



Antitumor effect of laticifer proteins of *Himatanthus drasticus* (Mart.) Plumel – Apocynaceae

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ABSTRACT

Ethnopharmacological relevance: *Himatanthus drasticus* (Mart.) Plumel – Apocynaceae is a medicinal plant popularly known as Janaguba. Its bark and latex have been used by the public for cancer treatment, among other medicinal uses. However, there is almost no scientific research report on its medicinal properties. **Aim of the study:** The aim of this study was to investigate the antitumor effects of *Himatanthus drasticus* latex proteins (HdLP) in experimental models.

Materials and methods: The *in vitro* cytotoxic activity of the HdLP was determined on cultured tumor cells. HdLP was also tested for its ability to induce lysis of mouse erythrocytes. *In vivo* antitumor activity was assessed in two experimental models, Sarcoma 180 and Walker 256 carcinosarcoma. Additionally, its effects on the immunological system were also investigated.

Results: HdLP did not show any significant *in vitro* cytotoxic effect at experimental exposure levels. When intraperitoneally administered, HdLP was active against both *in vivo* experimental tumors. However, it was inactive by oral administration. The histopathological analysis indicates that the liver and kidney were only weakly affected by HdLP treatment. It was also demonstrated that HdLP acts as an immunomodulatory agent, increasing the production of OVA-specific antibodies. Additionally, it increased relative spleen weight and the incidence of megakaryocyte colonies.

Conclusion: In summary, HdLP has some interesting anticancer activity that could be associated with its immunostimulating properties.

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1. Introduction

Himatanthus is a small Apocynaceae genus composed of 14 species (Plumel, 1991). The importance of the genus *Himatanthus* in traditional medicine is supported by reports where preparations were evaluated for mainly anti-tumor (Bolzani et al., 1999), anti-inflammatory (Miranda et al., 2000), anti-ulcerous (Baggio et al.,

2005), anti-spasmodic (Rattmann et al., 2005), antimicrobial (Souza et al., 2004), and antileishmanial (Castillo et al., 2007) activities.

Himatanthus drasticus (Mart.) Plumel – Apocynaceae is a medium-sized tree growing on firm ground in South America. It is a medicinal plant popularly known as janaguba, tiboma, jasmim-manga, raivosa, pau-de-leite, joanaguba, and sucuúba (Plumel, 1991). Its bark and latex have been used by the public mainly for cancer treatment, as an anti-inflammatory medication, and to stimulate the immune system (Amaro et al., 2006). Usually, latex plus water (janaguba milk) or decoctions of the bark or latex are taken at a dose of one cup about three times a day. Although several medicinal uses have been described, there are almost no scientific research reports to this regard.

In a preliminary study of the phytochemical and biological effects of *Himatanthus drasticus*, the ethanolic extract of stem bark was evaluated biologically. This extract was found to be toxic against brine shrimp, but showed no antimicrobial effect

Abbreviations: 5-FU, 5-fluorouracil; SBCAL, Sociedade Brasileira de Ciência em Animais de Laboratório; HDLP, *Himatanthus drasticus* latex protein; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; OVA, ovalbumin; PBMC, peripheral blood mononuclear cells; TGF- β , transforming growth factor- β ; TNF- α , tumor nuclear factor- α .

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against the pathogens tested *in vitro* (*Enterobacter*, *Streptococcus* and *Escherichia coli*). Additionally, it showed antinociceptive (writhing test in rats) affect (Colares et al., 2008). Leite et al. (2009) demonstrated the latex from *Himatanthus drasticus* to be a cytoprotective agent against ethanol-induced ulcer formation in mice.

The aim of this study was to investigate the antitumor effects of *Himatanthus drasticus* latex proteins (HdLP) in experimental models. In order to evaluate the toxicological aspects related to HdLP treatment, histopathological and morphological analyses of treated animals were also performed.

2. Material and methods

2.1. Reagents

5-Fluorouracil (5-FU), *O*-phenylenediamine dihydrochloride, Ficoll-Hypaque, phytohemagglutinin, resazurin, and ovalbumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA); rabbit antimouse total Ig (IgG, A, M) was from Serotec (Kidlington, Oxford, UK). All other reagents were of analytical grade.

