

***In vitro* and *In vivo* Anti-cancer Effects of *Tillandsia recurvata* (Ball Moss) from Jamaica**

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ABSTRACT

Objective: *Tillandsia recurvata*, also commonly known as Ball Moss, is endemic to Jamaica and some parts of the Caribbean and South America. The plant, despite being reported to be used in folk medicine, had not previously been evaluated for its anti-cancer potential. The aim of this study was to evaluate the anti-cancer activity of Ball Moss.

Methods: The anti-proliferation activity of the crude methanolic extract of the *T. recurvata* was evaluated in vitro in five different histogenic cancer cell lines (prostate cancer – PC-3, breast cancer, Kaposi sarcoma, B-16 melanoma and a B-cell lymphoma from a transgenic mouse strain) using the trypan blue assay. The crude extract was also evaluated in vivo in tumour-bearing mice. Immunohistochemistry staining with Apoptag was used for histology and determination of apoptosis.

Results: The crude methanolic extract of *T. recurvata* demonstrated anti-proliferation activity against all the cell lines, killing > 50% of the cells at a concentration of 2.5 µg/ml. Kaposi sarcoma xenograft tumours were inhibited by up to 75% compared to control in the in vivo study ($p < 0.05$). There was evidence of DNA fragmentation and a decrease in cell viability on histological studies. The methanolic extract showed no toxic effect in the mice at a dose of 200 mg/kg.

Conclusions: Our data suggest that *T. recurvata* has great potential as an anti-cancer agent and that one of its mechanisms of cell kill and tumour inhibition is by the induction of apoptosis.

Keywords: Ball moss, cancer, medicinal plant, *Tillandsia recurvata*

Efectos Anticancerosos *In vitro* e *In vivo* de la *Tillandsia recurvata* (Bola de Musgo) de Jamaica

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RESUMEN

Objetivo: La *Tillandsia recurvata*, también conocida como bola de musgo, es endémica en Jamaica, así como en algunas partes del Caribe y América del sur. Si bien se había reportado su uso como parte de la medicina popular, esta planta no había sido evaluada previamente en relación con su potencial para la lucha contra el cáncer. El objetivo de este estudio fue evaluar la actividad anticancerígena de la bola de musgo.

Métodos: La actividad antiproliferativa del extracto metanólico crudo de la *T. recurvata*, fue evaluada in vitro en cinco líneas celulares diferentes de cáncer histogénico (cáncer de próstata – PC-3, cáncer de mama, sarcoma de Kaposi, melanoma B-16 y un linfoma de células B de una cepa de ratón transgénico) usando el ensayo con azul de tripano. El extracto crudo también se evaluó in vivo en ratones portadores de tumor. La tinción inmunohistoquímica con ApopTag fue utilizada para la histología y determinación de la apoptosis.

Resultados: El extracto metanólico crudo de *T. recurvata* demostró la actividad proliferativa frente a todas las líneas celulares, matando > 50% de las células a una concentración de 2,5 µg/ml. Los tumores de xenoinjerto de sarcoma de Kaposi fueron inhibidos hasta un 75% en comparación con el control en el estudio in vivo ($p < 0.05$). Hubo evidencia de fragmentación de DNA y una disminución

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en la viabilidad celular en los estudios histológicos. El extracto metanólico no mostró ningún efecto tóxico en los ratones a dosis de 200 mg/kg.

Conclusiones: Nuestros datos sugieren que la *T. recurvata* tiene gran potencial como agente anticanceroso, y que uno de sus mecanismos de inhibición de tumores y muerte de las células tiene lugar mediante la inducción de la apoptosis.

Palabras clave: Bola de moho, cáncer, plantas medicinales, *Tillandsia recurvata*

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INTRODUCTION

Natural products are the most consistent successful source of drug leads (1–3). Plants supply most of the active ingredients of traditional medicinal products (4–6). The fight against cancer will continue to require the development of novel and improved chemotherapeutic agents. Drug discovery from plants involves a multidisciplinary approach combining botanical, ethnobotanical, phytochemical and biological technologies. Plants in particular have a long history of use in the treatment of cancer (7).

The genus *Tillandsia* is a group of epiphytic plants with approximately 500 species (8). There are no reports of traditional use of Ball Moss in Jamaica; however, the plant is used in folk medicine in Brazil, Bolivia, Mexico and the United States of America [USA] (9–13). Research has shown its potential medicinal value in treating hypoglycaemia, rheumatoid arthritis and liver infections (14) and the ground breaking anti-cancer effect of this plant was discovered in 2007 (15). In addition, research has identified several phytochemicals from the plant and these include: cycloartane triterpenoids, pentacyclic triterpenes, sterols and flavonoids (16, 17).

