

Antitumor activity of leaves of *Himatanthus drasticus* (Mart.) Plumel-Apocynaceae (Janaguba) in the treatment of Sarcoma 180 tumor

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Himatanthus drasticus, also known as janaguba, is used popularly in Brazil's Northeastern region in the treatment of cancer. However, no scientific reports are available. The present study is the first investigation on the antitumor activity of crude methanolic extract from *Himatanthus drasticus* leaves against Sarcoma 180 tumor and on its side effects including acute oral toxicity. The OECD 423 methodology was used to study acute oral toxicity, and the STOCK methodology to assess antitumor activity. The crude extract showed low toxicity at the tested doses (50, 300 and 2000 mg/kg) administered orally. The histopathological analyses demonstrated alterations in liver lung, spleen and kidney. It also showed activity against Sarcoma 180 tumor in male Swiss albino mice, evidencing tumor growth inhibition of 67.7% and 68% at 300 mg/kg and 400 mg/kg doses, respectively.

Uniterms: *Himatanthus drasticus*/antitumor activity. *Himatanthus drasticus*/acute oral toxicity. Janaguba/antitumor activity. Sarcoma 180/experimental study.

Himatanthus drasticus, conhecida popularmente como janaguba, tem uma longa história de emprego na cura do câncer no nordeste brasileiro, porém quase sem registro na literatura. O objetivo desse trabalho foi investigar a atividade antitumoral do extrato bruto metanólico das folhas de *Himatanthus drasticus* frente ao modelo experimental Sarcoma 180 e avaliar sua toxicidade aguda. A determinação da toxicidade aguda foi realizada segundo a metodologia da OECD 423 e o transplante do tumor sólido de sarcoma 180 foi realizado seguindo a metodologia de Stock. O extrato apresentou baixa toxicidade nas doses testadas (50, 300 e 2000 mg/kg) por via oral. A análise histopatológica apresentou alterações em nível hepático, pulmonar, baço e renal. A atividade antineoplásica apresentou inibição tumoral significativa em relação ao grupo controle nas doses de 300 mg/kg e 400 mg/kg de peso do animal com um percentual de inibição de 67.7% e 68% respectivamente. Na menor dose analisada, 200 mg/kg, o percentual de inibição tumoral foi apenas de 32.8%.

Unitermos: *Himatanthus drasticus*/atividade antitumoral. *Himatanthus drasticus*/toxicidade aguda. Janaguba/atividade antitumoral. Sarcoma 180/estudo experimental.

INTRODUCTION

Plants contain a wide variety of compounds that have important biological activity including anticancer activity (Ramakrishna *et al.*, 1984). Several drugs used

to treat cancer are derived from natural products, such as Paclitaxel, a diterpenoid from *Taxus brevifolia* (Souza, 2004), Vinca alkaloids such as vincristine and vinblastine from *Catharanthus roseus* (Almeida *et al.*, 2005), natural alkaloid camptothecin from *Camptotheca acuminata* (Silva *et al.*, 2003), amongst others. Therefore, the analysis of plants is key to developing purified and well-defined chemical compounds for dose-controlled medications.

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The toxicity determination of plant extracts is relevant because there is no single beneficial effect whereas biotransformation occurs, and these products can have toxic side effects which are harmful to human health (Hilary *et al.*, 2004).

The plant of the *Himatanthus* (Apocynaceae) genus is a medium-sized tree, growing on firm ground in South America from Southeastern regions of Brazil to the French Guiana (Plumel, 1991). In the Araripe National Forest, south of the State of Ceará, the species found is *H. drasticus*, known as Janaguba. Its wood stem latex is popularly used in the treatment of cancer in Brazil's Northeastern Region (Amaro *et al.*, 2006a).

To our knowledge, only three papers have been reported in the literature on *H. drasticus*. However, there are no studies describing its leaves or anti-tumoral activity (Amaro *et al.*, 2006a; Colares *et al.*, 2008).

In 2008, Colares *et al.* isolated and identified an ethanolic extract from *H. drasticus* stem bark, namely, triterpene lupeol cinnamate which is probably a substance with potential for anti-tumor activity. The active ingredient of *H. sucuuba* was also isolated (an iridoid fulvoplumierin) and this also showed activity against the human epidermoid carcinoma of the nasopharynx (Perdue, Blomster, 1978).

In this study, the acute oral toxicity and antitumor activity against Sarcoma 180 tumor of the crude methanolic extract from *Himatanthus drasticus* leaves was described.

EXPERIMENTAL

Plant material

Leaves of *Himatanthus drasticus* were collected in December of 2007 at Araripe National Forest, Jardim, Ceará, Brazil, and identified by Marlene Barbosa, PhD. A sample specimen (n° 48.961) was deposited at the herbarium Geraldo Mariz of the Federal University of Pernambuco (UFPE).

