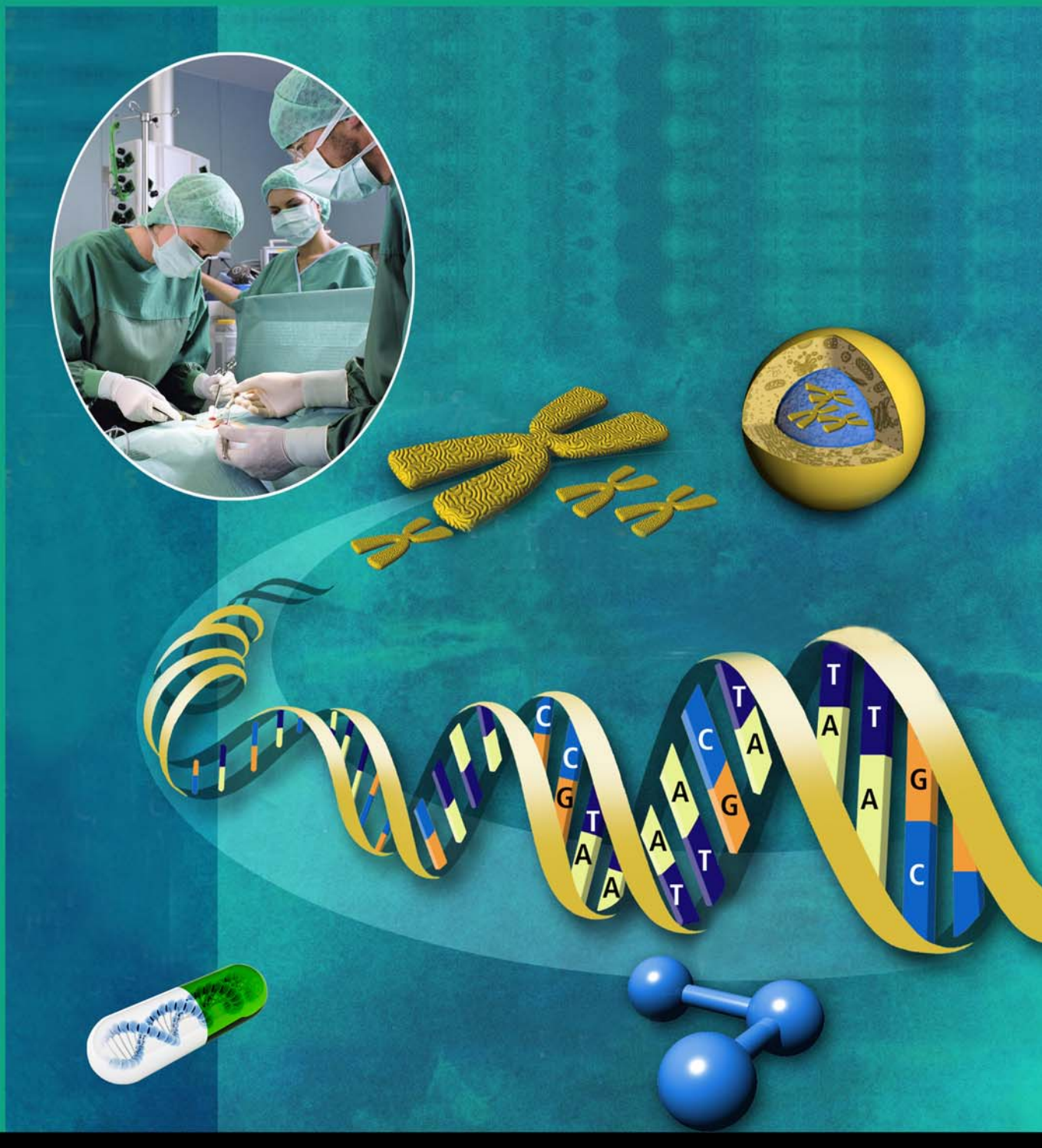
ISSN 2278 - 5221
Vol. 2, No. 3, July 2013

International Journal of Pharma Medicine and Biological Sciences

IJPMBS



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International Journal of Pharma Medicine and Biological Sciences

ISSN 2278 – 5221 www.ijpmbs.com
Vol. 2, No. 3, July 2013

Research Paper

TUMOR REDUCING AND ANTICARCINOGENIC ACTIVITY OF GREEN TEA AGAINST DALTON'S ASCITES LYMPHOMA CELLS

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The present study was carried out to evaluate the effect of Methanol Extract of Green Tea (MEGT) on Tumor growth and haematological parameters. After inoculation of DAL cells in to mice, treatment with MEGT (200 and 400 mg/kg) and standard drug 5-Fluorouracil (10 mg/kg) were continued for 9 days. The MEGT decreased the ascites fluid volume, viable cell count, and increased the non viable cell count. RBC, WBC, PCV, Haemoglobin content and differential count was estimated from the peripheral blood of normal, DAL control and extract treated animal groups. Haemoglobin and RBC level that generally goes down during the progression of tumor was found to improve in mice treated with MEGT. The total WBC counts which were significantly higher in the DAL control mice was found to be reduced in treated groups. This indicates that MEGT possesses protective action on the haemopoietic system. In short, the present work indicates that the MEGT exhibited significant effect on Tumor growth and haematological parameters against DAL cell lines.