This paper describes the anti-cancer properties of the Jamaican ball moss.

METHODS

Plant collection and preparation

T. recurvata (Fig. 1) was collected from trees and electrical poles in Kingston, Jamaica. A voucher specimen of the plant was identified at the Institute of Jamaica Herbarium where it was deposited with accession number: IJ 3411. The collected biomass was air dried under shade and pulverized into powder.

Extraction and isolation

1 kg of ball moss biomass was extracted twice with 5L of methanol. The filtrate was dried in a rotavapor to obtain a dark green residue (16.3 g). The extract was stored in a refrigerator at 4 °C until needed for use in the bioassay.

Biological assay

Cell lines and culture medium

Five different histogenic tumours (prostate cancer, PC-3; breast cancer, BC; Kaposi sarcoma, KS; B-16 melanoma and

a B-cell lymphoma) from a transgenic mouse strain were used in the study. The cell lines were obtained from American Type Culture Collection (ATCC) [Manassas, VA, USA] or from in-house cultures, specifically KS and the B-cell lymphoma from an HIV-1 transgenic mouse. The cells were maintained in minimum essential media supplemented with 10% fetal calf serum, 1% L-glutamine, 2% penicillin-streptomycin, and 0.2% gentamicin at 37 °C with 5% CO₂. Cell death/viability was measured by the trypan blue exclusion assay protocol (18).

Extract preparation and treatment

The dried crude extract of ball moss formulated was in dimethyl sulfoxide (DMSO) and normal saline (2:8). The drug concentration was 1 mg/ml and each mouse was administered 0.2 ml, giving a dose of 10 mg/kg. The vehicle was prepared using the same diluent concentrations without the extract.

In vivo maximum tolerable dose study

Mice used were purchased from Harlan Sprague Dawley (Indianapolis, Indiana) and maintained in a pathogen-free environment in the Institute of Human Virology Animal Facility in the University of Maryland School of Medicine. All experiments involving mice were approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine. To determine the maximum tolerable dose (MTD) for the experiment, 15 nude mice were divided into three equal groups. Two of the groups were respectively treated with 100 mg/kg and 200 mg/kg IP using a crude ball moss extract daily for five days and the third group was treated with the vehicle control. The MTD was found to be > 200 mg/kg and it was thus determined that it was safe to use any of the two concentrations or lower in the efficacy study.

In vivo efficacy study

From the above MTD studies, we used the 10 mg/kg for treatment of the tumours in the mice. All mice were maintained as described above. Thirty nu/nu (NIH) mice, four to six weeks old were inoculated with 3 x 10⁶ Kaposi sarcoma cancer cells in 33% matrigel/67% media with no fetal calf serum (FCS). When tumours reached ~100 mm³, the mice were divided into two groups of eight mice each so that the

mean tumour volume was similar. Dosing was initiated on the day of sorting. Mice were dosed daily for a total of 30 days orally. The body weight of mice was taken alongside tumour volumes twice a week and the tumour volumes determined using the following formula:

$L \times W^2/2$ (L = tumour length; W = tumour width)

Assessment of apoptosis

The Apoptag kit (Intergen, Purchase, NY) was used to determine effect on apoptosis according to the manufacturer's instructions. After light counterstaining with haematoxylin (H&E), nuclei that stained brown were scored as positive for apoptosis and those that stained blue were scored as negative.

RESULTS

In vitro anti-proliferation activity

The results of the *in vitro* anti-proliferation assay results are presented in Fig. 2. All the five cancer cell lines showed



Fig. 1: *Tillandsia recurvata* in its natural habitat growing on plants (left) and young shoot of the plant (right).

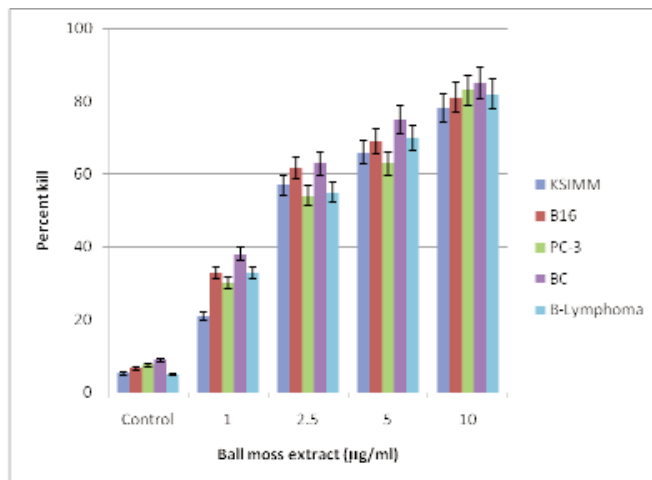


Fig. 2: Anti-proliferation activity of ball moss on different cancer cell lines (KSIMM = Kaposi sarcoma; B16 = mouse melanoma; PC-3 = prostate cancer; BC = breast cancer and B-cell lymphoma) *in vitro*, using the trypan blue assay after 48 hours. At least 50% of the cells were inhibited from proliferation at the concentration of 2.5 µg/ml. Breast cancer was the most sensitive cell line.

sensitivity to the ball moss extract with the breast cancer cell line showing the most sensitivity and the Kaposi sarcoma cell line showing lower sensitivity.

