2009), and also with chronic kidney disease (Targher et al., 2009). The measurement of urinary GGT is more adequate for detection of kidney damage (Braun et al., 1983), while the plasma values can indicate disturbances of excretory liver function (Nemesánszky and Lott, 1985). In this case, despite lacking reduction of plasmatic GGT by UT treatment in rats bearing W-256, the level of plasmatic urea was remarkably reduced by UT extracts. Plasmatic urea returned to baseline levels with all UT tested doses. These results may indicate that (a) liver ureagenesis was normalized and/or (b) that excretory kidney function was preserved by UT treatment. These are important statements considering that the hepatic metabolism seems to be particularly affected in tumor-bearing animals (Acco et al., 2007). For instance, W-256 tumor reduces ureagenesis from alanine in isolated perfused liver. Ureagenesis from exogenously supplied ammonia is also reduced by this tumor unless aspartate is the exogenous carbon source, but urea production from arginine is substantially increased, suggesting also increased polyamine synthesis (Corbello-Pereira et al., 2004). Plasmatic urea levels of tumor-bearing rats treated with UT were similar to that from baseline group ($40.7 \pm 12.3 \text{ mg dL}^{-1}$). For example, the UT intermediary dose (50 mg kg^{-1}) provoked ~82% of reduction in urea plasmatic level ($37.5 \pm 6.9 \text{ mg dL}^{-1}$) in comparison with control rats ($208.9 \pm 47.9 \text{ mg dL}^{-1}$). These data are compatible with the reduction of plasma AST, indicating that the hepatocytes integrity and maintenance of intracellular AST could reflect in normalization of urea production. This is relevant at least when aspartate is the substrate, because the availability of this aminoacid seems to be critical for urea synthesis in the liver of tumor-bearing rats, which is possibly unable to produce aspartate in sufficient amounts from endogenous sources (Corbello-Pereira et al., 2004). These results demonstrate that UT can also act controlling some important hepatic metabolic pathway, such as ureagenesis, in tumor-bearing rats.

5. Conclusions

The present work demonstrates a relevant antineoplastic effect of an UT hydroalcoholic extract on W-256, an *in vivo* solid tumor model. Furthermore, this effect is related with its antioxidant properties via ROS-regulating mechanisms and metabolism homeostasis, probably among the concomitant action of several other means. Our data indicates that the combined antineoplastic and antioxidant actions of UT are indeed promising for its application in neoplastic diseases, especially in solid tumors.

Acknowledgments

The authors would like to express their gratitude to Prof^a. Dr^a. Helena Cristina da Silva de Assis and Prof^a. Dr^a. Maria Consuelo de Andrade Marques for supporting some laboratorial materials and equipments used in the presented experiments.

