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Immunomodulatory properties of *Alternanthera tenella* Colla aqueous extracts in mice

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Abstract

Plants from the genus *Alternanthera* are thought to possess antimicrobial and antiviral properties. In Brazilian folk medicine, the aqueous extract of *A. tenella* Colla is used for its anti-inflammatory activity. The present study investigated the immunomodulatory property of *A. tenella* extract by evaluating the antibody production in male albino Swiss mice weighing 20-25 g (10 per group). The animals received standard laboratory diet and water *ad libitum*. The effect of *A. tenella* extract (5 and 50 mg/kg, *ip*) was evaluated in mice immunized with sheep red blood cells (SRBC 10%, *ip*) as T-dependent antigen, or in mice stimulated with mitogens (10 µg, *Escherichia coli* lipopolysaccharide, LPS, *ip*). The same doses (5 and 50 mg/kg, *ip*) of *A. tenella* extract were also tested for antitumor activity, using the Ehrlich ascites carcinoma as model. The results showed that 50 mg/kg *A. tenella* extract *ip* significantly enhanced IgM (64%) and IgG2a (50%) antibody production in mice treated with LPS mitogen. The same dose had no effect on IgM-specific response, whereas the 5 mg/kg treatment caused a statiscally significant reduction of anti-SRBC IgM-specific antibodies (82%). The aqueous extract of *A. tenella* (50 mg/kg) increased the life span (from 16 ± 1 to 25 ± 1 days) and decreased the number of viable tumor cells (59%) in mice with Ehrlich ascites carcinoma. The present findings are significant for the development of alternative, inexpensive and perhaps even safer strategies for cancer treatment.

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- Antitumor activity
- Antibody
- Immunomodulation
- Ehrlich ascites
- *Alternanthera*

Plants are invaluable sources of new drugs. There is an ever-growing interest in investigating different species of plants to identify their potential therapeutic applications. This increasing interest is due to a tremendous historical legacy in folk medicine use of plants as medicines (1) and their easy availability, cost effectiveness and presumed safety. In the recent past, scientific studies on plants used in ethnomedicine have led to the discovery of many valuable drugs such as pilocarpine and vincristine among others.

Alternanthera tenella Colla (Amaranthaceae), a herbaceous plant commonly known as "*enxuga*" or "*pérpetua do mato*", is fre-

quently found in northwest Brazil. *A. tenella* is used in folk medicine to treat fever, infections and genital inflammation (1). Some species of the *Alternanthera* genus have been reported to inhibit lymphocyte activation (2), to have antiviral (3) and hepatoprotective properties (4), antinociceptive effects, and analgesic activity (5). *A. tenella* was also found to have antibiotic activity in assays using Gram-positive or Gram-negative bacteria *in vitro* (6,7).

The aim of the present study was to examine the immunological effect of *A. tenella* aqueous extracts in mice, with emphasis on antibody production and the antitumor activity.

Male albino Swiss mice weighing 20-25 g were used. The animals received standard laboratory diet (Purina chow, Campinas, SP, Brazil) and water *ad libitum*.

A. tenella aerial parts (stem and leaves) were locally collected (Horto Comunitário Jambeiro, São Luís, MA, Brazil). A voucher specimen (No. 1.160) identified by specialists at the Emilio Goeldi Museum, Belém, PA, Brazil, has been preserved in the Ático Seabra Herbarium, Universidade Federal do Maranhão, São Luís, MA, Brazil.

The fresh aerial parts of *A. tenella* (350 g) were dried and powdered and the aqueous extract was prepared by the addition of 700 ml twice-distilled water. The crude extract was then concentrated in a rotary evaporator (40ºC under vacuum) and the resulting extract dissolved to a final concentration of 10 mg/ml in saline solution (0.87% NaCl).

