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Assessment of Anticancer Potential of Chilauni, *Schima wallichii* (DC.) Korth. in Mice Transplanted with Dalton's Lymphoma Ascite Tumor

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Abstract

The effect of various doses of chloroform, ethanol and aqueous extracts of Chilauni or *Schima wallichii* was studied in Dalton's lymphoma tumor bearing mice. The acute toxicity was determined by oral and intraperitoneal administration in normal non-tumor bearing mice. The oral administration studies have revealed that the chloroform and aqueous extracts of Chilauni were non-toxic up to 4g/kg body weight, whereas the ethanol extract was non-toxic up to 2g/kg body weight. The intraperitoneal administration of various doses of different extracts showed signs of toxicity in mice and a LD50 of 500,100 and 500 mg/kg body weight was recorded for chloroform, ethanol and aqueous extract, respectively. The administration of 10- 250 mg/kg b. wt. chloroform, ethanol and aqueous extracts of Chilauni into tumor bearing mice resulted in a dose dependent rise in the tumor free survival and a maximum effect was observed for 10 mg/kg ethanol extract, which increased the tumor free survival by 40% beyond 120 days, whereas chloroform and aqueous extracts of Chilauni were not that effective. However, 20 and 40 % long term tumor free survivors were observed up to 60 days for chloroform and aqueous extracts at a dose of 150 and 100 mg/kg, respectively. The administration of 10mg/kg body weight ethanol extract of Chilauni resulted in an increase in the average survival time up to 64.81 days and Median survival time up to 72.6 days. The mechanism of cell death was studied in tumorized mice injected with 10 mg/kg body weight of the ethanol extract of Chilauni, which resulted in a time dependent rise in the apoptotic and necrotic indices, and a maximum rise was observed at 36 h post treatment. The cytotoxic effect of ethanol extract of Chilauni may be due to its ability to induce DNA damage and apoptosis.

Keywords: *Schima Wallichii*; Mice; Dalton's Lymphoma; Acute Toxicity; Apoptosis

Introduction

Cancer is a threat to human health and it affects the lives of millions of people around the world. Cancer drains financial and emotional resources of a family in which cancer is detected. It is the second largest cause of death succeeding cardiovascular diseases. Globally, the number of cancer cases diagnosed in 2018 are 1,735,350 and 609,640 deaths are projected in United States alone and global burden will be much higher [1]. It has been estimated that the total annual economic cost of cancer in 2010 was approximately US $ 1.16 trillion and it is rising every year [2]. In the last five years 70 new high cost oncology drugs have been approved increasing the cost of cancer care [3]. The present cancer treatments modalities include surgery, radiotherapy, and chemotherapy or their combination. The application of chemotherapy puts cancer patients under a lot of stress as it may be responsible for further serious damage to their health and leads to therapy related second malignancies [4,5]. Therefore, using alternative treatments therapies against cancer is the main goal to develop agents, especially from plants to reduce the economic burden to the cancer patients.

Natural products have been used for centuries for the treatment of several ailments. Many bioactive compounds have been discovered from plants, animals and microbes, which synthesize natural products and secondary metabolites for various purposes. These products and secondary metabolites serve a major source of drugs to treat different diseases including cancer [6,7]. However, research on this aspect has been limited, and more and more pharmaceutical industries are interested in examining potential of natural products and secondary plant metabolites as sources of novel medicinal compounds [8]. In the 21st century, findings related to developing new drugs from natural plants and marine life have attracted more and more attention [9,10]. Different medicines from plants and natural products have been accepted by people from all over the world, looking forward to improving the quality of life, disease prevention, treatment of chronic diseases and geriatric diseases as well as Western medicine with helpless

mysterious illness [8]. New therapeutic strategies are not only directed to eliminate cancer cells by induction of apoptosis, but also include targeting the tumor microenvironment, avoiding angiogenesis, modulating the immune response or the chronic inflammation which are often associated with cancers [11-14]. Plants have a long history of use in the treatment of cancer and have played an important role as a source of effective anti-cancer agents, and it is significant that over 60% of currently used anti-cancer agents are derived in one way or the other from natural sources, including plants, marine organisms and micro-organisms [9,10].