References

- Acco, A., Alves da Silva, M.H., Batista, M.R., Yamamoto, N.S., Bracht, A., 2007. Action of celecoxib on the hepatic metabolic changes induced by the Walker-256 tumor in rats. *Basic and Clinical Pharmacology and Toxicology* 101, 294–300.
- Aebi, H., 1984. Catalase In Vitro, 105. Academic Press, pp. 121–126.
- Aguiar, J.L., Rojas, P., Marcelo, A., Plaza, A., Bauer, R., Reininger, E., Klaas, C.A., Mersfort, I., 2002. Anti-inflammatory activity of two different extracts of *Uncaria tomentosa* (Rubiaceae). *Journal of Ethnopharmacology* 81, 271–276.
- Aquino, R., De Feo, V., De Simone, F., Pizza, C., Cirino, G., 1991. Plant metabolites. New compounds and anti-inflammatory activity of *Uncaria tomentosa*. *Journal of Natural Products* 54, 453–459.
- Bacher, N., Tiefenthaler, M., Sturm, S., Stuppner, H., Ausserlechner, M.J., Kofler, R., Konwalinka, G., 2006. Oxindole alkaloids from *Uncaria tomentosa* induce apoptosis in proliferating, G0/G1-arrested and bcl-2-expressing acute lymphoblastic leukaemia cells. *British Journal of Haematology* 132, 615–622.
- Bastos-Pereira, A.L., Lugarini, D., de Oliveira-Christoff, A., Avila, T.V., Teixeira, S., Pires, A.D., Muscará, M.N., Cadena, S.M., Donatti, L., da Silva de Assis, H.C., Acco, A., 2009. Celecoxib prevents tumor growth in an animal model by a COX-2 independent mechanism. *Cancer Chemotherapy and Pharmacology* 65, 267–276.
- Bechtel, W., Bauer, G., 2009. Catalase protects tumor cells from apoptosis induction by intercellular ROS signaling. *Anticancer Research* 29, 4541–4557.
- Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry* 72, 248–254.
- Braun, J.P., Benard, P., Burgat, V., Rico, A.G., 1983. Gamma glutamyl transferase in domestic animals. *Veterinary Research Communications* 6, 77–90.
- Brigatte, P., Sampaio, S.C., Gutierrez, V.P., Guerra, J.L., Sinhorini, I.L., Curi, R., Cury, Y., 2007. Walker 256 tumor-bearing rats as a model to study cancer pain. *The Journal of Pain: Official Journal of the American Pain Society* 8, 412–421.
- Caballero, M., Arsenak, M., Abad, M.J., Caseri, I.M., Taylor, P.G., 2005. Effect of 3 plant extracts on b16-b16 melanoma cell growth and metastasis in C57bl/6 mice. *Acta Científica Venezolana* 56, 32–36.
- Calabrese, V., Cornelius, C., Mancuso, C., Lentile, R., Stella, A.M., Butterfield, D.A., 2010. Redox homeostasis and cellular stress response in aging and neurodegeneration. *Methods in Molecular Biology* 610, 285–308.
- Cerri, R., Aquino, R., De Simone, F., Pizza, C., 1988. New quinovic acid glycosides from *Uncaria tomentosa*. *Journal of Natural Products* 51, 257–261.
- Chen, Y., Wang, M., Rosen, R.T., Ho, C.T., 1999. 2-2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* thunb. *Journal of Agricultural and Food Chemistry* 47, 2226–2228.
- Cheng, A.C., Jian, C.B., Huang, Y.T., Lai, C.S., Hsu, P.C., Pan, M.H., 2007. Induction of apoptosis by *Uncaria tomentosa* through reactive oxygen species production, cytochrome c release, and caspases activation in human leukemia cells. *Food and Chemical Toxicology* 45, 2206–2218. An International Journal Published for the British Industrial Biological Research Association.
- Choi, H.R., Choi, J.S., Han, Y.N., Bae, S.J., Chung, H.Y., 2002. Peroxynitrite scavenging activity of herb extracts. *Phytotherapy Research* 16, 364–367.
- Corbello-Pereira, S.R., Danronqui, E., Constantini, J., Silva, M.H.R., Yamamoto, N.S., Bracht, A., 2004. The urea cycle and related pathways in the liver of Walker-256 tumor-bearing rats. *Biochimica et Biophysica Acta* 1688, 187–196.
- Corti, A., Duarte, T.L., Giommarelli, C., 2009. Membrane gamma-glutamyl transferase activity promotes iron-dependent oxidative DNA damage in melanoma cells. *Mutation Research* 669, 112–121.
- De Almeida Salles Perroud, A.P., Ashimine, R., De Castro, G.M., Guimarães, F., Vieira, K.P., Aparecida Vilella, C., Samico Cavalcanti, T.C., De Lima Zollner, R., 2006. Cytokine gene expression in Walker 256: a comparison of variants A (aggressive) and AR (regressive). *Cytokine* 36, 123–133.
- De Martino, L., Martinot, J.L., Franceschelli, S., Leone, A., Pizza, C., De Feo, V., 2006. Proapoptotic effect of *Uncaria tomentosa* extracts. *Journal of Ethnopharmacology* 11, 91–94.