For the antibody assays, *A. tenella* extract was injected intraperitoneally (*ip*) in two different doses (5 and 50 mg/kg) 48 h before immunization with sheep red blood cells (SRBC, 0.5 ml of a 10% saline suspension) or 48 h before *ip* stimulation of the animals with 10 µg of *Escherichia coli* lipopolysaccharide (LPS; Sigma, St. Louis, MO, USA).

Spleen cells were obtained from whole spleens either 5 days after immunization with SRBC, or 2 days after stimulation with *E. coli* LPS (N = 10 mice/group). The cell suspension obtained was washed three times in balanced salt solution prepared as previously described (8).

Spleen cells (10^6) were plated with 25 µl SRBC or SRBC conjugated to protein A (Sigma). Fifty microliters of guinea pig serum, 250 µl of bacto-agar (Difco Laboratories, Detroit, MI, USA) and 25 µl of goat anti-mouse IgM or IgG2a antibody (Sigma) were also added. After 4-h incubation at 37ºC and 18-h incubation at 4ºC the number of antibody-secreting cells was determined (8). For each stimulation, three experiments were performed.

Antitumor activity was assessed by treating the mice with the extract before the *ip* injection of Ehrlich ascites carcinoma cells.

Ehrlich ascites cells were maintained as tumors in the peritoneal cavity of Swiss albino mice obtained from the Central Animal House of Universidade Federal do Maranhão. For the experimental procedures, 5 ml of ascitic fluid from mice inoculated 8 days before was collected and centrifuged at 140 *g* for 10 min. Male Swiss albino mice weighing 20-25 g (10 per group) were injected with 10^6 tumor cells *ip* for the induction of ascites tumor 48 h after *A. tenella* extract treatment (5 or 50 mg/kg body weight, *ip*) and a control group treated only with saline was used for comparison. The effects of *A. tenella* on tumor growth and host survival were estimated by peritoneal tumor cell count, animal weight, and percent increase in life span of the tumor hosts (N = 10 mice/group). The Trypan blue exclusion test was used to determine the percentage of living cells, which were counted in a Neubauer chamber. Cell viability was always found to be 90% or higher.

Results are reported as means ± SEM for plaque-forming cell results and as means ± SD for cancer studies. Statistical analyses were carried out by the Student *t*-test and the difference was considered statiscally signifi-

cant when $P < 0.05$.

Mice treated with the lower dose of *A. tenella* extract (5 mg/kg) 48 h before immunization showed a significant reduction in anti-SRBC IgM-secreting cells when compared to control (from 1730 ± 354 to 310 ± 51). In contrast, the higher dose of the same extract (50 mg/kg) had no significant effect (from 1730 ± 354 to 1480 ± 185) on plaque-forming cell numbers compared to control (Figure 1A).

In mice stimulated with LPS, only the higher dose (50 mg/kg) of *A. tenella* extract significantly enhanced IgM and IgG2a antibody production compared to control (from 103 ± 15 to 284 ± 19 and from 106 ± 15 to 209 ± 22, respectively), whereas the lower dose had no effect (from 103 ± 15 to 128 ± 10 and from 106 ± 15 to 111 ± 14, respectively) (Figure 1B). No statistically significant differences between *A. tenella*-treated animals and controls were observed in total numbers of nucleated spleen cells or spleen weight.

The present experiments revealed a strong dose-dependent effect of *A. tenella* extract on antibody production in the spleen. A low dose of plant extract significantly inhibited IgM antibody production in mice immunized with SRBC, suggesting that the extract contains substance(s) with an inhibitory action on B lymphocyte function.

At a higher concentration (50 mg/kg), however, the same aqueous extract of *A. tenella* enhanced IgM and IgG2a antibody production in mice stimulated with *E. coli* LPS. These results suggest that the aqueous extract of *A. tenella* was able to reduce antibody production to T-dependent antigen. In addition, the extract can exert a stimulatory effect on antibody production induced by the mitogens. The nature of the substance(s) responsible for these effects is unknown. Isolation and purification of the components present in the *A. tenella* extract are needed to characterize the nature of the active compound(s).