The first agents to advance into clinical use were the vinca alkaloids, vinblastine (VLB) and vincristine (VCR), isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae), which was used by various cultures for the treatment of diabetes [15,16]. Plants did also provide several other modern chemotherapeutic molecules including podophyllotoxins, taxols, camptotheicin, doxorubicin and bleomycin that are in frequent clinical use to treat different types of malignant neoplasia [9,10]. However, the adverse effects of modern isolated molecules are severe and their use has been associated with the development of second malignancies [4,5,17]. Therefore, search for novel drugs is still an interesting avenue for cancer therapy due to the fact that chemotherapeutic drug resistance is becoming more and more frequent [18,19].

Schima wallichii (DC.) Korth. or Chilauni (family, Theaceae) is an evergreen tree growing luxuriously in the warm temperate to subtropical climates. It is widely found across southern and South East Asia, and stretch from the Eastern Himalaya of Nepal to Eastern India across Indochina, Southern China, Taiwan and the Ryukyu Islands. It is commonly known as needle wood tree and it grows up to 10-20 m high [20]. Locally, it is called "khiang" in Mizo language. *Schima wallichii* is credited to possess several medicinal properties. The leaves and the stem bark are normally used traditionally as a medicine. The bark is used as an antiseptic to treat cuts and wounds. It is a vermicide, mechanical irritant and used to cure gonorrhea [21]. Decoction of bark is useful to cure fever and it is also effective against head lice. The bark juice is given to disinfest the animals from liver flukes [22]. The sap from the stem is used for curing ear infection. Fruit decoction is used by the people of Western Mizoram, India against snakebite [22,23]. The young plants, leaves and roots are also used medicinally against fever and the bark is anthelmintic and rubefacient [24]. The leaves of *Schima wallichii* are known to have antitumor and antimutagenic properties [25,26]. The astringent corollas are used to treat uterine disorders and hysteria [27]. Recently the stem bark of *Schima wallichi* has been reported to scavenge various free radicals and also exhibit anticancer activity *in vitro* [28,29]. The antineoplastic activity of Chilauni has not been investigated systematically until now in vivo, therefore the present study was undertaken to obtain an insight into the antineoplastic activity of *Schima wallichii* in the Swiss albino mice transplanted with Dalton's Lymphoma.

Materials and Methods

Chemicals

Dimethylsulphoxide (DMSO), ethidium bromide, acridine orange, and crystal violet, were obtained from Sigma Aldrich Chemical Co. Kolkata, India. Sodium hydroxide (NaOH), sodium chloride (NaCl), disodium hydrogen phosphate (Na_2HPO_4), sulphuric acid (H_2SO_4), ammonium oxalate, methanol, acetic acid, petroleum ether, chloroform, ethanol and hydrochloric acid (HCl), were procured from Merck India Limited, Mumbai. Doxorubicin was requisitioned from Getwell Pharmaceuticals, Gurgaon, India.

Collection and Preparation of the plant extract

The non-infected stem bark of *Schima wallichii* (DC.) Korth. or Chilauni (family: Theaceae) was collected from Bazar veng, Lunglei, Mizoram, India during the months of April and May. The authentication and identification of *Schima wallichii* was done by the Botanical Survey of India, Shillong, Meghalaya (BSI/ERC/Tech// Identification/2017/570). The stem bark was washed with water to remove dust and other extraneous material, shade dried at room temperature in clean and hygienic conditions. The dried bark was then powdered using an electrical grinder. The dried powder of *Schima wallichii* was extracted sequentially with petroleum ether, chloroform, ethanol and distilled water in order of increasing polarity using a Soxhlet apparatus. The liquid extracts were filtered and concentrated by evaporating their liquid contents to dryness under reduced pressure. The concentrated extracts were stored at -80 °C until use.

Preparation of Drug and mode of administration

The various extracts of *S. wallichii* were dissolved in appropriate solvent/s before use. The chloroform (SWC) extract was dissolved in 5% ethanol in distilled water, 1% absolute ethanol was used for dissolving the ethanol extract (SWE), whereas doubled distilled water was used for dissolving aqueous extract (SWA) and doxorubicin. The weight of the animals from different groups were taken and recorded. According to the body weight of the animals, each animal received treatments orally and intraperitoneally depending on the experimental design.