- DeWeese, T.L., Hruszkewycz, A.M., Marnett, L.J., 2001. Oxidative stress in chemoprevention trials. *Urology* 57, 137–140.
- El-Najjar, N., Chatila, M., Moukadem, H., Vuorela, H., Ocker, M., Gandesiri, M., Schneider-Stock, R., Gali-Muhtasib, H., 2009. Reactive oxygen species mediate thymoquinone-induced apoptosis and activate ERK and JNK signaling. *Apoptosis*, doi:10.1007/s10495-009-0421-z. *An International Journal on Programmed Cell Death*, Epub ahead of print.
- Fang, J., Nakamura, H., Iyer, A.K., 2007. Tumor-targeted induction of oxystress for cancer therapy. *Journal of Drug Targeting* 15, 475–486.
- Fazio, A.L., Ballém, D., Cesari, I.M., Abad, M.J., Arsenak, M., Taylor, P., 2008. An ethanolic extract of *Uncaria tomentosa* reduces inflammation and B16-BL6 melanoma growth in C57BL/6 mice. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 7, 217–224.
- Gao, R., Yuan, Z., Zhao, Z., Gao, X., 1998. Mechanism of pyrogallol autoxidation and determination of superoxide dismutase enzyme activity. *Bioelectrochemistry and Bioenergetics* 45, 41–45.
- García Giménez, D., García Prado, E., Sáenz Rodríguez, T., Fernández Arche, A., De la Puerta, R., 2010. Cytotoxic effect of the pentacyclic oxindole alkaloid mitraphylline isolated from *Uncaria tomentosa* bark on human Ewing's sarcoma and breast cancer cell lines. *Planta Medica* 76, 133–136.
- García Prado, E., García Gimenez, M.D., De la Puerta Vázquez, R., Espartero Sánchez, J.L., Sáenz Rodríguez, M.T., 2007. Antiproliferative effects of mitraphylline, a pentacyclic oxindole alkaloid of *Uncaria tomentosa* on human glioma and neuroblastoma cell lines. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 14, 280–284.
- Goldberg, D.M., 1980. Structural, functional, and clinical aspects of gamma-glutamyltransferase. *CRC Critical Reviews in Clinical Laboratory Sciences* 12, 1–58.
- Habig, W.H., Papst, M.J., Jakoby, W.B., 1974. Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249, 7130–7139.
- Hanigan, M.H., Frierson, H.F., Swanson, P.E., De Young, B.R., 1999. Altered expression of gamma-glutamyl transpeptidase in human tumors. *Human Pathology* 30, 300–305.
- Jagadeesan, G., Kavitha, A.V., 2006. Recovery of phosphatase and transaminase activity of mercury intoxicated *Mus musculus* (Linn.) liver tissue by *Tribulus terrestris* (Linn.) (Zygophyllaceae) extract. *Tropical Biomedicine* 23, 45–51.
- Jiang, Y., Woolard, A.C.S., Wolff, S.P., 1991. Lipid hydroperoxide measurement by oxidation of Fe²⁺ in the presence of xilenol orange. Comparison with the TBA assay and an iodometric method. *Lipids* 26, 853–856.
- Jumes, F.M.D., Lugarini, D., Bastos-Pereira, A.L., Oliveira, A., Christoff, A.O., Linde, G.A., Valle, J.S., Colauto, N.B., Acco, A., 2010. Effects of *Agaricus brasiliensis* mushroom in Walker-256 bearing rats. *Canadian Journal of Physiology and Pharmacology* 88, 21–27.
- Kaneko, K., 1989. *Clinical Biochemistry of Domestic Animals*, 4 ed. Academic Press, San Diego, p. 932.
- Laus, G., Keplinger, D., 1994. Separation of stereoisomeric oxindole alkaloids from *Uncaria tomentosa* by high performance liquid chromatography. *Journal of Chromatography A* 662, 243–249.
- Lemaire, I., Assinewe, P., Cano, P., Awang, D.V., Arnason, J.T., 1999. Stimulation of interleukin-1 and -6 production in alveolar macrophages by the neotropical liana, *Uncaria tomentosa*. *Journal of Ethnopharmacology* 64, 109–115.
- Maellaro, E., Dominici, S., Del Bello, B., Valentini, M.A., Pieri, L., Perego, P., Supino, R., Zunino, F., Lorenzini, E., Paolicchi, A., Comperti, M., Pompella, A., 2000. Membrane gamma-glutamyl transpeptidase activity of melanoma cells: effects on cellular H₂O₂ production, cell surface protein thiol oxidation and NF-κB activation status. *Journal of Cell Science* 113, 2671–2678.
- Mizuno, M., Minato, K., Ito, H., Kawade, M., Terai, H., Tshuchida, H., 1999. Antitumor polysaccharide from the mycelium of liquid cultured *Agaricus blazei* mill. *Biochemistry and Molecular Biology International* 47, 707–714.
- Moran, E.C., Kamiguti, A.S., Cawley, J.C., Pettitt, A.R., 2002. Cytoprotective antioxidant activity of serum albumin and autocrine catalase in chronic lymphatic leukaemia. *British Journal of Haematology* 116, 316–328.
- Nemesánszky, E., Lott, J.A., 1985. Gamma-glutamyltransferase and its isoenzymes: progress and problems. *Clinical Chemistry* 31, 797–803.
- Peichoto, M.E., Teibler, P., Ruiz, R., Leiva, L., Acosta, O., 2006. Systemic pathological alterations caused by *Philodryas patagoniensis* colubrid snake venom in rats. *Toxicol* 48, 520–528.
- Pelicano, H., Carney, D., Huang, P., 2004. ROS stress in cancer cells and therapeutic implications. *Drug Resistances Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy* 7, 97–110.
