

HIGH DOSES OF *UNCARIA TOMENTOSA* (CAT'S CLAW) REDUCE BLOOD GLUCOSE LEVELS IN RATS

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ABSTRACT

Objective: *Uncaria tomentosa* (UT) is a medicinal plant employed worldwide for its anti-inflammatory and immunomodulatory activities. Thus, the purpose of the present studies was to verify if UT could promote toxic and/or immunotoxic effects in rats following exposure for 90 days.

Methods: Forty male rats were orally treated with 15, 75 or 150mg/kg of UT. At the end, the rats were sacrificed for evaluations of the biochemistry, haematology, histopathology, status of the lymphoid and non-lymphoid organs.

Results: The results revealed an increase in the levels of ALT in the animals treated with 75mg/kg and a reduction in the glycaemic levels of rats treated with 75 and 150mg/kg of UT. However, only rats treated with the higher dose showed a slight centrilobular hepatic vacuolation; thus, ALT data alone are not suggestive of a hepatic adverse effect of UT following long-term treatment. UT promoted a reduction in blood glucose levels of the rats.

Conclusion: This effect could present an important risk for diabetic humans, who are susceptible to the development of hypoglycaemia and who might use UT for purposes other than anti-diabetes. In closing, these studies demonstrated that, while UT has no immunotoxic effect, long-term UT treatment at high doses can promote hepatotoxicity and hypoglycaemia.

Keywords: Cat's claw, Phytotherapeutic, Long-term study, Immunotoxicology.

INTRODUCTION

Plants used for medicinal purposes merge over time with human development; it is well known that early civilisation employed medicinal plants, as seen in the Ebers Papyrus, which dates earlier than 2,000 B.C [1]. Currently, the world population is not only continuing to employ medicinal plants but it has begun to make extensive use of phytotherapies. This trend is at least partially due to the belief that natural products are innocuous.

Among these phytotherapeutic plants, there is a vine found in the tropical forests of the Amazonian region and belonging to the Rubiaceae family; this plant is named *Uncaria tomentosa* (Willd.) DC (Rubiaceae) and is known as "Cat's claw" [2]. *Uncaria tomentosa* (UT) is usually commercialised as a phytotherapeutic and is employed worldwide for the treatment of many different diseases and conditions, such as arthritis, rheumatism [3], gastrointestinal injuries [4], wounds, abscesses, asthma, fever and even contraception [2]. In fact, in 1994, a conference of the World Health Organization (WHO) recognised and classified UT as a true "medicinal plant" due to its broad use in folk medicine [5, 6].

Phytochemical studies using UT revealed that this plant possesses approximately 50 potentially bioactive chemical constituents [7]; they are classified as triterpenoids, steroids, esters, glycosides of quinovic acid, procyanidins and indolic and oxindole alkaloids [8, 9]. These compounds are able to act individually or synergistically, triggering the effects of UT [10].

Among these constituents, many reports have revealed that oxindole pentacyclic alkaloids show immunomodulatory properties [2, 11]. Others have reported a UT induced increase of granulocyte-macrophage colony-stimulating factor [12], lymphocyte proliferation [2, 11, 13] and, of special interest, anti-inflammatory effects [14-16].

Despite the numerous studies that have revealed these therapeutics properties, no UT research is currently focused on the possible toxicity of this plant and its phytotherapies. In addition, it is important to highlight that immunomodulatory effects do not always reflect a positive attribute. Thus, the aim of the present study was to verify the toxicity of UT and to employ immunotoxicological

protocols to verify the scope of the safety of UT. The utilised protocols will include those suggested by many regulatory agencies, such as the Food and Drug Administration (FDA), the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (US-EPA).