2.2. Plant material, latex collection and extraction of *Himatanthus drasticus* latex proteins (HdLP)

Latex was obtained in May 2010 from uncultivated plants located in the vicinity of Fortaleza, State of Ceará, Brazil. Botanical material was identified at the Herbarium Prisco Bezerra (Departamento de Biologia, Universidade Federal do Ceará). A voucher was registered under the code 40408.

The latex was obtained after cutting stem and allowing it to flow into tap water in order to give an equal mixture of volumes. The samples were initially centrifuged ($5000 \times g$) at 10°C for 25 min. The pellet was discarded and the soluble phase was dialyzed against distilled water for 60 h at 25°C with water being renewed three times daily. Finally, the dialyzed material was centrifuged as previously done, and clean supernatant was recovered, freeze dried and used in all further determinations. This fraction, comprising almost all soluble latex protein was called HdLP.

2.3. Animals

A total of 56 Swiss mice (female, 25–30 g) and 40 Wistar rats (female, 180–220 g), obtained from the central animal house of Universidade Federal do Ceará – Brazil, were used. Animals were housed in cages with free access to food and water. All animals were kept under a 12:12 h light–dark cycle (lights on at 6:00 a.m.). Animals were treated according to the ethical principles for animal experimentation of SBCAL (Sociedade Brasileira de Ciência em Animais de Laboratório), Brazil. The Animal Studies Committee of Universidade Federal do Ceará approved the experimental protocol (number 08/08).

2.4. Cells

The cytotoxicity of HdLP was tested against HL-60 (leukemia), MDA-MB-435 (melanoma), SF-295 (brain), and HCT-8 (colon) human cancer cell lines, all obtained from the National Cancer Institute, Bethesda, MD, USA. Cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 $\mu\text{g}/\text{ml}$ streptomycin and 100 U/ml penicillin, and incubated at 37°C with a 5% CO_2 atmosphere.

In order to get healthy human peripheral blood mononuclear cells (PBMC), heparinized blood (from healthy, non-smoker donors who had not taken any drug at least 15 days prior to sampling) was

collected, and PBMC were isolated by a standard method of density-gradient centrifugation over Ficoll-Hypaque. PBMC were washed and resuspended. Cells were grown under the same conditions as above plus the addition of phytohemagglutinin (4%).

Sarcoma 180 tumor cells had been maintained in the peritoneal cavity of Swiss mice and Walker 256 carcinosarcoma tumor cells had been maintained by intramuscular inoculation of the medial side of the thigh of Wistar rats in the Laboratory of Experimental Oncology from the Universidade Federal do Ceará since the mid-1980s.

2.5. *In vitro* cytotoxic evaluation of HdLP

2.5.1. Determination of the effect of HdLP on cultured tumor cells

Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan product (Mossman, 1983). For all experiments, cells were seeded in 96-well plates (10^5 cells/well for adherent cells or 0.5×10^5 cells/well for suspended cells in 100 μl of medium). After 24 h, the test substance (0.39–50 $\mu\text{g}/\text{ml}$), dissolved in saline, was added to each well (using the HTS—high-throughput screening—Biomek 3000; Beckman Coulter Inc., Fullerton, CA, USA) and incubated for 72 h. 5-FU was used as the positive control. At the end of incubation, the plates were centrifuged and the medium was replaced by fresh medium (150 μl) containing 0.5 mg/ml MTT. Three hours later, the formazan product was dissolved in 150 μl DMSO and the absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter Inc., Fullerton, CA, USA). The drug effect was quantified as the percentage of control absorbance of reduced dye at 595 nm.

