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FURTHER ON THE PHARMACOLOGY OF *TRICHOPUS ZEYLANICUS*

P. PUSHPANGADAN, S. RAJASEKHARAN, A. SUBRAMANIAM, P.G. LATHA, D.A. EVANS and R. VALSA RAJ

Division of Ethnopharmacology and Ethnomedicine, Tropical Botanic Garden and Research Institute (T.B.G.R.I), Palode, Thiruvanthapuram – 695 562, Kerala, India.

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ABSTRACT: *Oral administration of Trichopus zeylanicus to mice (0.5 ml of 2% water suspension / mouse) for 7 consecutive days markedly increased the number of thymocytes splenic lymphocytes, total blood leucocytes and peritoneal macrophages without any effect on Haemoglobin content and body weight. This increase in the proliferation of lymphocytes and macrophages could be one of the mechanism of T.zeylanicus induced immunomodulation. Treatment with T. zeylanicus protected mice from tumour cell growth when challenged with 0.5 million of EAC ascetic tumour cells / mouse. Studies on the gastrointestinal function of this drug showed that the drug slightly reduced intestinal motility as judged from charcoal movement.*

INTRODUCTION

The medical value of *Trichopus zeylanicus* was brought to light by the ethnopharmacological studies carried out by Pushpangadan and co-workers (Pushpangadan *et al*[1,2] Sharma *et al*[3]). The studies of the discoverer's group revealed the powerful immunostimulating properties of the alcoholic extract of this plant (Pushpangadan *et al*[2]). The plant powder or alcoholic extract of it (leaf, stem, seed, etc) increased delayed type hypersensitivity response in mice to sheep red blood cells (particular antigen) suggesting stimulation of cell mediated immunity. So it was thought worthwhile to determinate the *in vivo* effect of *T.zeylanicus* on the number of lymphocytes and macrophages.

Immunomodulators may protect animals and humans to some extent from tumour and cancer cell growth by the tumouricidal property of immune system known as immunological surveillance against cancer (Burett, 1970[4]; Ray and Roychaudhuri 1983[5]). Since *T.zeylanicus* possess immuno – enhancing property, we have studies the effect of *T.zeylanicus* as ascetic tumor cell growth in mice.

It has been shown earlier that *T. zeylanicus* induces an increase in the resistance of rodent against a variety of stress (Sharma *et al*[3]). The drug reduced the gastric secretory clume and acid output in pylorus ligated rat stomach. In a follow up study, now we report the effect of the drug on the motility of mouse intestine.

One of the remarkable properties of *T. zeylanicus* is its ability to increase stamina (Pushpangadan *et al*[1]). The drug in the form of a suspension in water or its alcoholic extract increases the swimming performance of normal and adrenalectomised rats significantly. In the present study, various extracts of the plant were tested for their effect on swimming performance of mice.

MATERIALS AND METHODS

Animals: Adult male swiss albino mice (25 – 30g) and Charles Foster rats (100 – 150g) were used. They were fed with standard rodent pellet and water *ad lib* and maintained under standard laboratory conditions.

Preparation of *T.zeylanicus* extracts or water suspension.

Fresh whole plants were dried at room temperature in the laboratory and powered. 2% water suspension of the powder was used. Based on our preliminary studies a dose of 0.5 ml / mouse was given orally to mice unless otherwise stated.

To determine the efficacy of various extracts on swimming performance *T. zeylanicus* powder was extracted with methanol or acetone or water at room temperature with constant shaking for 4 hrs. The extract was filtered through Whatman filter paper and the filtrate was dried under vaccum and weighed. Acetone or methanol extracted material was dissolved in 10% Tween 80.

Immunological Studies

Mice were divided into 2 groups of six animals each. The experimental group received a daily oral dose of 0.5 ml *T. zeylanicus* water suspension for 7 days. The other group received normal saline and served as control.

Preparation of Thymocytes and Splenic Lymphocytes

Thymus glands and spleens were removed and carefully trimmed off from adjoing tissues and weighed. Single cell suspension of thymocytes as well as splenic lymphocytes were prepared in cold RPMI 1640 medium as described elsewhere(Ford 1978)[6]. Cell counts were made using haemocytometer.

Leucocyte Count

Blood was drawn from tail vein and total leucocyte count was made with haemocytometer. The number of granulocytes as a percentage of total leucocytes was also determined.

Estimation of Haemoglobin

Haemoglobin content was measured using haemometer with permanent coloured glass and comparison standards.

Collection of Peritoned Macrophages

Peritoned exudates cells were collected using RPMI 1640 medium containing heparin using the method of Hudson and Hay 1980[7]. Macrophages were separated by adhering on glass surfaces at 37^0C in heparin free medium and counted using haemocytometer.