- Petterino, C., Argentino-Storino, A., 2006. Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. *Experimental and Toxicologic Pathology* 57, 213–219.
- Pilarski, R., Zieliński, H., Ciesiołka, D., Gulewicz, K., 2006. Antioxidant activity of ethanolic and aqueous extracts of *Uncaria tomentosa* (Willd.) DC. *Journal of Ethnopharmacology* 8, 18–23.
- Piscoya, J., Rodriguez, Z., Bustamante, S.A., Okuhama, N.N., Miller, M.J., Sandoval, M., 2001. Efficacy and safety of freeze-dried cat's claw in osteoarthritis of the knee: mechanisms of action of the species *Uncaria guianensis*. *Inflammation Research: Official Journal of the European Histamine Research Society* 50, 442–448.
- Pompella, A., De Tata, V., Paolicchi, A., Zunino, F., 2006. Expression of gamma-glutamyltransferase in cancer cells and its significance in drug resistance. *Biochemical Pharmacology* 71, 231–238.
- Portugal, J., Bataller, M., Mansilla, S., 2009. Cell death pathways in response to anti-tumor therapy. *Tumori* 95, 409–421.
- Reinhard, K.H., 1997. *Uncaria tomentosa* (Willd) DC: cat's claw, uña de gato, oder Katzenkralle. *Zeitschrift für Phytotherapie* 18, 112–121.
- Riva, L., Coradini, D., Di Fronzo, G., De Feo, V., De Tommasi, N., De Simone, F., Pizza, C., 2001. The antiproliferative effects of *Uncaria tomentosa* extracts and fractions on the growth of breast cancer cell line. *Anticancer Research* 21, 2457–2461.
- Rizzi, R., Re, F., Bianchi, A., De Feo, V., De Simone, F., Bianchi, L., Stivala, L.A., 1993. Mutagenic and antimutagenic activities of *Uncaria tomentosa* and its extracts. *Journal of Ethnopharmacology* 38, 63–77.
- Ruhl, C.E., Everhart, J.E., 2009. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. *Gastroenterology* 136, 477–485.
- Sandoval, M., Charbonnet, R.M., Okuhama, N.N., Roberts, J., Krenova, Z., Trentacosti, A.M., Miller, M.J., 2000. Cat's claw inhibits TNF-alpha production and scavenges free radicals: role in cytoprotection. *Free Radical Biology and Medicine* 29, 71–78.
- Sandoval, M., Okuhama, N.N., Zhang, X.J., Condezo, L.A., Lao, J., Angeles, F.M., Musah, R.A., Bobrowski, P., Miller, M.J., 2002. Anti-inflammatory and antioxidant activities of cat's claw (*Uncaria tomentosa* and *Uncaria guianensis*) are independent of their alkaloid content. *Phytomedicine* 9, 325–337.
- Sandstrom, P.A., Buttkie, T.M., 1993. Autocrine production of extracellular catalase prevents apoptosis of the human CEM T-cell line in serum-free medium. *Proceedings of the National Academy of Sciences of the United States of America* 90, 4708–4712.
- Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry* 25, 192–205.
- Sheng, Y., Pero, R.W., Amiri, A., Bryngelsson, C., 1998. Induction of apoptosis and inhibition of proliferation in human tumor cells treated with extracts of *Uncaria tomentosa*. *Anticancer Research* 18, 3363–3368.
- Sheng, Y., Li, L., Holmgren, K., Pero, R.W., 2001. DNA repair enhancement of aqueous extracts of *Uncaria tomentosa* in a human volunteer study. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 8, 275–282.
- Skulachev, V.P., 2000. Mitochondria in the programmed death phenomena; a principle of biology: "it is better to die than to be wrong". *IUBMB Life* 49, 365–373.
- Targher, G., Kendrick, J., Smits, G., Chonchol, M., 2009. Relationship between serum gamma-glutamyltransferase and chronic kidney disease in the United States adult population. In: *Findings from the National Health and Nutrition Examination Survey 2001–2006*. Nutrition, Metabolism & Cardiovascular Diseases., doi:10.1016/j.numecd.2009.05.012, Epub ahead of print.
- Tome, M.E., Baker, A.F., Powis, G., Payne, C.M., Briehl, M.M., 2001. Catalase-overexpressing thymocytes are resistant to glucocorticoid-induced apoptosis and exhibit increased net tumor growth. *Cancer Research* 61, 2766–2773.
- Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions* 10, 1–40.
- Vandenabeele, P., Vanden Berghe, T., Festjens, N., 2006. Caspase inhibitors promote alternative cell death pathways. *Science's STKE: Signal Transduction Knowledge Environment* 358, 44.
- Vicentino, C., Constantin, J., Aparecido Stecanella, L., Bracht, A., Yamamoto, N.S., 2002. Glucose and glycogen catabolism in perfused livers of Walker-256 tumor-bearing rats and the response to hormones. *Pathophysiology* 8, 175–182.
- Von Eynatten, K., Bauer, G., 2001. Central and ambivalent role of hydrogen peroxide during intercellular induction of apoptosis. *International Journal of Oncology* 18, 1169–1174.
- Wirth, C., Wagner, H., 1997. Pharmacologically active procyanidines from the bark of *Uncaria tomentosa*. *Phytomedicine* 4, 265–266.
- Wurm, M., Kacani, L., Laus, G., Keplinger, K., Dierich, M.P., 1998. Pentacyclic oxindole alkaloids from *Uncaria tomentosa* induce human endothelial cells to release a lymphocyte-proliferation-regulating factor. *Planta Medica* 64, 701–704.