MATERIALS AND METHODS

The phytotherapeutic and its chemical compounds (dosage preparations)

A dried bark extract of UT was purchased (Lot# UNX01/0311, certified to contain 0.65% of total alkaloids) as this is usually employed as the raw material for the production of the phytotherapeutic obtained from the bark of the stems of the plant; quality control certification of this material was also provided by the manufacturer: "Santosflora: Herbs, Spices and Dry Extracts, Ltd." (Tatuape, Sao Paulo, Brazil).

The dried bark powder was weighed (200mg) and extracted with 60% ethanol (10mL) in an ultrasonic bath (20min, 30°C). An aliquot of 2mL was cleaned up by solid-phase extraction (SPE) on polyamide before analysis. The cartridge was conditioned with 2mL of methanol and then 5mL of water. After sample introduction, the alkaloids were eluted with 2mL of 60% ethanol and transferred to a 5mL volumetric flask.

The analytical method was previously described by Bertol et al.[17]. The nano LC system used consisted of a Shimadzu Proeminence Nano HPLC System (Shimadzu, Japan) with an autoinjector for solvent delivery and sample introduction. All analyses were made with a Zorbax XDB C18 column (150mm×4.6mm, 3.5µm). The mobile phase was 35mM triethylammonium acetate buffer (0.2%v/v), pH 6.9 (A) and acetonitrile (B). Gradient composition (A:B): 0-18min (65:35); 18-32min (50:50). Flow rate was 0.8mL/min and detection was at 245nm. Myrtraphylline was used as standard of pure alkaloid. Peaks were assigned on the basis of their retention times compared to standard (myrtraphylline). In the absence of reference compounds, the results for the tetracyclic compounds allowed a percentage calculated in terms of the total amount of the oxindole alkaloids. For quantification of all alkaloids, a five point calibration curve of mitraphylline was used (8-200µg/mL).

The major alkaloids of UT are represented by six stereoisomers of pentacyclic alkaloids, namely, pteropodine, isopteropodine, speciophylline, uncarine F, mytraphylline and isomytraphylline. The two major tetracyclic alkaloids identified as ryncophylline and isoryncophylline and two minor tetracyclic alkaloids, corynoxine and isocorynoxine are also described.

The results indicate that pteropodine (20%), isopteropodine (12%) and isomytraphylline (10%) were the most abundant alkaloids in the bark extract. Speciophylline (11%), uncarine F (4%) and mytraphylline (18%) correspond to 33% of pentacyclics alkaloid content, totaling 75%. The content of tetracyclics alkaloids was 25%, determined as ryncophylline (15%), isoryncophylline (10%). Corynoxine and isocorynoxine were detected but it was not possible to quantify them, as they are considered trace alkaloids (Figure 1). These results are in accordance to the literature [18].

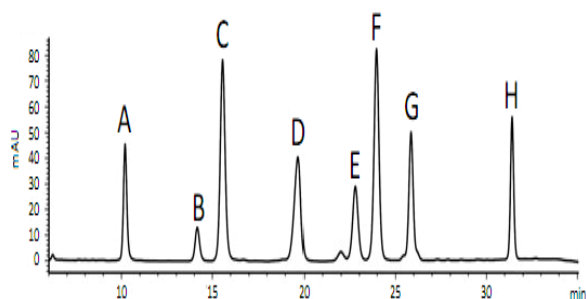


Fig. 1: HPLC chromatogram of alkaloid-rich fraction from the barks of *Uncaria tomentosa* L. (Rubiaceae) whereby eight alkaloids were identified, including: (A) speciophylline; (B) uncarine F; (C) mytraphylline; (D) ryncophylline; (E) isomytraphylline; (F) pteropodine; (G) isorhynchophylline; (H) isopteropodine.

Animals

Male adult Wistar Hannover rats (60 days of age) were obtained from our colony in the Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo. The animals were used in accordance with the ethical principles and procedures adopted by the Bioethics Committee of the University of São Paulo (protocol 2500/2011, approved on 15/02/2012). All rats were housed separately in cages measuring 40x50x20cm, and the cage bottoms were lined with sterilised wood shavings. Cages were checked daily over the entire experimental period for soft faeces, food waste and mortality. The animals received food and water *ad libitum* and were maintained under controlled condition of temperature (22-25°C), relative humidity (50-65%) and lighting (12/12h light/dark cycle).