Determination of Anti-tumour Effect

Mice were divided into 2 groups. One group received daily *T. zeylanicus* water suspension (oral; 0.5 ml/mouse) for 7 days and then the mice were challenged with Ehrlich Ascitic carcinoma (EAC) cells (0.5 million cells / mouse) in the peritoned cavity. The drug treatment was continued for another 20 days. The control (tumour-control) received only the tumour cells. Twenty days after tumour challenge, animals were killed and peritoneal cavity was examined carefully for the presence of tumour cells and number of cells were determined by haemocytometer counting.

Assessment of Intestinal Motility

Mice fasted for 20 hrs were divided into 3 groups of 10 mice each. Group I and II received 2 different doses of *T. zeylanicus* water suspension. Group III received 0.5 ml of water and served as control. 30 min. after the drug administration, all mice were given 5% suspension of finely ground charcoal in 50% gum acacia. The animals were killed 30 min. after charcoal administration. The distance traversed by charcoal as a percentage of total intestinal length was measured.

Swimming Endurance Test

Animals were divided into 5 groups of 10 animals each. Groups I and II were given 0.2 ml of 10% Tween 80 and water respectively and served as control. Groups III, IV and V were given a single dose (200 mg/kg. Body wt.) of water, methanol and acetone extracts respectively in 0.2 ml water or 10% Tween 80. After 1 hr. all mice were put to swimming in plastic buckets filled with water (tem. 26^0C) and were allowed to swim till exhausted and drowned which was taken as the end point. Swimming time for each animals was recorded.

RESULTS

Effect of *Trichopus zeylanicus* on lymphocytes and macrophages in mice.

Trichopus zeylanicus treatment (0.5 ml of 2% suspension/mouse) for seven consecutive days markedly increased the weight of thymus and spleen but that of liver and body was unaltered (Table.1). The cell counts in thymus as well as spleen showed an increase in the treated animals as compared to controls (Table.2).

The viability of the cells were not altered when tested by trypan blue method. Total leucocyte count in the blood was also significantly enhanced in the treated animals. The increase in granulocytes was more than that in total leucocytes. In controls, 17.8% of total leucocytes were granulocytes whereas in the treated animals it was 34.2%. The number of peritoneal macrophages was dramatically increased in the treated animals. The blood haemoglobin content was unaltered by the treatment.

Table 1 : Effect of *Trichopus zeylanicus* on the weight of thymus, spleen, liver and body of mice.

Weight (mg)

	Control	Treated
Thymus	38.5 ± 3.5	60 ± 5.0 * (156)
Spleen	61.0 ± 6.0	90 ± 4.5* (148)
Liver	929 ± 84	994 ± 106
Body weight (g)	27.4 ± 2.5	28 ± 3.1

Values are mean ± SD; n=6 *P<.001 *Trichopus zeylanicus* (2% water suspension; 0.5 ml/mouse) was given orally for 7 days.

Table 2 : Effect of *Trichopus zeylanicus* on the number of lymphocytes and macrophages in mice.

	Control	Treated
Thymus (cells / organ x 10^{-6})	183 ± 16.3	320 ± 24 (174)
Spleen (cells / organ x 10^{-6})	312 ± 13	456 ± 49 (146)
Total Blood Leucocytes (cells / ml x 10^{-6})	8.9 ± 1.6	15.8 ± 2.0 (177)
Granulocytes (% of total leucocytes)	17.8 ± 2.5	34.2 ± 3.7 (192)
Haemoglobin (g/100 ml blood)	15.8 ± 2.5	16.6 ± 0.6
Peritonial macrophages (cells / mouse x 10^{-6})	7.9 ± 0.36	26.6 ± 4.6 (337)

Water suspension of powdered *T. zeylanicus* was administered (oral; 0.5 ml) daily for 7 days. Values are mean ± S.D. of 6 animals. * P < .001. Values in paranthese represent % of control.

Effect of *T. zeylanicus* on Ascitic Tumour Cell Growth in mice

Treatment of mice with *Trichopus zeylanicus* before and after tumour challenge with EAC ascetic tumour cells (0.5 million / mouse) completely protected 60% of mice from the tumour cell growth and the number of tumour cells were dramatically reduced in the rest of the drug treated mice as compared to untreated tumour challenged mice (Table.3). In the tumour control mice (untreated mice), full tumour growth was observed in all animals.

Table 3 : Effect of *Trichopus zeylanicus* on ascetic tumour cell growth in mice

	Control (tumour control)	Drug Treated
No. of animals used (n)	10	10
No. of animals carrying tumour cells, 20 days after tumour challenge	10	4
Tumour cell count x 10^{-6} (cells / mouse)	88 (n=10)	7.8 (n=4)

Animals were treated with the drug for 1 week and then 0.5 million ascetic cells were injected into the peritoneal cavity. The drug treatment was continued for 20 more days. Control animals (tumour control) received sham injection.