Extract preparation

The leaves were dried at room temperature in fresh air for seven days, and then cut into small fragments. The sample material (500g) was macerated with methanol at room temperature (30 ± 2 °C). Three extractions were made allowing 48h for each macerated extraction. Solvent was evaporated at 60 °C under reduced pressure and dried in a high vacuum for 10 h affording the crude extract (100.6 g).

Animals

60 day-old male Swiss albino mice weighing between 25 and 35 g were housed under controlled temperature conditions (22 ± 2 °C) and 12h light/dark cycles. Food and water were available *ad libitum* before and throughout the experiments. All studies were approved by the Research Ethics Committee (CEEA) of the Federal University of Pernambuco (n° 23076.03566/2008-71) in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals" (NIH publication no. 86-23, 1985).

Acute oral toxicity

The acute toxicity of the methanolic extract from the leaves of *Himatanthus drasticus* was evaluated in mice using the up-and-down procedure (OECD, 423). Male mice, (weighing from 25-35 g, aged from 6-8 weeks) received a single initial gavage containing a 50 mg/kg dose of *Himatanthus drasticus* leaf methanolic extract. Three animals were used for each step. The dose level used as the starting dose was selected from one of the four fixed levels: 50, 300 and 2000 mg per kg of body weight. The animals were continuously observed for toxicity development during the first 4 hours of administration of each dose. Finally, the number of survivors was noted after 24 hours, and these animals were then kept alive for a further 14 days while observations were made on a daily basis. The experiment was then repeated after the end of the first trial.

Histopathological studies

Macroscopic analysis and histopathological examination of the bodies of those animals subjected to acute toxicity at higher doses were performed. The liver, kidneys, spleen and lung tissues were excised and rinsed with an ice-cold normal saline solution in order to remove blood and debris adhering to the tissues. These tissues were then fixed in a 10% formalin solution for 24 h. The fixative was removed by washing specimens thoroughly with running tap water overnight. After dehydration through a graded series of alcohols, the tissues were cleaned in methyl-benzoate, and embedded in paraffin wax. Sections were cut 7 μ m-thick and stained with hematoxylin and eosin (Michelany, 1980). After dehydration and cleaning, the slides were mounted and observed under light microscope for details.

Antitumor activity

The transplant of the solid tumor of Sarcoma 180 was carried out according to the methodology of Stock

et al. (1955). The treatment was initiated 48 hours post-tumor transplant. The animals were divided into groups ($n = 5$): standard group (Cyclophosphamide, CTX, 10 mg/kg), control group (NaCl 0.9%) and treated groups I, II and III, which received the methanolic extract from janaguba, at 200, 300 and 400 mg/kg doses, respectively. All substances had been managed by oral administration during the previous seven days. On the eighth day, the animals were sacrificed and their tumors removed and weighed. Subsequently, the difference between the treated groups and control group was calculated to give the index of tumor inhibition in accordance with the formula below.

$$TWI\% = C - T \times 100/C$$

Where:

TWI% = Tumor weight inhibition (%)

C = Mean tumor weight in control group animals

T = Mean tumor weight in treated group animals

Statistical analysis

The results are presented as mean \pm S.E.M. and statistical significance between the groups was analyzed by mean of variance analysis followed by Student's *t* test. *P* values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Acute oral toxicity

In the study for acute toxicity, the animals were treated with a single dose of the extract in question for a period of approximately 24 h (Simões, 2003). The oral administration of the methanolic extract from *H. drasticus* leaves at doses of 50 and 300 mg presented no alteration in the animals during the first 30 minutes of observation. The animals remained without changes after 14 days. Within the first 15 minutes of 2000 mg/kg dose administration, the animals presented excitability and agitation including exophthalmia, agitation, vibrissa, stereotyped and circle movements. They also presented increased respiratory rate, position in claw, snout edema, lack of flee attempt. After this period, depressive reactions such as lowering of the posterior train and prostration became evident. After 14 days, no mortality or changes in behavior were observed. The excitability followed by depressive behaviors suggests a reduction in the amount of stimulant neurotransmitters during the excitation phase (Fai *et al.*, 2005). During the observation period, animals displayed no variation in their general appearance. These results obtained for methanolic

extract from *H. drasticus* leaves were in accordance with the norms established for OECD 423, and presented low toxicity for oral administration at 2000 mg/kg. The general results for acute oral toxicity are shown in Table I.

Histopathological studies

Animal bodies were macroscopically preserved. Histopathological examination of the livers presented hepatic congestion at sinusoids and the portal vessel, pericentre globular micro-steatosis, Kuffe cell proliferation, hepatocyte diffuse necrosis and mononuclear infiltrate. In the kidneys, the findings were glomerular atrophy, capsule thickening, renal cortico-medullary congestion, hemorrhage and mononuclear infiltrate. The pulmonary findings were alveolar interstitial thickening, intense pulmonary congestion, mononuclear infiltrate, pneumocyte-type II proliferation, pulmonary emphysema, edema and vessel thickening. In the spleen, the authors noted red pulp congestion, white pulp hyperplasia and apoptotic cells at the germinal center.