PBMC cell growth was determined by the Alamar blue assay (Ahmed et al., 1994). For all experiments, cells were seeded in 96-well plates (0.3×10^6 cells/well for suspended cells in 100 μl of medium). After 24 h, the test substance (0.39–50 $\mu\text{g}/\text{ml}$), dissolved in saline, was added to each well (using the HTS – high-throughput screening – Biomek 3000 – Beckman Coulter, Inc., Fullerton, CA, USA) and incubated for 72 h. 5-FU was used as the positive control. Twenty-four hours before the end of incubation, 10 μl of stock solution (0.312 mg/ml) of Alamar Blue (Resazurin) was added to each well. The absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter®) and the drug effect was quantified as the percentage of control absorbance at 570 nm and 595 nm. The absorbance of Alamar Blue in culture medium is measured at a higher wavelength and lower wavelength. The absorbance of the medium is also measured at the higher and lower wavelengths. The absorbance of the medium alone is subtracted from the absorbance of medium plus Alamar Blue at the higher wavelength. This value is called AO_{HW} . The absorbance of the medium alone is subtracted from the absorbance of medium plus Alamar Blue at the lower wavelength. This value is called AO_{LW} . A correction factor R_0 can be calculated from AO_{HW} and AO_{LW} , where $R_0 = \text{AO}_{\text{LW}}/\text{AO}_{\text{HW}}$. The percent Alamar Blue reduced is then expressed as follows: % reduced = $A_{\text{LW}} - (A_{\text{HW}} \times R_0) \times 100$.

2.5.2. Determination of the effect of HdLP on mouse erythrocytes

The test was performed in 96-well plates using a 2% mouse erythrocyte suspension in 0.85% NaCl containing 10 mM CaCl_2 , following the method described by Jimenez et al. (2003). HdLP was tested at concentrations ranging from 8 to 200 $\mu\text{g}/\text{ml}$. After incubation at room temperature for 30 min and centrifugation, the supernatant was removed and the hemoglobin released was measured spectrophotometrically as the absorbance at 540 nm.

2.6. *In vivo* antitumor evaluation of HdLP

2.6.1. Determination of the effect of HdLP on tumor growth in mice and rats

2.6.1.1. Sarcoma 180 tumor protocol. Ten-day-old Sarcoma 180 ascites tumor cells (2×10^6 cells per 500 μ l) were implanted subcutaneously into the left hind groin of mice (as described by Bezerra et al., 2008). One day after inoculation, HdLP (10 or 20 mg/kg, by intraperitoneal administration; 50 or 100 mg/kg, by oral administration) was dissolved in saline and administered for 7 days.

2.6.1.2. Walker 256 carcinosarcoma tumor protocol. Eight-day-old Walker 256 carcinosarcoma tumor cells (2×10^6 cells per 500 μ l) were implanted subcutaneously into the left hind groin of rats (as described by Moraes et al., 1997). One day after inoculation, HdLP (10 or 20 mg/kg, by intraperitoneal administration) was dissolved in saline and administered daily for 7 days.

On day 8, the animals were sacrificed by cervical dislocation. The tumors, livers, spleens, and kidneys were excised, weighed, and fixed in 10% formaldehyde. Percent inhibition (%) was calculated by the following formula: percent inhibition (%) = $[(A - B)/A] \times 100$, where *A* is the tumor weight average of the negative control, and *B* is that of the treated group. Body weights were determined at the start and on the last day of treatment. 5-FU (25 mg/kg/day) was used as the positive control. Negative control was treated with the vehicle used for diluting the test substance (saline).

2.6.2. Toxicological analyses – histopathology and morphological analyses

After fixation with formaldehyde, tumors, livers, spleens, and kidneys were grossly examined for size or color changes and hemorrhage. Next, portions of the tumor, liver, spleen, and kidney were cut into small pieces, followed by staining of the histological sections with hematoxylin and eosin. Histological analysis was performed by light microscopy. The presence and extent of liver, kidney or spleen lesions attributed to drugs were determined.

2.7. Immunomodulatory analyses

2.7.1. Subcutaneous immunization

Two groups of eight Swiss mice were immunized subcutaneously with a single dose of ovalbumin (OVA) (2 mg/kg) or OVA (2 mg/kg) plus HdLP (40 mg/kg). The mice were bled from the retro-orbital plexus to obtain serum samples prior to immunization and at 7, 14 and 21 days after immunization.