Effect of *T. zeylanicus* on Intestinal Motility

As shown in Table. 4, *Trichopus zeylanicus* (water suspension) administration induced slight concentration dependent decrease in bowel movement as judged from movement of charcoal. At a higher dose (1 ml / mouse) there was about 30% decrease in the movement of charcoal whereas at a lower dose (0.5 ml / mouse) there was a slight decrease but it felt short of statistical significance.

Table 4 : Effect of *Trichopus zeylanicus* on intestinal motility in mice

	Distance moved by charcoal (% of total length of small intestine)
Control	81 ± 5.9
Treated (0.5 ml / mouse)	72 ± 8.1 (88)
Treated (1.0 ml / mouse)	57 ± 6.7* (70)

2% (W/V) *T. zeylanicus* water suspension was used. The drug was given 30 min. prior to charcoal administration. Values are mean ± SD. N=6, *P<0.01. Values in parenthesis represent percentage of control values.

Extractability of *T. zeylanicus* Anti-Fatigue Principle

The water extract of *T. zeylanicus* was found to have no significant effect on the swimming performance of mice at a dose of 200 mg/kg., whereas at this dose methanol and acetone extract showed 54 and 40% increase respectively in the swimming performance as compared to control (Table 5). This shows that the agent which induces the anti-fatigue effect is not extractable in water.

Table 5: Effect of different extracts of *Trichopus zeylanicus* on swimming endurance in mice

	Swimming Time (min.)
Control	206 ± 26.0
Water extract	250 ± 55.0 (121)
Methanol extract	318 ± 29.0 **(154)
Acetone extract	288 ± 23.0 *(140)

10 – 15 mice were used in each group. The extracted material was orally given (0.2 ml/mouse) at a dose of 200 mg/kg body weight, 1 hr before the commencement of swimming performance.

Values are mean ± SD **P < 0.001; * P<0.01

Values given in parentheses represent percentage of control values.

DISCUSSION

In our earlier studies *Trichopus zeylanicus* was found to enhance both cell mediated and humoral immunity. The observed increase in lymphocytes and macrophages in the present study suggests that component(s) of *Trichopus zeylanicus* directly or indirectly stimulate proliferation of macrophaes and lymphocytes. This could be one of the mechanisms of *T. zeylanicus* induced immunostimulation. The action of the drug appears to be specific to cells involved in immunological reactions because the weight of liver and body as well as haemoglobin content are unaffected by the treatment. The drug may directly act on the lymphocytes and macrophages or it may stimulate the release of immunomodulatory cytokines which in turn activate these cells leading to increased proliferation. These possibilities remain to be studied.

It is the interest to note that polymorphonuclear leucocytes were increased in the treated mice more than that of total leucocytes. Similarly there was more than 200% increase in peritoneal macrophages whereas the increase in total leucocytes was 77%.

Polymorphonuclear neutrophils and macrophages are the most important phagocytic cells. (Babior 1978[8]; Esparza et al 1983[9]; Silverstein et al 1981[10]). Further activated macrophages can release Tumour Necrosis Factor and Interleukins. (Fiers 1991[11]; Dinarello et al 1989[12]). These cytokines have tumouricidal as well as immunomodulatory properties.

The observed anti-tumour effect of *T. zeylanicus* could be mediated by the enhancement of immune function. Host immune system can kill tumour cells by the following mechanisms : 1. Antibody dependent cell mediated cytotoxicity 2. Activated macrophase mediated killing of tumour cells. 3. Complement mediated lysis of tumour cells. 4. Cytotoxic T. cell mediated tumour killing and 5. Natural killer cell mediated killing of tumour cells

(Ray and Roychaudhuri 1983[5]). A strong immune system can kill tumour or cancer cells especially when the cancer volume is small (Burett 1940[4]). Thus it is likely that regular consumption of *T. zeylanicus* could protect people from cancer.

The bowel movement decrease induced by the drug is worth noting when *T. zeylanicus* is used as an anti-fatigue or a therapeutic agent.

One of the important properties of *T. zeylanicus* is its ability to boost stamina. The present study on swimming performance test indicates that the active principle responsible for this effect is not water soluble. Studies are in progress to isolate this factor.

The present studies show for the first time novel pharmacological effects of *T. zeylanicus*, stimulation of lymphocyte and macrophage proliferation in mice, protection from ascetic like tumour cell multiplication and decrease in intestinal motility. Studies are on in this laboratory at the molecular and cellular level on the mechanism of the immunoenhancing and anti-fatigue property of *Trichopus zeylanicus.*

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