Reagents

Ethylenediaminetetraacetic acid (EDTA) 10% 3 mM and phormol 10% were supplied by Merck (Sao Paulo, SP, Brazil). Archote (Curitiba, PR, Brazil) provided the ethanol. The RPMI culture medium-1640 and the trypan blue were obtained from Gibco (Sao Paulo, SP Brazil).

Laborlab (Sao Paulo, SP, Brazil) supplied assay kits for albumin, total protein, alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), γ -glutamyl transpeptidase (GGT), glucose, cholesterol, triglycerides and *high*-density lipoproteins (HDL), urea and creatinine. Keyhole limpet hemocyanin (KLH) was obtained from Merck (Darmstadt, Germany). Dichlorofluorescein diacetate (DCFH-DA) was obtained from Molecular Probes. Lipopolysaccharide (LPS 0127:B8), peroxidase, phorbol myristate acetate (PMA), and propidium iodide (PI) were purchased from Sigma Chemical Co (San Diego, California, USA). RPMI culture medium-1640 and trypan blue were obtained from Gibco (São Paulo, SP, Brazil).

Toxicological and immunotoxicological evaluations

Dried extract of UT was weighed, suspended and mixed in distilled water to achieve final concentrations of 15, 75 and 150mg of dry extract of UT/mL for use.

Forty rats were randomly divided into one control group and three experimental groups. Rats from the experimental groups were treated once daily with 15 (usual therapeutic dose), 75 and 150mg/kg of body weight by gavage for 90 consecutive days. On day 91, all rats were deeply anaesthetised with xylazine and ketamine (5 and 50mg/kg of body weight, respectively) for blood collection from the caudal cava vein. After euthanasia by cervical dislocation, lymphoid organs (thymus and spleen) and bone marrow from the left femur were removed for lymphohematopoietic organ and cellularity analysis.

Blood collection was performed with and without EDTA for the evaluation of haematological and serum biochemical analyses, respectively. The haematological parameters were assessed using automatic Horiba® ABX equipment; differential white blood cell analyses were performed manually.

Serum biochemistry was analysed using a Celm-SBA200-biochemistry analyser with the corresponding reagents. The measured markers were albumin, total protein, ALP, ALT, AST, GGT, glucose, cholesterol, triglycerides, HDL, urea and creatinine. Tissue samples of liver, Peyer's patches, mesenteric lymph nodes, adrenal glands and kidney were collected from 5 animals/group and fixed in 10% formalin, routinely embedded in paraffin, cut into 5- μ m thick sections and stained with haematoxylin and eosin for histopathology.

In addition, brain, heart, liver, lungs and kidney were collected from 5 animals/group, weighed in an analytical balance and then placed in an oven at 60°C for 24 hours to determine their relative dry weight. After the relative organ weight analysis of thymus and spleen, the thymus was fixed in phormol for histopathology and morphometric evaluation.

A fragment of a known weight of splenic tissue were prepared, washed in cold RPMI-1640 culture medium and resuspended in 5 mL of the culture medium for a splenocyte count; the remaining fragment was fixed for histopathology. Bone marrow cell suspensions were produced by flushing the marrow cavity of the left femur of each rat with ice-cold RPMI-1640 medium using a sterile syringe with a 26-gauge needle. Viability of white blood cells from the spleen and bone marrow was assessed by a trypan blue dye exclusion test, and cell numbers were determined with a Countess Automated Cell Counter equipment (Invitrogen, SP, Brazil). The histological sections of the rat's thymuses underwent morphometric analysis in an Image-J system, Version 1.43 m (Wayne Rasband, National Institute of Mental Health, USA). The ratio of cortical to medullar areas (C/M) was determined with the formula $C/M = \text{Total cortex area} / [(\text{Total area}) - (\text{Total medulla area})]$.