2.7.2. Measurement of specific antibody

Specific antibodies in serum were detected by enzyme-linked immunosorbent assay (ELISA). In order to evaluate the ability of HdLP to increase the response elicited by OVA, total Ig antibodies against OVA were determined using OVA (50 μ g/well)-coated plates. The plates were incubated at 37 °C for 1 h and washed three times with PBS-0.05% Tween. The plates were blocked with 5% non-fat milk in 10 mM potassium phosphate buffer, pH 7.2, with 0.9% NaCl (PBS) for 2 h at 37 °C and washed once, after which 100 μ l of the appropriate serum diluted in PBS was added and the plates re-incubated for 2 h at 37 °C. The plates were washed again three times with PBS-0.05% Tween and treated with peroxidase-conjugated rabbit antimouse total immunoglobulins (100 μ l/well, 1:1000 final dilution) for 2 h at room temperature. The plates were subsequently washed three times with PBS-Tween. The reaction was developed by the addition of orthophenylenediamine followed by incubation for 10 min at 37 °C. The intensity of the resulting color was read at 450 nm using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter Inc., Fullerton, CA, USA).

Table 1

Effect of *Himatanthus drasticus* latex proteins (HdLP) on human cell lines.

| Cell line | Histotype | HdLP IC ₅₀ (μ g/ml) | 5-FU IC ₅₀ (μ g/ml) |
|------------|-------------|-------------------------------------|-------------------------------------|
| HL-60 | Leukemia | >50 | 12.59 9.72–16.29 |
| HCT-8 | Colon | >50 | 0.36 0.18–0.71 |
| SF295 | Brain | >50 | 0.38 0.23–0.63 |
| MDA-MB-435 | Melanoma | >50 | 1.36 0.98–1.88 |
| PBMC | Lymphocytes | >50 | >5 |

Data are presented as IC₅₀ values and 95% confidence interval from three independent experiments, performed in duplicate. 5-Fluorouracil (5-FU) was used as the positive control.

2.8. Statistical analysis

Data are presented as mean \pm SEM or IC₅₀ values and their 95% confidence intervals (CI 95%) obtained by nonlinear regression. The differences between experimental groups were compared by ANOVA (analysis of variance) followed by Student–Newman–Keuls ($P < 0.05$). All statistical analyses were performed using the GRAPH-PAD program (Intuitive Software for Science, San Diego, CA, USA).

3. Results

3.1. *In vitro* evaluation of HdLP cytotoxicity

The *in vitro* effects of HdLP against human tumor cell lines were determined and showed no cytotoxic activity at the tested concentrations (Table 1). 5-FU, used as the positive control, showed IC₅₀ values ranging from 0.36 to 12.59 μ g/ml for HCT-8 and HL-60, respectively.

HdLP was also tested for its ability to induce lysis of mouse erythrocytes. HdLP was not hemolytic even at the highest concentration tested (200 μ g/ml, data not shown).

3.2. *In vivo* antitumor evaluation of HdLP

The effects of HdLP on mice transplanted with Sarcoma 180 tumor or rats transplanted with Walker 256 carcinosarcoma are presented in Fig. 1. HdLP, given intraperitoneally, was active against both experimental tumors showing a very similar profile. However, it was inactive by oral administration.

In mice inoculated with Sarcoma 180, the average tumor weight of the control was 2.52 ± 0.14 g on day 8. Intraperitoneal HdLP reduced tumor weight to 1.60 ± 0.18 and 1.66 ± 0.18 g at doses of 10 and 20 mg/kg/day, respectively (Fig. 1A). These reductions gave inhibition rates of 36.46% and 34.22%. At 25 mg/kg/day, 5-FU reduced tumor weight by 48.02% within the same period. Oral HdLP had no effect on tumor weight (data not shown).

In rats inoculated with Walker 256 carcinosarcoma, the average tumor weight of the control was 8.36 ± 0.55 g on day 8. Intraperitoneal HdLP reduced tumor weight to 3.52 ± 0.94 and 3.74 ± 0.95 g at doses of 10 and 20 mg/kg/day, respectively (Fig. 1B). These reductions gave inhibition rates of 57.91% and 55.23%. At 25 mg/kg/day, 5-FU reduced tumor weight by 56.34% within the same period.