Animal care handling

The guidelines issued by the World Health Organization (WHO), Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi, India) were strictly followed for handling and care of animals. Swiss albino mice purchased from Pasteur Institute, Shillong were bred and maintained in a controlled environment of temperature (24-25 °C), 50% humidity and a light and dark cycle of 12 h each. About five animals were housed in a sterile polypropylene cage which contained sawdust (procured

locally) as bedding material. For experimentation, normally six to eight weeks old Swiss albino mice of both genders weighing around 25-30 g were selected. The animals were fed with commercially available food pellets and allowed free access to water. The animal experiments were carried out according to NIH and Indian National Science Academy, New Delhi, India guidelines. The Institutional Animal Ethics Committee of Mizoram University, Aizawl, India approved the entire study vide letter No. MZU/IAEC/14-15/10.

Determination of acute toxicity

The acute toxicity of all extracts of *S. wallichii* was determined by administering 0, 2 or 4 g/kg b. wt. of chloroform, ethanol or aqueous extract orally or 0.1, 0.5, 2, 3, 4 or 5 g/kg body weight of chloroform, ethanol or aqueous extract of *S. wallichii* intraperitoneally according to the guidelines of Organization for Economic Co-operation and Development (OECD). The mice of both sexes (5 males and 5 females) were categorized into different groups by random sampling technique. Usually ten animals were utilized for each dose of each extract. The animals were fasted for 18 hours (both food and water withdrawn) prior to administration of different extracts of *S. wallichii* [30,31]. The weights of the animals were recorded before and after fasting to estimate their weight loss. The selected animals were divided into four groups according to the extract administered. The SWC group received chloroform extract, the SWE group received ethanol extract, and the SWA group received aqueous extract. The control group received sterile physiological saline (SPS). The animals were provided with food immediately after administration of different extracts. The animals under treatment were observed for first two hours and every 6 hours until 24 hours, and daily thereafter for a total period of 14 days for the development of toxic symptoms. If mortality was observed in 3-4 animals, then the dose administered was assigned as toxic dose. The behavior of the animals was observed and recorded and the LD_{50} for each extract was calculated using probit analysis.

Tumor Model

A Dalton's lymphoma ascites (DLA) tumor was used for the entire study as it provides most convenient model system to study antitumor activity within a short time [32]. DLA was procured from North-Eastern Hills University (NEHU), Shillong, India and was maintained in 4-6 weeks old Swiss albino mice by serial intraperitoneal transplantation. Usually one million viable DLA cells were inoculated intraperitoneally into each animal under aseptic conditions and the day of inoculation was taken as day 0.

Experimental design

The anticancer activity of different extracts of Chilauni (*Schima wallichii*) was determined in Dalton's lymphoma tumor bearing mice that were divided into the following groups: -

SPS group: This group of tumorized mice received 0.01 ml/g body weight of sterile physiological saline and served as the negative control group.

SWC group: The animals of this group were administered with 50, 100, 150, 200 and 250 mg/kg body weight of the chloroform extract of *Schima wallichii* intraperitoneally.

SWE group: This group of animals was intraperitoneally injected with 10, 20, 30, 40 and 50 mg/kg body weight of the ethanol extract of *Schima wallichii*.

SWA group: This group of animals was given 50, 100, 150, 200 and 250 mg/kg body weight of the aqueous extract of *Schima wallichii* intraperitoneally.

The tumor bearing animals were given treatment once daily on 1 day after tumorization and for subsequent 9 days [33]. Each group consisted of ten animals for each extract dose and 170 animals were used for this experiment. The animal survival was monitored daily up to 120 days, since the survival of animals up to 120 days is approximately equivalent to 5 years in humans [34]. The deaths, if any, of the tumor bearing mice were recorded daily and the survival was determined. The tumor response was assessed by calculating median survival time (MST) and average survival time (AST). The MST and AST were calculated from the animals dying within 120 days and those surviving beyond 120 days were excluded from the study [33]. The increase in median life span (% IMLS), increase in average life span (% IALS) was also calculated using the formulae:

MST= First death + Last death in the group/2
AST= Sum of animals dead on different days/No. of animals
IMLS (%) = MST of treated mice – MST of control x 100/MST of control
IALS (%) = AST of treated mice – AST of control x 100/AST of control
The optimum dose for each extract was determined and the optimum dose as well as ethanol extract which increased the longest tumor free survivors was selected for other assays.