Keywords: Green tea, Anticarcinogenic, Daltons ascites lymphoma, Methanolic extract

INTRODUCTION

A large number of plants possessing anti cancer properties have been documented (Gupta *et al.*, 2004; Shimizu *et al.*, 2004; Akai *et al.*, 2005; and Kim *et al.*, 2005). Green tea, a plant with established medicinal properties was chosen for this study. Several of their constituents have shown potent anticancer properties in many models based on the studies conducted throughout the world. Many of the beneficial properties of green tea were attributed to its most abundant catechin, EGCG. But recent reports showed that green tea catechins have the potential to affect absorption and metabolism of ions because flavonoids interact with a variety of metal ions (Mira *et al.*, 2002). Moreover EGCG of green tea extract is cytotoxic and higher consumption of green tea can exert acute cytotoxicity in liver cells, a major metabolic organ in the body (Schmidt *et al.*, 2005). Another study found that higher intake of green tea might cause oxidative DNA damage

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of hamster pancreas and liver (Takabagashi *et al.*, 2004). Therefore based on the ethno medical claims, the present study was intended to highlights the recent research on the efficacy of crude extract of green tea against Dalton's Ascites Lymphoma (DAL) cells.

MATERIALS AND METHODS

Plant Collection

Fresh tea leaves were collected from Tea estate, Valpparai.

Preparation of Extracts

The plucked tea leaves were steamed, rolled and then dried. Dried leaves were powdered using an electrical grinder. Fine powder was obtained by sieving 100 mg of soxhlet extract was dissolved in 2 ml of Dimethyl Sulfoxide (DMSO) and then mixed with 100 ml distilled water and this formed 1000 ppm solution. From the stock solution, solutions of required concentration were prepared and used in this study.

Experimental Animals

Inbred female Swiss Albino mice of 2 months age, weighing 20 ± 5 g, purchased from Government Veterinary College, Mannuthi, Thrissur, India, were used for the study. The mice were obtained from the stock in breed colony, which was maintained by mating brothers and sisters. They were housed at room temperature of 22 °C under 12 hr light/12 hr dark cycle in the animal house. Mice were fed with commercial pellet diet and water ad libitum freely throughout the study. All animal procedures were performed after approval from the Institution of Animal Ethical Committee (IAEC No. KMCRET/phd/06/2010-11) and in accordance with the recommendations for the proper care and use of laboratory animals.

Treatment Procedure

Animals were divided in to five groups. All the groups were inoculated with DAL cells (1 x 10^6 cells/mouse) except those from the normal control group. The treatments were given intraperitoneally at 24 hour after tumor inoculation and continued for 14 consecutive days.

The designation of the animal groups and treatment details are as follows.

Group I → Normal control

Group II → DAL control

Group III → DAL + Positive control (5-FU 10 mg/kg)

Group IV → DAL + MEGT 20 mg/kg

Group V → DAL + MEGT 40 mg/kg

MEGT : Methanolic extract of green tea

5-FU : 5 Fluoro Uracil

Group V → DAL + MEGT 400 μg/kg

MEGT : Methanolic extract of green tea.

After the final dose, 5 animals of each group were sacrificed to study the tumor growth parameters (Mean survival time, % increase in lifespan, viable, non-viable cell, tumor volume, and tumor packed cell volume) and hematological parameters. Blood was collected for estimation of RBC, WBC, Differential cell count and Percentage of Haemoglobin.

Statistical Analysis

Values were expressed as mean ± S.E.M. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's test using SAS (Version 9.1) software.

RESULTS

The mean survival time as well as the percentage increase in lifespan was found to be decreased

Table 1: Effect of MEGT on the Tumor Growth Parameters

Parameters	Normal Control	DAL Control	Positive Control (5-FU)	DAL + MEGT 200 mg	DAL + MEGT 400 mg
Mean Survival Time (days)	21.50 + 0.43	42.50 +0.76	$30.33+ 1.02^{b}$	$34.16+ 1.92^{b}$	$26 + 1.82^{NS}$
Increased Life Span (%)	0.00 + 0.00	97.67	41.08	58.91	20.93
Tumor Volume (ml)	0.00 + 0.00	22.8 + 4.4	$10 + 4.6^{b}$	$20.6 + 5.7^{NS}$	$14.6 + 3.2^{c}$
Tumor PCV (ml)	0.00 + 0.00	6.9 + 2.3	$5.1 + 1.50^{b}$	$7.1 + 0.8^{NS}$	$5.4 + 1.02^{b}$
Viable Cell Count (10^6 cells/ml)	0.00 + 0.00	9.77 + 0.17	$2.28 + 0.07^{a}$	$4.5 + 0.21^{b}$	$4.03 + 0.43^{b}$
Non Viable Cell Count (10^6 cells/ml)	0.00 + 0.00	0.84 + 0.10	$5.75 + 0.07^{a}$	$4.7 + 0.19^{b}$	$3.6 + 0.26^{b}$

Note: Values are expressed as the mean $\pm$ S.E.M. (n = 6); Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test. $^{a}p < 0.001$, $^{b}p < 0.01$, $^{c}p < 0.05$, NS – Non Significant. Calculated by comparing treated group with DAL control group.