Histopathological analysis of the tumors extirpated from Sarcoma 180 control mice or Walker 256 carcinosarcoma control rats showed groups of large, round and polygonal cells, with pleomorphic shapes, hyperchromatic nuclei and binucleation. In the tumors extirpated from treated animals, extensive areas of coagulative necrosis were observed (data not shown).

3.3. Toxicological analyses

After treatment with HdLP, no significant changes in the weight of livers or kidneys were seen in mice inoculated with sarcoma 180 or rats inoculated with Walker 256 carcinosarcoma (Table 2).

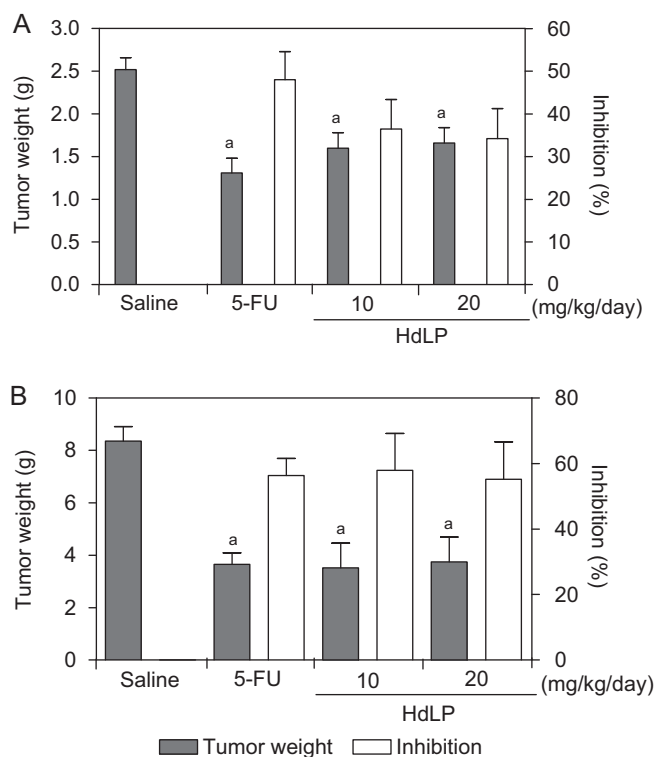


Fig. 1. Effect of *Himatanthus drasticus* latex proteins (HdLP) on mice transplanted with sarcoma 180 tumor (A) and rats transplanted with Walker 256 carcinosarcoma tumor (B). 5-Fluorouracil (5-FU, 25 mg/kg/day) was used as the positive control. Negative control was treated with the vehicle used for diluting the tested substance (saline). The animals were treated by intraperitoneal administration, starting one day after tumor implantation, for seven consecutive days. Data are presented as mean \pm S.E.M. of eight animals. ^a $P < 0.05$ compared with saline group by ANOVA followed by Student–Newman–Keuls.

However, spleen weights were significantly increased when compared to the control group, in both experimental tumors ($P < 0.05$). Body weight gain was significantly reduced after treatment with HdLP (20 mg/kg/day) or 5-FU (25 mg/kg/day) in mice inoculated with sarcoma 180.

Histopathological analyses of kidneys removed from animals treated with HdLP showed intense swelling of tubular epithelial cells, presence of hyaline cylinder and focal tubular necrosis, but the structure of the glomeruli was essentially preserved (data not shown). In the liver, histopathological analysis showed that animals treated with HdLP had numerous inflammatory foci, intense

ballooning degeneration of hepatocytes, microvesicular steatosis, and necrosis (data not shown). In the spleen, HdLP-treated animals showed a discreet increase in megakaryocyte colonies, which suggests an immunomodulatory activity (Fig. 2).

3.4. Immunomodulatory analyses

To investigate the effect of HdLP on the induction of humoral immune response in mice immunized with a single dose of OVA (2 mg/kg) or OVA (2 mg/kg) plus HdLP (40 mg/kg), the OVA-specific antibody levels in serum were measured prior to immunization and at 7, 14 and 21 days after, at a dilution of 1:3200 by ELISA. The results are shown in Fig. 3. HdLP significantly increased the amount of OVA-specific total Ig in the sera of treated animals at the dose of 40 mg/kg compared to the OVA control ($P < 0.05$).