Micronucleus Assay

The ability of SWE to induce DNA damage in Dalton's lymphoma was studied by performing a separate experiment where 1 x 10^6 Dalton's lymphoma cells were transplanted into 5-8 weeks old mice and allowed to develop the tumor for 1day. Thereafter, these

animals were given a nine days treatment of 10 mg/kg body weight of *Schima wallichii* ethanol extract (SWE) or 0.5 mg/kg body weight doxorubicin intraperitoneally. One hour after the last drug/s administration, each of the tumorized mice was injected with 150 µg of cytochalasin-B so as to suppress cytokinesis in the proliferating tumor cells. The mice were euthanized at 6, 12, 24 and 48 h after last-drug treatment and the tumor cells were collected in individual tubes. The tumor cells were washed with ammonium chloride to lyse erythrocytes and centrifuged at 1000 rpm. The micronuclei were prepared according to the modified method of Fenech and Morley [35]. In brief, cells were washed with sterile PBS and pelleted by centrifugation at 1000 rpm for 5 min. The cell pellet was disturbed and treated with mild hypotonic solution (0.75% ammonium oxalate) at 37 °C, centrifuged once again and the resultant cell pellet was allowed to fix in Carnoy's fixative 3:1 (Methanol: Acetic acid) overnight. The cells were centrifuged and the resultant pellet was resuspended in a small volume of fixative. The cells were spread on to precleaned coded slides to avoid observer's bias. The cells were stained with 0.025% acridine orange (BDH, England, Gurr Cat. no. 34001 9704640E) in Sorensen's buffer (pH 6.8) and subsequently washed twice in the buffer to remove excess stain. The slides were mounted in Sorensen's buffer and observed under a DM-2500 fluorescent microscope (Leica Microsystems, Wetzlar, Germany) equipped with 450–490 nm BP filter set with excitation at 453 nm using a 20 X N Plan objective. Usually one thousand mononucleated or binucleated cells (a total of 5000 each) with well-preserved cytoplasm were scored for each post-treatment time in each group. The frequency of mononucleated cells bearing micronuclei (MNMNC) as well as binucleated cell bearing micronuclei (MNBNC) was determined. The micronucleated cells were scored according to the earlier descried criteria [36,37].

Apoptosis Assay

The ability of SWE to induce apoptosis in Dalton's lymphoma cells was performed to investigate induction of DNA damage, where grouping and other conditions were exactly similar to that described for micronucleus assay except that tumor bearing mice were euthanized at 2, 6, 12, 24 and 48h after last drug treatment. The tumor cells were aspirated and washed with ammonium chloride to lyse the erythrocytes and cells were pelleted by centrifugation. The cells were washed again with sterile PBS and spread on to clean coded slides and stained with freshly prepared ethidium bromide and acridine orange (1:1) (Sigma Aldrich Chemical Co. Bangalore, India) stain and observed under a DM-2500 fluorescent microscope (Leica Microsystems, Wetzlar, Germany). The number of live, necrotic and apoptotic cells were counted. The viable cells were recognized by green fluorescing nuclei having organized structure, whereas the early apoptotic cells showed highly condensed or fragmented yellow chromatin in the nuclei and membrane blebbing. The late apoptotic cells were conspicuous by orange stained chromatin with nuclei that were highly condensed and fragmented. The necrotic cells showed bright orange chromatin in round nuclei [38]. Only cells with yellow, condensed, or fragmented nuclei were counted as apoptotic cells in a blinded, non-biased manner. A total of 1000 cells were counted for each animal and a total of 5000 cells were counted for each group at each as assay time. The percentage of apoptotic, and necrotic cells was calculated as follows:

Apoptotic index (%) = Number of apoptotic cells scored X 100/Total number of cells counted.
Necrotic index (%) = Number of necrotic cells scored X 100/Total number of cells counted.

Statistical Analyses

The statistical analyses were carried out using Origin Pro 8 SRO v8.0724 (B724), Northampton, MA, USA. The significance for survival analysis was determined by Kaplan Meier test and Mann Whitney "U" test was applied for micronucleus and apoptosis assays. The results were confirmed by repetition of the experiments. Test of homogeneity was applied to determine any statistical differences between the repeat experiments. Since no significant differences were observed the data of all experiments were combined and expressed as mean ± standard error of the mean (SEM). A p value of < 0.05 was considered statistically significant.

Results

The results have been expressed as the mean ± standard error of the mean (SEM) and are presented in Tables 1-10 and Figures 1-8.