Antitumor activity

Laboratory animals represent a valuable experimental system for the understanding of the mechanism of cancer in humans. The majority of the currently accepted concepts of tumorigenesis are strongly influenced by cancer development models in mice. Mice organisms are accessible models and possess similar systems to humans (Kamb, 2005). Sarcoma 180 is an original mouse tumor and one of the most frequently used cell lineages in the research of antitumor activity (Lee *et al.*, 2003; Magalhães *et al.*, 2006).

The antitumor activity of the methanolic extract from *H. drasticus* leaves was tested by oral administration against the Sarcoma 180 experimental model. Figure 1 shows the mean weight of the tumors in the control group (NaCl 0.9% Solution), standard group (cyclophosphamide, CTX, 10mg/kg), treated I (200 mg/kg), treated II (300 mg/kg) and treated III (400 mg/kg) groups. The standard group presented significant tumor growth inhibition of 70.4% in comparison to the control group. The treated groups also presented a reduction in tumor growth in relation to the control group; however, the doses of 300 mg/kg and 400 mg/kg also demonstrated marked tumor growth inhibition activity of 67.7% and 68%, respectively. At the 200 mg/kg dose, tumor growth inhibition was 32.8%.

The macroscopic analysis of the animals of the control group revealed hemorrhagic ascites; solid and invasive tumor, adhered to epidermis and well vascularized.

TABLE I – Toxic effects for methanolic extract from *H. drasticus* leaves in male Swiss albino mice (*Mus musculus*)

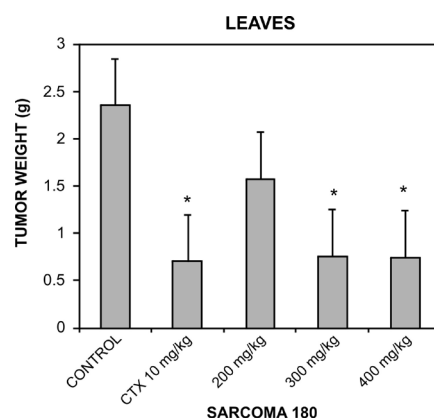
EFFECTS	DOSES (mg/kg)		
	50	300	2000
STIMULANT			
Tachycardia	-	-	-
Tachypnea	-	-	+
Piloerection	-	-	++
Agitation	-	-	+
Exophthalmia	-	-	+
Stereotyped Movements	-	-	+++
Circle Movements	-	-	++
Vibrissa Movements	-	-	+
Tail Curl	-	-	+
Fine/Coarse Tremors	-	-	-
Position in Claw	-	-	+
Flee Reaction	-	-	+++
DEPRESSIVE			
Lowering of the posterior member	-	-	+
Apnea	-	-	-
Alteration in gait	-	-	-
Sleepiness	-	-	-
Prostration	-	-	++
OTHERS			
Fecal Elimination	-	-	+
Diuresis	-	-	+
Abdominal Movements	-	-	-
Petechiae	-	-	+
Snout Edema	-	-	++
Cyanosis	-	-	-
Photophobia	-	-	-
Reflux	-	-	-

Data obtained within first 30 minutes of oral administration.

(-) No Effect; (+) Mild Effect; (++) Moderate Effect; (+++) Severe Effect.

However, in the treated groups, only the Treated I group (200 mg/kg) presented ascites, solid and invasive tumor, adhered to epidermis and well vascularized. The Treated II (300 mg/kg) and Treated III (400 mg/kg) groups presented well described solid tumors which were less vascularized than control group tumors.

The mechanism underlying janaguba antitumor activity has not yet been elucidated. It is known that the *Himatanthus* genus has iridoid and triterpene in its chemical constitution, and one of these substances is likely responsible for the activity. Detailed studies are being carried

**FIGURE 1** – Mean tumor weight. *p < 0.05 comparing treated groups with control group (normal saline solution). Student's *t* test (n = 5/group).

to clarify the antitumor activity of leaves of *H. drasticus* as well as to determine new routes of drug administration.

Based on these results, we can conclude that the methanolic extract from *H. drasticus* leaves possesses low toxicity and significant antitumor activity at the tested doses administered orally. The results for antitumor activity were very good in relation to control cyclophosphamide, the synthetic drug widely used in chemotherapy protocols. The extract was also found to be safe in terms of acute toxicity in experimental animal models whereas further studies are necessary on its chronic toxicity, mutagenicity and carcinogenicity to ascertain the safety and benefits of the plant.

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