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SHORT COMMUNICATION

Antitumour Activity of *Hibiscus tiliaceus* Linn. Roots

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ABSTRACT

The aim of this study was to evaluate antitumour activity of the roots of *Hibiscus tiliaceus* Linn. against Dalton's Ascitic Lymphoma (DAL) in Swiss albino mice. A significant enhancement of mean survival time (MST) of *H. tiliaceus* treated tumour bearing mice was found with respect to control group. *H. tiliaceus* treatment was found to enhance peritoneal cell counts. When these *H. tiliaceus* treated animals underwent intraperitoneal (i.p.) inoculation with DAL cells, tumour cell growth was found to be inhibited. The results indicated that, *H. tiliaceus* treated group were able to reverse the haematological parameters, protein and Packed Cell Volume (PCV) consequent to tumour inoculation with in fourteen days after the transplantation.

Keywords: *Hibiscus tiliaceus, Roots and Antitumour*

The plant *Hibiscus tiliaceus* Linn. (Malvaceae) is a fast growing tree commonly grows along the seashore and back mangroves of China, Iran, India, Malaysia and South Africa. H. tiliaceus leaves are used to treat fever, cough and the fresh roots are used to treat dysentery, microbial infection, skin boils and chest congestion. Fresh flower boils in milk is used to treat ear infections [1]. It is also used as anticonvulsive, anti phlogistic, diuretic, antioxidants, hepatoprotective and hypoglycemic [2].The phytochemical review indicated the presence of hibiscusin, hibiscus amide, vanillic acid, P-hydroxybenzoic acid, syringic acid, P-hydroxybenzaldehyde, scopoletin, N-trans- feruloyltyramine, N-cis-feruloyltyramine, β-sitosterol, stigmasterol, β-sitosteronone, stigmasta-4,22-diene-3-one, gemlofuran, hibiscolactone, hibiscones, hibiscoquinones, lapachol, gossypol, gossypetin, mansonones, hyperoside, kaempferol, quercetin, and 3-O-galactosides of quercetin and kaempferol, gossypetin glucoside, gossypitin and gossytrin, cyanidin-3-sophoroside, paracoumaric and fumaric acid [3,4]. The present study is focused on evaluation of antitumour activity of the roots of *H. tiliaceus* against Dalton's Ascitic Lymphoma in mice.

MATERIALS AND METHODS

The roots of H. tiliaceus were collected from Ramnathapuram, Tamilnadu, India and were authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Center, Tamilnadu. A voucher specimen has been deposited for the future reference (Ref No.12 C1). The roots were shade dried, coarsely powdered and macerated with water [5] and the yield was 5.2 % of the crude plant. This *H. tiliaceus* extract (HTE) was used for the present study.

Swiss albino mice weighing 20-25 g were obtained from the animal house of M.S University, Rajakamangalam, Tamil Nadu. The protocol was approved by CPCSEA and IAEC No: (MSU/F-106(f)/264) dated on 26/07/04. They were housed in standard microlon boxes and were given standard laboratory diet and water. Dalton's Ascitic Lymphoma (DHA) cells were obtained through the courtesy of Cancer Research Institute, Adayar, Chennai, India. DAL cells were maintained by weekly intraperitoneal inoculation of 10^6 cells/mouse.

Animals were inoculated (i.p.) with 2×10^5 cells/mouse in phosphate buffered saline on day 0 and treatment with HTE was started 24 h after inoculation, at a dose of 200 mg/kg/day i.p. (group 1). The negative control group (group-2) was treated with same volume of 0.9% sodium chloride. All the treatments were continued for 9 days. Median survival time (MST) for each group was noted. The animals surviving more than 60 days were considered to be cured [6]. The antitumour efficacy of HTE (200 mg/kg/day i.p. for 9 days) was compared with that of 5-fluorouracil (5-FU 20 mg/kg/day i.p. for 9 days). MST was noted with reference control. Survival times of treated groups (T) were compared with those of control group (C).

Table 1. Effect of inoculation with *Hibiscus tiliaceus* and 5-FU-treated DAL

Treatment	Median Survival Time (d)	Increase in Life Span (T/C %)
H. tiliaceus (200 mg/kg, i.p.)	32	139.1*
5 FU (20 mg/kg, i.p.)	40	173.9
Sodium chloride (0.9%)	23	100

i. Numbers of animals in each group were 10 and days of drug treatment were 9.
ii. *p<0.01 when compared to the control.

Table 2. Effect of *Hibiscus tiliaceus* treatment on peritoneal cell count in normal mice.