Extract/ Group	Sex	Dose (g/kg body weight)	Body weight (g)			Survival
			Before fasting	After fasting	Loss (18 h)	
Control (SPS)	M	0	30	27	3	> 14 days
			32	29.8	2.2	> 14 days
			28.2	25.0	3.2	> 14 days
	F		30	25.9	4.1	> 14 days
			25.8	22.2	3.6	> 14 days
			27	24	3	> 14 days

Extract/ Group	Sex	Dose (g/kg body weight)	Body weight (g)			Survival
			Before fasting	After fasting	Loss (18 h)	
Chloroform	M	4	31.6	29.9	1.7	> 14 days
			35	31	4	> 14 days
			29.6	27	2.6	> 14 days
			33	30.2	2.8	> 14 days
			30.3	27.4	2.9	> 14 days
	F		25	22	3	> 14 days
			28.5	26.7	1.8	> 14 days
			29.4	27.3	2.1	> 14 days
			25.7	22.8	2.9	> 14 days
			25.3	24	1.3	> 14 days
Ethanol	M	2	35.2	33.3	1.9	> 14 days
			35.5	33.2	2.3	> 14 days
			31.6	29.9	1.7	> 14 days
			27.2	25.0	2.2	> 14 days
			32.2	29.8	2.4	> 14 days
	F		25.8	23.7	2.1	> 14 days
			26.4	23.5	2.9	> 14 days
			25.8	23.7	2.1	> 14 days
			29.3	27.0	2.3	> 14 days
			27.0	25.4	1.6	> 14 days
Aqueous	M	4	35.2	32.6	2.6	> 14 days
			32.5	30.4	2.1	> 14 days
			35	31.6	3.4	> 14 days
			27.2	23.8	3.4	> 14 days
			25.9	24.1	1.8	> 14 days
	F		30	27.5	2.5	> 14 days
			28.8	25.2	3.6	> 14 days
			30	27.5	2.5	> 14 days
			29.5	26.7	2.8	> 14 days
			33.0	30.5	2.5	> 14 days

N=10 for each dose of each extract.

Table 1: Acute toxicity of different extracts of *Schima wallichii* in Swiss albino mice after oral administration

Acute toxicity

The oral administration of the different extracts of *Schima wallichii* showed no signs of toxicity up to 4g/kg body weight for chloroform and aqueous extracts whereas administration of 2g/kg body weight ethanol extract also did not reveal any toxic effect (Table 1). The acute toxicity assay after the intraperitoneal administration was carried out by up and down method. This mode of administration exerted toxic effects at 2 g/kg body weight for chloroform extract where 30% animals succumbed to death (Tables 2 and 5). Administration of ethanol extract was highly toxic as 30% animals died after administration of 500 mg/kg body weight of this extract (Tables 3 and 5). The intraperitoneal administration of 3 g/kg body weight of aqueous extract led to a 30% mortality and this was least toxic when compared to other extracts (Tables 4 and 5). The probit analysis resulted into the LD50 of 100mg/kg body weight for ethanol extract, whereas it was 500 mg/kg b. wt. for the chloroform and aqueous extracts, respectively (Tables 5and 6).

Dose (g/kg)	Mortality (%) on different days														Total	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
5	100	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Paralysis, lethargy, died within 1h.

Dose (g/kg)	Mortality (%) on different days														Total	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
4	100	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Lethargy, died within 3h.
3	-	-	20	20	-	-	20	-	-	30	-	-	-	-	90	Inactive, died before day 10.
2	-	-	-	-	20	-	30	-	-	20	-	-	-	-	70	Inactive, died within day 10.
1	-	-	-	-	-	20	20	-	-	-	-	20	-	-	60	Active,4 survived after day 12
0.5	-	-	-	-	-	-	-	10	-	10	-	20	10	-	50	Active, 5 died within day 13.

N = 10 for each dose of each extract.

Table 2: Acute toxicity of chloroform extract of *Schima wallichii* after intraperitoneal administration in mice

Dose (g/kg)	Mortality (%) on different days														Total	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
5	100	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Paralysis, lethargy, semiconscious, died within 1h.
4	100	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Paralysis, lethargy, died within 4h.
3	80	-	-	20	-	-	-	-	-	-	-	-	-	-	100	Lethargic, dullness, died within day 4.
2	50	-	20	-	-	20	-	-	-	-	-	-	-	-	90	Lethargic, dullness, died before day 7.
1	20	-	20	-	-	40	-	-	-	-	-	-	-	-	80	Dullness, died before day 7.
0.5	20	-	-	-	-	-	-	-	20	-	20	10	-	-	70	Inactive, died within day 12.
0.25	-	20	-	-	-	-	20	-	-	-	-	20	-	-	60	Inactive, died within day 12.
0.1	-	-	-	-	-	-	10	-	-	40	-	-	-	-	50	Active and only 5 died.