An immunosuppressive and immunostimulatory activity for the same compound was previously described by Wagner and Proksch (9) when they studied the biological activity of tylophorin isolated from *Tylophora indica necasthmatica*. Extracts of a diverse range of plants have been shown to possess immunomodulatory properties by presenting simultaneous immunostimulatory and immunosuppressive effects (10-12).

The effect of *A. tenella* extract (5 or 50 mg/kg body weight, *ip*) on the survival time of Ehrlich ascites carcinoma-bearing mice is summarized in Figure 2A. The mean survival time was 16 ± 1 days for the Ehrlich ascites carcinoma control group, 20 ± 2 days for the 5 mg/kg group and 25 ± 1 days for the 50 mg/kg group (Figure 2A). The viable tumor cell count was significantly inhibited only in the 50 mg/kg group (Figure 2B). This inhibition was 59% compared to the control and 62% compared to the 5 mg/kg group. No variation in animal weight was observed

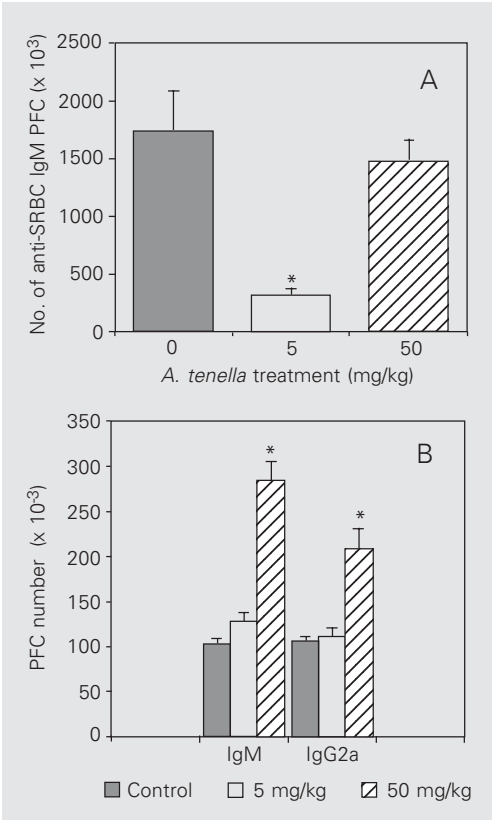

Figure 1. Effect of *Alternanthera tenella* extract (5 and 50 mg/kg) on IgM and IgG2a production assayed by the plaque-forming cell (PFC) assay. *A*, Mice immunized with sheep red blood cells (SRBC), and *B*, mice treated with 10 µg LPS 48 h after *A. tenella* treatment. Data are reported as means ± SEM for 10 animals. *$P < 0.001$ compared to control (Student *t*-test).

until 8 days after tumor inoculation when the weight of the 5 mg/kg group was significantly lower than the control (Figure 2C).

The present study demonstrated that the aqueous extract of *A. tenella* also has a tumor inhibitory activity on Ehrlich ascites cells. Furthermore, the increase in life span of tumor-bearing mice caused by *A. tenella* treatment is a positive result, since the plant extract demonstrated an increased survival time effect even in groups whose tumor cell counts were not significantly reduced. The exact mechanism by which *A. tenella* mediates its antitumor effect is not known.