4. Discussion

The present work reports the antitumor effects of HdLP on mice transplanted with Sarcoma 180 and rats transplanted with Walker 256 carcinosarcoma. These models are animal-originated tumors frequently used in *in vivo* cancer therapy research (Moraes et al., 1997; Lee et al., 2003; Bezerra et al., 2008; Lins et al., 2009; Chen et al., 2010). HdLP inhibited the growth of both experimental tumors, with no effect on cell proliferation *in vitro* at the concentrations tested. Additionally, its immunoadjuvant activity was also demonstrated. This is the first report describing the antitumor and immunomodulatory properties of HdLP.

HdLP did not show any significant *in vitro* cytotoxic effect at the experimental exposure levels, but it did show an *in vivo* antitumor effect. In the preclinical anticancer drug-screening program used in this study, an extract that displayed IC_{50} values below 30 μ g/ml was considered promising (Suffness and Pezzuto, 1990). HdLP showed IC_{50} values greater than 50 μ g/ml for all tumor cell lines tested, suggesting that the *in vivo* antitumor activity was not related to direct antiproliferative effects.

As previously cited, the ethanolic extract of stem bark of *Himatanthus drasticus* was active in the brine shrimp lethality test. Its extract showed a highly significant activity in this assay ($LC_{50} = 257$ ppm). The authors attributed these effects to the triterpene lupeol cinammate (Colares et al., 2008). On the other hand, the effect of its latex proteins has never been evaluated against tumor cell lines.

In the genus *Himatanthus*, the latex of *Himatanthus sucuuba* is used in its raw form as an antitumor agent (Van der Berg, 1984). Chemical studies have indicated the presence of lupeol acetate,

Table 2
Effect of *Himatanthus drasticus* latex proteins (HdLP) on organ weights.

| Drug | Dose (mg/kg/day) | Liver (g/100 g body weight) | Kidney (g/100 g body weight) | Spleen (g/100 g body weight) |
|-----------------------------------|------------------|-----------------------------|------------------------------|------------------------------|
| Healthy mice | | | | |
| Saline | – | 4.61 \pm 0.19 | 1.07 \pm 0.03 | 0.26 \pm 0.02 |
| Mice transplanted with S180 | | | | |
| Saline | – | 4.66 \pm 0.06 | 1.08 \pm 0.02 | 0.73 \pm 0.02 |
| 5-FU | 25 | 4.38 \pm 0.09 | 1.08 \pm 0.04 | 0.35 \pm 0.05 ^a |
| HdLP | 10 | 4.88 \pm 0.14 | 1.17 \pm 0.03 | 1.09 \pm 0.06 ^a |
| HdLP | 20 | 4.97 \pm 0.13 | 1.19 \pm 0.04 | 1.10 \pm 0.05 ^a |
| Healthy rats | | | | |
| Saline | – | 4.54 \pm 0.11 | 1.01 \pm 0.06 | 0.50 \pm 0.01 |
| Rats transplanted with Walker 256 | | | | |
| Saline | – | 4.83 \pm 0.11 | 0.89 \pm 0.02 | 0.43 \pm 0.01 |
| 5-FU | 25 | 4.95 \pm 0.06 | 1.04 \pm 0.04 | 0.34 \pm 0.03 ^a |
| HdLP | 10 | 4.84 \pm 0.30 | 0.88 \pm 0.06 | 0.50 \pm 0.06 |
| HdLP | 20 | 5.24 \pm 0.20 | 0.94 \pm 0.17 | 0.65 \pm 0.04 ^a |

Data are presented as mean \pm SEM of eight animals. 5-Fluorouracil (5-FU) was used as the positive control. Negative control was treated with the vehicle used for diluting the tested substance (saline). The animals were treated by intraperitoneal administration, starting one day after tumor implantation, for seven consecutive days.

^a $P < 0.05$ compared with saline group by ANOVA followed by Student–Newman–Keuls.