Delayed-Type Hypersensitivity assay and serum immunoglobulin evaluation

Forty rats were randomly distributed into 4 groups, one control and three experimental groups. All animals were sensitized with KLH as described by Exon et al. [19]. Briefly, KLH (5mg/mL) was injected into the caudal tail fold in a 200 μ L volume of sterile water on experimental days 75 and 82. At the end of the experimental period (Day 89), all of the rats were challenged with heat-aggregated KLH (80°C for 1 hr) in 0.1mL of saline (20mg/mL).

The challenge antigen was injected into one footpad, while the other footpad received sterile saline. Swelling was measured with a micrometer and calculated by subtracting the thickness of the saline-injected left footpad from that of the KLH-injected right footpad. Swelling was then plotted as the difference between the measurements. Before euthanasia, rats were anesthetized for blood collection from the abdominal cava vein in order to determine serum immunoglobulin (IgG) levels. Serum IgG was analysed using a Celm-analyser® with corresponding reagents (Laborlab).

Evaluation of innate immunity

Another 40 rats were also distributed into 4 groups, one control and 3 experimental groups treated with UT. On experimental day 85, all rats received an intraperitoneal injection of 5mL of sterilized solution of thioglycolate 3% to induce macrophage elicitation in this

cavity. On the last experimental day, rats were killed to perform the macrophage analyses. For that, 10mL of ice cold PBS was slowly injected into the peritoneal cavity using a 26G1/2 needle. After washing the cavity with PBS, the macrophages were collected, and 100µL of the solution obtained was used to analyze the phagocytic activity and the oxidative macrophage burst response. The method of analysis was adopted from that outlined by Hasui et al. [20]. To analyze the oxidative burst response, cells obtained from each rat (2×10^5 cells/tube) were incubated for 30 minutes at 37°C with either dichlorofluorescein diacetate (DCFH-DA), DCFH-DA + phorbol myristate acetate (PMA) or DCFH-DA + *Staphylococcus aureus* conjugated to propidium iodide (SAPI). To analyze the phagocytic activity, the cells were incubated for 30 minutes at 37°C with either SAPI or DCFH-DA and SAPI.

Ten thousand neutrophils were then acquired using flow cytometry (FACS Calibur FACSCalibur flow cytometer equipped with Cell Quest Pro® software (Becton Dickinson [BD] Immunocytometry System), and the data were analyzed using FlowJo 7.2.2® software (Tree Star Inc, Ashland, KY).

Statistical analysis

The data were analysed using GraphPad Prism 5.03® software (GraphPad Software, Inc., San Diego, California, USA) by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. Kruskal-Wallis analysis of variance followed by the Dunn test for multiple comparisons was employed for the nonparametric data. Data are expressed as the mean \pm standard error of the mean (SEM), and differences between the control and the experimental groups were considered to be statistically significant when *P < 0.05 and **P < 0.01.

RESULTS

Toxicity signs, food consumption, body weight gain

During the experimental period, none of the rats showed signs of toxicity, such as soft faeces, depression, piloerection, neurological signs or mortality. Table 1 shows the total food consumption and the total body weight gain of rats treated with or without UT; no significant differences were observed in the signs of toxicity among the groups of rats analysed.