Table 2: Effect of MEGT on Haematological Parameters

Parameters	RBC (10^6 cells/«l)	Hb (g/dl)	WBC 10^3 cells/«l	PCV (%)	WBC Differential Count (%)		
					Lymphocyte	Mono	Eosino
Normal Control	8.72±0.27	15.45±39	16.38±2.67	45.9±1.25	39.8±3.10	9.42±0.9	14.4±0.9
DAL Control	4.08±1.17	9.10±0.34	25.13±1.48	21.11±6.6	12.3±4.24	8.42±0.53	8.5±0.88
Positive Control (5-FU)	8.79 ± 0.17^{a}	17.57 ± 0.9^{a}	15.64 ± 2.24^{c}	53.42 ± 3.4^{a}	18.7 ± 2.47^{NS}	9.9 ± 1.15^{NS}	20.9 ± 4.22^{a}
MEGT (200 mg)	7.53 ± 0.65^{b}	12.3 ± 1.19^{c}	12.2 ± 1.24^{b}	32.4 ± 5.9^{NS}	15.5 ± 46^{NS}	9.8 ± 0.77^{NS}	14.8 ± 0.4^{NS}
MEGT (400 mg)	7.83 ± 0.50^{b}	12.8 ± 0.84^{c}	13.37 ± 1.22^{c}	33 ± 4.58^{NS}	36.1 ± 3.08^{a}	10.2 ± 0.7^{NS}	12.2 ± 0.9^{NS}

Note: Values are expressed as the mean + S.E.M. (n = 6); Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test. $^{a}p < 0.001$, $^{b}p < 0.01$, $^{c}p < 0.05$, NS – Non Significant. Calculated by comparing treated group with DAL control group.

in treatment groups (Table 1). A significant reduction of tumor volume was found in all the treatment groups. Maximum reduction was found in the positive control followed by higher dose (MEGT 400 mg). Similar result was showed by tumor PCV. Viable cell count was found to be decreased in treatment groups while the non viable cell count was significantly increased in all the treatment groups.

Inoculation of DAL cells resulted in a significant increase in the level of total WBC when compared with the normal animals (Table 2). Administration of extract reversed these changes and this was comparable with the results produced by the standard drug. Hemoglobin and RBC level that generally goes down during the progression of tumor was found to improve in mice treated with MEGT. With regard to packed cell volume, though the treatment with MEGT increased the volume when compared with that of DAL control the study was found to be statistically non significant. Similar result was observed in the case of monocytes and eosinophils. The data pertaining to lymphocyte count with the addition of MEGT restored to the near normal level when compared to that of the DAL control.

DISCUSSION

Several of green tea constituents have shown potent anticancer properties in many models based on the studies conducted throughout the world. The present study was carried out to investigate the antitumor potential of MEGT against DAL bearing mice. The MEGT treated animals at the doses of 200 and 400 mg/kg

brought back the haematological parameters to more or less normal levels.

In DAL bearing mice, a regular rapid increase in ascites tumor volume was noted. Ascites fluid is the direct nutritional source for tumor cells and a rapid increases in ascites fluid with tumor growth would be means to meet the nutritional requirement of tumor cells (Prasad and Giri, 1994).

The reliable criteria for judging the value of any anticancer drug are the prolongation of the life span of animals (Clarkson and Durchenal, 1965). The MEGT decreased the ascites fluid volume, viable cell count, and increased the percentage of life span.

The most common problems encountered in cancer chemotherapy are myelosuppression and anaemia (Price, 1958; and Marklund *et al.*, 1982). Similar results were observed in the present study in animals of the DAL tumor control group. This is mainly due to reduction in RBC and haemoglobin production and this may occur either due to the iron deficiency or due to haemolytic or other myelopathic conditions. Treatment with MEGT brought back the haemoglobin content, RBC and WBC counts near to normal levels. This indicates that the extract have a protective effect on the hemopoitic system.

Another criterion for judging the anti cancer effect of plant extract is reduction in viable cell count towards normal. It may be due to the extracts stimulate the growth and activity of immune cells by the production of inter leukins, which target tumor cells and cause lysis of the tumor cells by indirect cytotoxic mechanism. Furthermore, the reduced PCV and increased survival time of the mice suggest that the extracts might have exerted a delay in vascular permeability to the cells (Bhist *et al.*, 2010).

CONCLUSION

The outcome of the present investigation indicate that methanolic extract of green tea showed a protective effect on the tumor growth and haematological parameters against Daltons ascites lymphoma induced tumor in mice.

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ISSN 2278-5221
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