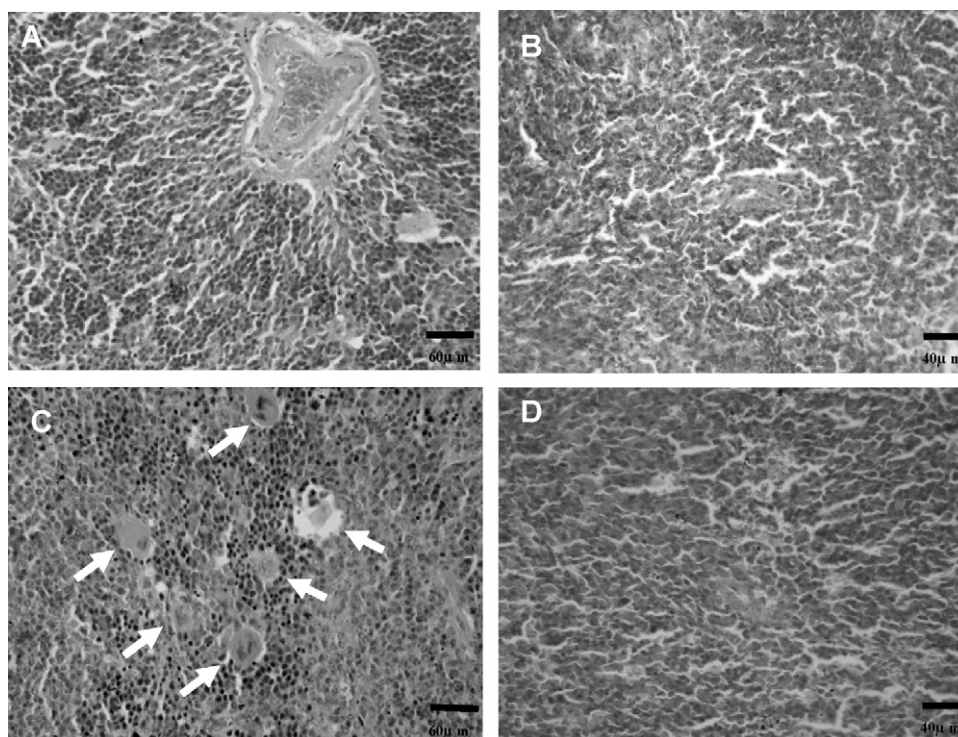


Fig. 2. Effect of *Himatanthus drasticus* latex proteins (HdLP) on spleens of mice transplanted with sarcoma 180 tumor. The animals were treated by intraperitoneal administration, starting one day after tumor implantation, for seven consecutive days. Photomicrographs show the histopathology of the spleens from saline-treated (panel A), 5-FU-treated (25 mg/kg/day, panel B), HdLP-treated (10 mg/kg/day, panel C), and HdLP-treated (20 mg/kg/day, panel D) animals, analyzed by light microscopy (400 \times). White arrow shows megakaryocyte.

alpha-amyrin, and lupeol cinnamates (Miranda et al., 2000). The ethanolic extract of leaves of *Himatanthus obovatus* showed strong cytotoxic activity in cancer cell lines (Mesquita et al., 2009). Additionally, the ethanolic extract of leaves of *Himatanthus attenuatus* showed significant toxicity against *Artemia salina* (Jiménez et al., 2001). In the family Apocynaceae, the laticifer proteins of *Calotropis procera* (Ait) was shown to exhibit selective cytotoxic effects on human cancer cell lines. It was shown to inhibit DNA synthesis, probably affecting topoisomerase I activity, leading to apoptosis in tumor cells (Oliveira et al., 2007). Recently, its *in vivo* anticancer potential was also evaluated, reinforcing the potential of laticifer proteins in treating neoplasia (Oliveira et al., 2010).

Furthermore, HdLP acts as an immunomodulatory agent, raising the production of OVA-specific antibodies. Additionally, it was demonstrated that HdLP increased relative spleen weight, and increased megakaryocytic nests, corroborating the hypothesis that it acts through immune stimulation. Thus, these data suggest that the mechanism of beneficial therapeutic effects elicited by HdLP seemed to be attributed to the potentiation of host-defense through the enhancement of immunity, in agreement with its traditional use.