Table 1: Total food consumption and body weight gain of rats treated with 0, 15, 75 and 150mg/kg of dry extract of *Uncaria tomentosa*, during 90 days, by gavage

	Groups			
	Control (n=10)	15mg/kg (n=10)	75mg/kg (n=10)	150mg/kg (n=10)
Food Consumption (g)	2368.9 \pm 80.2	2536.6 \pm 83.8	2491.5 \pm 65.3	2483.2 \pm 67.5
Body weight (g)	80.2 \pm 7.4	101.6 \pm 4.7	93 \pm 8.5	86.6 \pm 13.7

Table 2: Haematological parameters and differential white cell counts of rats treated with 0, 15, 75 and 150mg/kg of dry extract of *Uncaria tomentosa*, during 90 days, by gavage

	Groups			
	Control (n=10)	15mg/kg (n=10)	75mg/kg (n=10)	150mg/kg (n=10)
Erythrocytes ($10^6/\text{mm}^3$)	8.2 \pm 0.1	8.0 \pm 0.2	8.0 \pm 0.1	8.1 \pm 0.2
Leukocytes ($10^3/\text{mm}^3$)	5.8 \pm 0.5	5.9 \pm 0.5	6.6 \pm 0.3	6.7 \pm 0.5
Neutrophils (%)	22.4 \pm 1.1	23.7 \pm 2.1	23.2 \pm 2.3	22.4 \pm 1.9
Lymphocytes (%)	72.7 \pm 1.0	74.5 \pm 2.2	74.3 \pm 2.3	75.1 \pm 2.1
Monocytes (%)	4.0 \pm 0.6	2.2 \pm 0.7	2.6 \pm 0.4	2.0 \pm 0.5
Eosinophils (%)	0.7 \pm 0.3	0.6 \pm 0.3	0.9 \pm 0.3	0.4 \pm 0.2
Basophils (%)	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0

Table 3: Serum biochemistry analysis of rats treated with 0, 15, 75 and 150mg/kg of dry extract of *Uncaria tomentosa*, during 90 days, by gavage

Groups	*AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (mg/dL)	Pt (g/dL)	Alb (g/dL)	Glu (mg/dL)	Chol (mg/dL)	Trig (mg/dL)	HDL (mg/dL)
Control (n=8)	64.9 \pm 5.4	29.2 \pm 2.7	81.9 \pm 5.1	1.3 \pm 0.2	6.3 \pm 0.1	3.2 \pm 0.0	125.1 \pm 11.7	93.5 \pm 5.2(8)	44.8 \pm 9.3	77.3 \pm 11.6
15mg/kg (n=10)	71.6 \pm 3.9	33.0 \pm 2.3	95.1 \pm 5.8	1.1 \pm 0.2	6.6 \pm 0.1	3.2 \pm 0.0	141.9 \pm 5.1	99.7 \pm 3.6	37.1 \pm 6.1	84.1 \pm 10.0
75mg/kg (n=10)	70.1 \pm 3.3	47.2 \pm 4.0**	93.8 \pm 6.6	1.4 \pm 0.2	6.3 \pm 0.1	3.2 \pm 0.0	100.3 \pm 6.1*	91.5 \pm 2.1	49.5 \pm 5.6	78.5 \pm 12.3
150mg/kg (n=10)	72.7 \pm 4.6	39.4 \pm 2.0	98.8 \pm 4.9	1.1 \pm 0.1	6.3 \pm 0.1	3.2 \pm 0.0	94.6 \pm 4.0*	92.7 \pm 4.0	38.6 \pm 6.1	82.3 \pm 13.8

*AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transpeptidase; Pt = total proteins; Alb = albumin; Glu = glucose; Chol = cholesterol; Trig = triglycerides; HDL = high density lipoproteins. Urea and creatinine levels were not shown. *P < 0.05; **P < 0.01 versus control group. Dunnett's test.

Haematological and biochemical parameters

The statistical test employed to evaluate differences in the complete blood counts did not reveal any significant differences among the groups for all the parameters evaluated (Table 2).

Conversely, in relation to the serum biochemistry, the statistical analysis revealed an increase in the ALT levels of rats treated with 75mg/kg of the UT extract compared to untreated animals (P < 0.01). In addition, rats treated with 75 and 150mg/kg of the dry extract of UT showed diminished glycaemia levels when compared to the untreated rats (P < 0.05). No other biochemical parameters,

including renal function, showed statistically significant differences among the groups of rats (Table 3).