Apoptosis assay

The five tumours that were passed *in vivo* were tested and on histological examination, all tumours showed necrosis on H&E. Histologically, using the immunohistochemical staining (Apoptag), all tumours showed clear evidence of brown staining, demonstrating apoptosis. A representative sample of the histology and the Apoptag staining are demonstrated in Fig. 3.

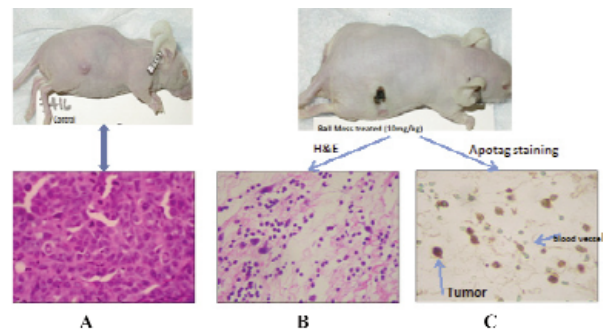


Fig. 3: The histological analysis of Kaposi sarcoma (KS) tumour growth of the control and experimental group. The haematoxylin section in the control mice (A), shows the typical microscopic growth of KS noting numerous blood vessels (note the highly angiogenic vessels). In the treatment group (B), note the necrosis of the tumour with loss of blood vessels. In treatment group (C), note the brown staining tumour cells and the thin endothelial cells (indicating apoptosis).

In vivo anti-tumour activity

Treating of mice injected with Kaposi sarcoma cells resulted in significant arrest of tumour growth compared to control ($p < 0.05$). Figure 4 presents the results of the *in vivo* efficacy

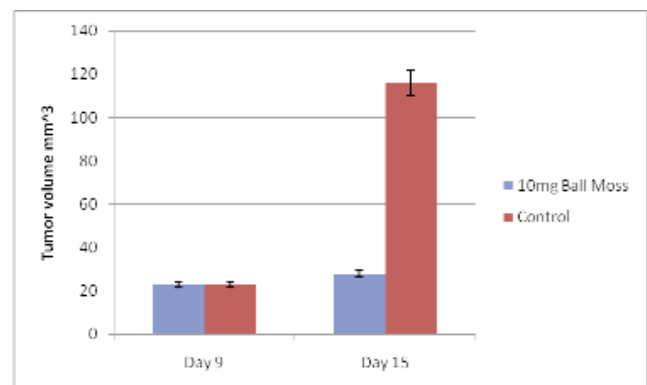


Fig. 4: Effect of ball moss extract on Kaposi sarcoma *in vivo* in nude mice. Nude mice were injected with 3×10^6 Kaposi sarcoma cells subcutaneously on the right flank. Tumours were monitored and measurements started once visible signs of tumour growth were observed. The ball moss group was treated by IP injection of 10 mg/kg of extract. By day 15, most of the tumours in the treatment group had regressed or dried up while the control group tumours continued to grow.

studies. The concentration used was selected based on the MTD study results where ball moss was found to be safe at up to 200 mg/kg by oral gavage. Haematoxylin staining of tumour cells harvested from treated and control mice showed significant difference in cell morphology.

DISCUSSION

On the basis of the observed *in vitro* and *in vivo* activities of ball moss, our hypothesis that this plant possesses unique compounds based on the environment that it grows in has been confirmed in these preliminary set of studies. The plant extract has demonstrated activity against five major cancer cell lines *in vitro* as well as *in vivo* activity against Kaposi sarcoma which is one of the HIV-related cancers currently plaguing the AIDS community.

Efforts to determine the mechanism of action has indicated that ball moss exhibit its activity against cancer cells by triggering the process of apoptosis (Fig. 3). Apoptosis or programmed cell death involves the activation of a set of cysteine proteases known as “caspases” (19).

The study presented here demonstrated the anti-cancer properties of *T. recurvata* extract against tumours of different histogenic origins. This observation of a crude extract showing strong anti-cancer properties supports the importance of studying this plant with unique bioactive metabolites. We are currently in the process of determining the specific compound(s) responsible for the anti-cancer activity demonstrated by the plant extract in this study.

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