Interestingly, the immunostimulant potential of the genus *Himatanthus* has been evaluated. The latex of *Himatanthus sucubus* increased nitric oxide (NO) and tumor nuclear factor- α (TNF- α) and decreased transforming growth factor- β (TGF- β) production in macrophages, suggesting an immunomodulatory activity (Soares et al., 2010). In the family Apocynaceae, experimental studies with proteins of the latex of *C. procera* suggested that the active LP fraction (PII) displays *in vivo* inhibition of tumor growth, probably by mediating an increased immunopharmacological response (Oliveira et al., 2010).

Hepatotoxicity and nephrotoxicity are very common side effects of cancer chemotherapeutic drugs. Hepatic dysfunction induced by vincristine and etoposide (King and Perry, 2001) and renal toxic-

ity induced by cisplatin (Pinzani et al., 1994) are such examples. In the present study, the integrity of liver and kidneys in animals subjected to HdLP treatment were evaluated by histopathological analyses.

The liver of HdLP-treated animals showed numerous inflammatory foci, intense ballooning degeneration of hepatocytes, microvesicular steatosis, and necrosis, suggesting intrinsic hepatotoxicity. However, regeneration of hepatic tissues occurs in many diseases. This occurs even when hepatocellular necrosis is present.

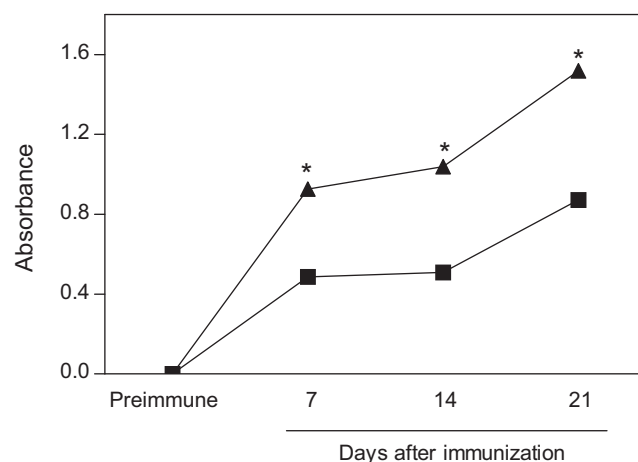


Fig. 3. Effect of *Himatanthus drasticus* latex proteins (HdLP) on the induction of humoral immune response. Mice were immunized subcutaneously with a single dose of OVA (2 mg/kg, ▲) or with OVA (2 mg/kg) plus HdLP (40 mg/kg, ■), and the production of ovalbumin-specific total Ig antibodies was determined. Sera were collected prior to immunization and 7, 14 and 21 days after immunization. Antibodies were detected by ELISA at a dilution of 1:3200. Data are presented as mean \pm SEM of eight animals. * $P < 0.05$, ANOVA followed by Student–Newman–Keuls.

In hepatic degeneration, regeneration is complete when the connective tissue is preserved (Scheuer and Lefkowitz, 2000; Kummar et al., 2004). The hepatic alterations observed in HdLP-treated animals could be considered reversible (McGee et al., 1992; Scheuer and Lefkowitz, 2000; Kummar et al., 2004).

The kidneys removed from animals-treated with HdLP showed intense swelling of tubular epithelium cell, presence of hyaline cylinder and focal tubular necrosis, but the structure of the glomeruli was essentially preserved. Necrosis of the renal tubule epithelium may often occur as a consequence of the administration of various cancer chemotherapeutic drugs (Olsen and Solez, 1994). It is worth mentioning that the histopathological analyses of HdLP-treated animals demonstrated that the interstitial tissues were preserved, suggesting that regeneration is possible (Curran, 1990; Olsen and Solez, 1994).

In summary, the histopathological analysis indicates that the liver and kidney were only weakly affected by HdLP treatment. Anyway, the HdLP-induced systemic toxicity effect needs to be more extensively evaluated to assess the safety. Then, further studies must be done to well-defined and understand the underline mechanism involved in HdLP toxic effect.

5. Conclusion

Briefly, this work showed that HdLP exhibited antitumor effects against experimental tumors without substantial toxicity. In addition, this activity seems to be related to its immunostimulant properties.

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