Treatment	Number of peritoneal cells/mouse × 10^6
Control	$5.6 \pm 0.7 \times 10^6$
Treated once	$9.2 \pm 1.3 \times 10^6$
Treated twice on two consecutive days	$13.4 \pm 2.3^* \times 10^6$

i. Number of animals in each group were 5 and the dose was 200 mg/kg/day, i.p.,
ii. *p<0.01 when compared to the control.
iii. Values are expressed as mean ± S.E.M.

Table 3. Effect of *Hibiscus tiliaceus* on Haematological parameters

Treatment	Hb (g %)	Total RBC cells/ml $\times 10^{10}$	Total WBC cells/ml $\times 10^6$	Protein (g %)	PCV (mm)	Differential count (%)		
						Lymphocytes	Neutrophils	Monocytes
Normal mice	16.8 ± 0.7	1.58 ±0.4	6.8 ± 0.6	8.9 ± 0.4	17 ± 0.8	68 ± 2.2	30 ± 2.2	2 ± 1.0
Tumour bearing mice (14days)	12.3 ± 0.6	1.18 ±0.4	16.3 ± 0.8	13.2 ± 0.6	26 ± 0.3	26 ± 0.3	68 ± 5.1	1 ± 0.7
H. tiliaceus (200 mg/kg) treated mice	14.5 ± 0.6	1.38 ± 0.3	8.3 ± 0.5*	9 ± 0.5	20 ± 0.2	58 ± 2.1*	41 ± 2.4*	2 ± 0.6

i. Number of animals in each group were 5 and the dose administered was 200 mg/kg/day, i.p.,
ii. *p<0.01 when compared to control.
iii. Values are expressed as mean ± S.E.M.

Three groups of normal mice (n=5) were used for determining the effect of HTE on normal peritoneal cells. First group was treated once with 200 mg/kg i.p. of HTE while the second group received the same treatment for 2 consecutive days. The third group was untreated and served as negative control. Peritoneal exudate cells were counted after 24 h of treatment and compared with negative control group.

In order to detect the influence of HTE on the haematological status of DAL bearing mice, comparisons were made among three groups (n=5) of mice on day 14 after transplantation. Out of three selected groups, group 1 was normal mice, group 2 and group 3 were tumour bearing mice, in which group 3 were treated with HTE 200 mg/kg/day i.p. (9 days), while group 2 kept as untreated. Blood samples were taken from the tail vein and the haematological parameters such as White Blood Cell count (WBC), Red Blood Cell (RBC), Hemoglobin (HB), Protein and Packed Cell Volume (PCV) were determined [6,7]. The average of five determinations was computed.

RESULTS

The effect of HTE on the survival of tumour bearing mice showed the MST for the control group was 23 days, while it was 32 days and 40 days for the groups treated with HTE (200 mg/kg/day, i.p.) and 5-FU (20 mg/kg/day, i.p.), respectively, as shown in Table 1. The average number of peritoneal exudate cells per normal mouse was found to be $5.6 \pm 0.7 \times 10^6$ (200 mg/kg, i.p.), HTE treatment increased the number of peritoneal cells as shown in Table 2. Single treatment enhanced peritoneal cells to $9.2 \pm 1.3 \times 10^6$, while two consecutive treatments enhanced the number to $13.4 \pm 2.3 \times 10^6$.Haematological parameters (Table 3) of tumour bearing mice on day 14 were found to be significantly altered from the normal group. The total WBC count, protein and PCV were found to be increased with a reduction in haemoglobin content. The total RBC count showed a medium change. In the differential count of WBC, the percentage of neutrophils increased while the lymphocyte count decreased. At the same time interval, HTE (200 mg/ kg/day, i.p.) treatment could restore those altered parameters to normal.

DISCUSSION

The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of the animal and disappearance of leukemic cells from blood [8, 9]. The acquired results illustrate the antitumour effect of HTE against DAL in Swiss albino mice. A significant enhancement of MST and peritoneal cells counts were observed (Tables 1 and 2).

To evaluate whether HTE treatment indirectly inhibited cell growth, the effect of HTE treatment was examined on the peritoneal exudate cells of normal mice. Normally each mouse contains about 5×10^6 intraperitoneal cells, 50 % of which are macrophages. HTE treatment was found to enhance peritoneal cell counts. These results demonstrated the indirect effect of HTE in DAL

cells probably medicated through enhancement and activation of macrophages or through some cytokine products inside the peritoneal cavity produced by HTE treatment.

Analysis of the haematological parameters showed a minimum toxic effect in mice which was considered as cured [6] by HTE treatment. Fourteen days after transplantation, HTE treated group was able to reverse the changes in the haematological parameters consequent to tumour inoculation.

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