Lymphoid organ evaluation and relative dry weight of non-lymphoid organs

In relation to lymphoid organ evaluation (relative weight of spleen and thymus; thymus morphometry and bone marrow and spleen cellularity), no significant differences were found among all the parameters evaluated. (Table 4). When the dry relative weight of non-lymphoid organs was examined, no significant differences were observed between the treated and untreated rats (data not shown).

Table 4: Relative weight of the spleen and thymus; the thymus morphometry (cortex/medulla ratio) and the bone marrow and spleen cellularity

	Groups			
	Control (n=10)	15mg/kg (n=10)	75mg/kg (n=10)	150mg/kg (n=10)
Thymus (x10 ⁻³ g/100g of BW ^a)	54.6 ± 2.9	59.3 ± 2.7	56.3 ± 1.6	59.6 ± 3.4
Spleen (x10 ⁻³ g/100g of BW)	216.0 ± 13.7	221.8 ± 10.6	235.7 ± 7.0	234.7 ± 10.3
Spleen cellularity (x10 ⁷ /g of spleen)	8.3 ± 0.3	7.9 ± 0.5	7.6 ± 0.4	6.6 ± 0.8
Bone marrow cellularity (x10 ⁶ /cells)	0.4 ± 0.03	0.3 ± 0.05	0.4 ± 0.03	0.4 ± 0.01
C/M ratio ^b	0.4 ± 0.08	0.28 ± 0.10	0.37 ± 0.07	0.45 ± 0.10

^aBW= body weight. ^bC/M ratio = cortex/medulla ratio.

Histopathology

Histopathological evaluation of tissue samples revealed only the occurrence of mild vacuolation of hepatocytes; this effect was mainly observed in the centrilobular region from animals treated with 150mg/kg (Figure 2).

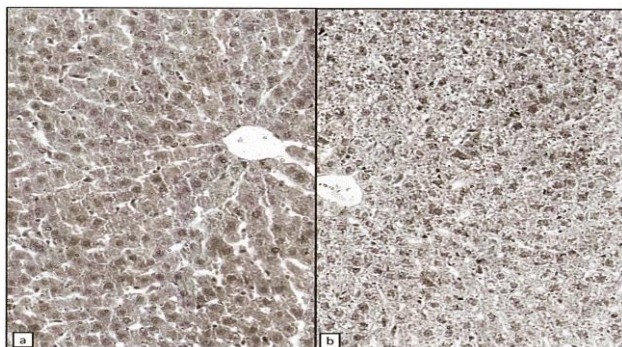


Fig. 2: Liver tissues of rats treated or not treated with *Uncaria tomentosa* dried extract over 90 days by gavage. (a) Liver section of untreated rat. (b) Note the presence of vacuolation of hepatocytes in the centrilobular zone from the rat treated with 150mg/kg. Hematoxylin and eosin stain, 20X microscope objective lens

Delayed-type hypersensitivity assay and serum immune globulin levels

Even though there was an observable reduction in tumour development in animals treated with different doses of UT as shown by the DTH assay, the treatments were not statistically different (Figure 3, $P > 0.05$). In addition, there were no statistical differences in the levels of various immunoglobulins (*i.e.* IgG) between the groups of rats (data not show).

Evaluation of innate immunity

Only animals treated with 75mg/kg of UT exhibited an increase in the effectiveness of peritoneal macrophage phagocytosis when compared with the control group (Figure 4).