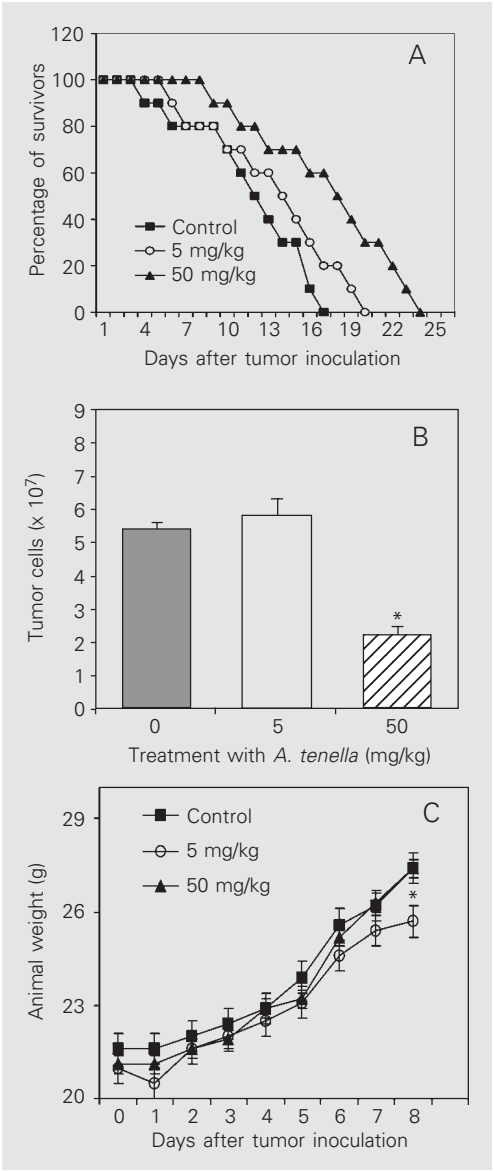

Figure 2. Effect of *Alternanthera tenella* extract (5 and 50 mg/kg) on Ehrlich tumor development when given 48 h before tumor cell inoculation (10^6). *A,* Survival of tumor-bearing mice treated with *A. tenella* extract. *B,* Reduction in total number of tumor cells recovered from the peritoneal cavity. *C,* Mean daily weight of *A. tenella*-treated and control animals. Data are reported as means ± SD for 10 animals per group. *P < 0.05 compared with control (Student *t*-test).

Some compounds with an immunomodulatory activity like fatty acids (10), flavonoids (12,13), polysaccharides (14,15), and triterpenes (16) are also found in the *Alternanthera* genus (3-5,7,17). Most studies concerning the immunomodulatory activities of plants have been carried out using crude extracts (11,16). In some, combinations of various herbs or herbs in combination with minerals have been used taking into consideration Ayurvedic (12), Unani (17) or Chinese (18) traditional formulations. Although it may be rational to use a single plant or its single constituents, it has been a general experience that the total plant extract shows more efficacy compared to single constituents (18).

The present findings are significant for the development of alternative, inexpensive and perhaps even safer strategies for cancer treatment. Although in this study the *A. tenella* extract was more effective in inducing a reduction in the total number of tumor cells (Figure 2B) than in prolonging the life of tumor-bearing animals, the finding that *A. tenella* groups presented an increased life span when compared to control (Figure 2A) might be considered a relevant observation for the eventual development of new strategies for the treatment of some forms of cancer. It is also important to note that the beneficial effect was observed with a low concentration of crude extract (5 mg/kg), in contrast to the high concentrations of plant extracts employed in other experiments (100, 200 and 300 mg/kg (Ref. 19) or 250 and 500 mg/kg (Ref. 20)) dealing with tumor growth inhibition in mice (15,19,20).

A. tenella extract seems to have the potential to interfere with the process of immune activation either by inhibiting or stimulating antibody production, depending on its concentration, and also by potentially reducing the number of tumor cells. The bi-directional effect of *A. tenella* Colla extract observed in this study is of high interest if we

consider that *A. tenella* can act in a prostimulatory manner on lymphocytes, as shown after stimulation with a mitogen. In contrast, *A. tenella* had an up-regulating effect after immunization with T-dependent antigens.

The present results support the hypothesis that water-soluble components derived from *A. tenella* may have an important effect on the immune system of mice, indicating that plant extracts are able to effectively modulate immunological interactions. This finding is of special interest since the biologically active compounds seem to be highly soluble in aqueous solution.

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