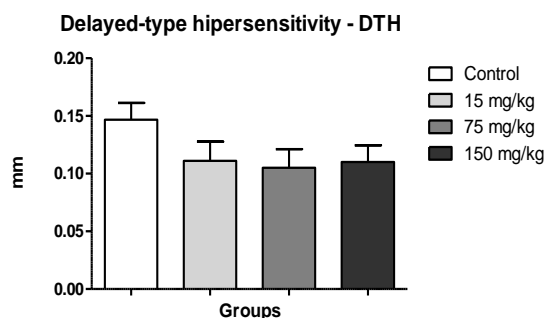


Fig. 3: Delayed-type hypersensitivity (DTH) of rats treated with 0, 15, 75 e 150mg/kg of dry extract of *Uncaria tomentosa*, during 90 days, by gavage

Effectiveness of phagocytosis

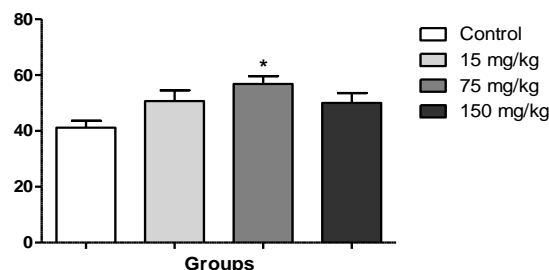


Fig. 4: Evaluation of innate immunity of rats treated with 0, 15, 75 and 150mg/kg of dry extract of *Uncaria tomentosa*, during 90 days, by gavage (mean ± SEM). * $P < 0.05$. Dunnett's test

DISCUSSION

UT is a phytotherapeutic widely employed in the treatment of many diseases, such as inflammatory and rheumatic processes, cancer and other health disturbances [21-23]. The therapeutic properties described in the manufacturer's package and advertising material focus on anecdotal reports about the safety of medicinal plants because the material is derived from a natural plant source. This in turn has led people to believe that UT has no harmful activity even when administered at high doses for prolonged periods. Thus, in the present study, we aimed to establish an exposure scenario similar to that used in folk medicine. Initially, to better mimic the effects of UT, it was necessary to assess whether the content of total alkaloids is consistent with that found in the literature [17, 18]. After chemical studies and the determination of the alkaloid content, we administered the usual therapeutic dose and a dose ten times higher for three months to verify the safety of UT and to identify its possible immunotoxic effects on rats.

The lower dose used in our study was based on the recommendation given by "Santosflora: Herbs, Spices and Dry Extracts, Ltd", and this dose was determined to be 6 capsules of 150mg/day of dried extract of UT. Considering an adult man with a 60kg body weight (the standard weight accepted by Brazilian ANVISA - National Agency of Health Surveillance), the recommended amount to be ingested is 900mg/day; thus, the lower dose employed here was 15mg/kg daily.

When *in vivo* studies examine the toxic effect of xenobiotics on the immune system, it is important to verify whether the doses used do not cause exacerbated toxicity and/or clinical symptoms in the hosts. It is well known that health disturbances such as fever [24, 25] or stress [26] can be deleterious to the immune system. One of the most significant signs of toxicity observed in animal models is anorexia and reduced body weight [27]. In the present study, no reduction of food consumption or body weight gain were observed among the groups of rats treated with the UT extract; this indicates that the doses employed here were acceptable for immunotoxic evaluation.

To standardise toxicological protocols worldwide, several regulatory agencies, including the FDA, OECD and US-EPA, proposed *in vivo* and *in vitro* models for the evaluation of the safety of pharmaceutical and other chemical compounds in humans. These studies are similar to

those employed here and were performed over a period of 90 consecutive days. Usually, these agencies preferentially use rats of both genders. However, we chose to avoid the possible influences of female hormones such as oestrogen, which is a known immunomodulator [28, 29]. Thus, we used only male rats for our studies of the possible toxic and immunotoxic effects of the UT extract.

Alteration in blood cell counts (red and white cells) is one of the first indications of a possible immunotoxic effect of a compound. The haemogram evaluated here did not reveal any deleterious effect caused by UT in these parameters. Moreover, the assessment of the bone marrow of animals treated with the phytotherapeutic did not reveal any alterations in its cellularity or in the cellularity of the spleen. In fact, despite previous reports of an *in vitro* lymphoproliferative effect [2, 11, 13] and an increased percentage of human lymphocytes [2], no alterations in immune function were observed. Nevertheless, it is important to emphasise that, at this point, no immunological challenge was performed on the rats that could have revealed any abnormal lymphoproliferative activity.

During an immune response, B lymphocytes can switch the expression of immunoglobulin class (isotype) from IgM to IgG. This Ig class switch is based on a deoxyribonucleic acid (DNA) recombination event that results in an exchange of the gene segments coding for the constant region of the Ig heavy chain, although the Ig heavy chain variable region is retained. This process changes the effectiveness of the corresponding antibody, resulting in enhanced antigen specificity [30].

Generally, the switch takes place after a committed B lymphocyte has been stimulated by a mitogen or antigen, as with the KLH sensitization here employed [31, 32]. The DTH assay did not show statistical differences due to UT treatment, and there were no changes in serum IgG levels observed between the groups of rats, indicating that UT did not promote any immunomodulator effects on either cell- or humoral immune responses.

In spite of the anti-inflammatory effects attributed to UT in the literature [14-16], the results presented here are not conclusive, since only rats treated with 75mg/kg of UT showed immunomodulatory effects on macrophage activity. In addition, the isolated increase in phagocytosis is not sufficient to reveal possible UT anti-inflammatory properties.

When the biochemical parameters were evaluated, an enhanced level of ALT from rats exposed to 75mg/kg of UT extract was observed. Furthermore, no differences were observed in AST, ALP, GGT levels and liver dry weight. It is important to highlight that, besides the occurrence of ALT in tissues, such as the muscle, heart, and kidneys [26], no adverse effects on the relative weight or histology of these organs from treated animals was found. Even though serum ALT value has long been used as surrogate marker of liver injury [33], and considered a more specific and sensitive indicator of hepatocellular injury than AST in rats, dogs and non-human primates [34], liver histopathology only revealed a slight presence of centrilobular vacuolation in rats exposed to the higher dose of UT. In addition, no occurrence of necrotic lesions was observed in any treated animals. Thus, it appears that the increased serum ALT activity found in this study does not have biological significance.

In addition to the biochemistry results, it was found that rats treated with 75 and 150mg/kg of dry extract of UT showed a reduction in blood glucose. The folk use of the *Uncaria* species worldwide is mainly for the treatment of inflammatory disorders; scientific reports about its hypoglycaemic effect are rare. Recently, an *in vitro* study performed with Malaysian *Uncaria* species extracts showed an alpha-glucosidase inhibitory activity similar to the potent glucosidase antagonist, 1-deoxyojirimycin [35]. Moreover, in their study, [36] used an aqueous-ethanol extract of UT in a streptozocin-induced diabetes model to reveal a reduction in the glycaemic levels and incidence of diabetes in the treated mice. This effect is related to ursolic acid, a pentacyclic triterpene acid found in *Uncaria hirsute*, which has many biological activities, including an anti-diabetic activity [37]. The UT species also contains ursolic acid as a chemical

constituent with biological activity [7, 10], suggesting a mechanism by which this plant could possess an anti-diabetic activity.

CONCLUSION

It is important to raise the question: how can a decrease in blood glucose levels be beneficial to a non-diabetic individual or especially to a diabetic patient who employs insulin and UT to treat an unrelated disease(s)? It is well known that hypoglycaemia can present as an adverse effect, which can range from mild dizziness with trembling and heart palpitations to more serious effects, such as unconsciousness and even seizures, coma and death. The latter effects pertain mainly to diabetic patients [38]. Thus, attention must be paid to this potentially serious adverse effect. The hypoglycaemic activity of UT in non-diabetic rats emphasises the high degree of attention that must be used by diabetics that also ingest UT.

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