N=10 for each dose of extract.

Table 3: Acute toxicity of ethanol extract of *Schima wallichii* after intraperitoneal administration in mice

Dose (g/kg)	Mortality (%) on different days														Total	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
5	100	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Paralysis, lethargy, died within 1h.
4	100	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Paralysis, lethargy, died within 3h.
3	-	-	20	-	-	40	-	-	10	-	-	-	-	-	70	Loss of appetite, inactive, died within day 9.
2	-	20	-	-	-	40	-	-	-	-	-	-	-	-	60	Inactive, died before day 7.
1	-	-	-	20	-	-	-	-	20	-	-	20	-	-	60	Inactive, died within day 12
0.5	-	-	-	-	-	-	-	-	-	30	-	20	-	-	50	Active,5 died on day 12.

N = 10 for each dose of extract.

Table 4: Acute toxicity of aqueous extract of *Schima wallichii* after intraperitoneal administration in mice

Extracts	Dose (mg/kg)	Survival %	LD50 (mg/kg)
Chloroform	5000	0	500
	2000	30	
	500	50	
Ethanol	5000	0	100
	500	30	
	100	50	
Aqueous	5000	0	500
	3000	30	
	500	50	

N = 10 for each dose of each extract.

Table 5: The LD50 of different extracts of *Schima wallichii* after intraperitoneal administration in mice (Probit analysis)

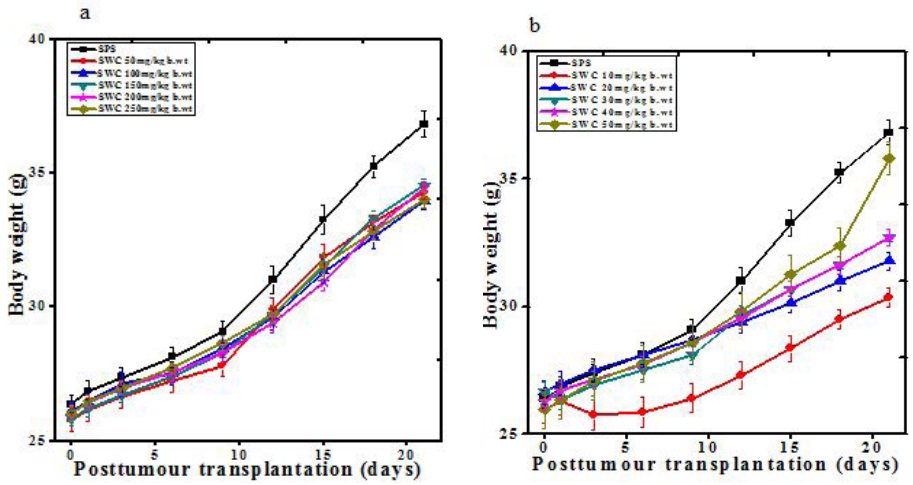
Treatment	Dose (mg/kg body weight)	Mean body weight (g)±SEM								
		Post tumor transplantation time(days)								
		0	1	3	6	9	12	15	18	21
SPS	--	26.37±0.32	26.88±0.35	27.39±0.37	28.13±0.37	29.08±0.39	31±0.51	33.26±0.53	35.24±0.39	36.82±0.48
Chloroform	50	25.77±0.43	26.16±0.43	26.64±0.42	27.24±0.41	27.82±0.40	29.91±0.43	31.88±0.48	33.18±0.27	34.27±0.25
	100	26.12±0.33	26.48±0.31	27.13±0.44	27.53±0.44	28.45±0.48	29.62±0.46	31.34±0.44	32.63±0.43	33.97±0.35
	150	25.86±0.28	26.21±0.31	26.72±0.29	27.41±0.28	28.27±0.31	29.72±0.36	31.46±0.39	33.32±0.26	34.54±0.22
	200	26.06±0.26	26.46±0.27	26.98±0.27	27.58±0.32	28.27±0.31	29.41±0.34	30.93±0.34	32.89±0.31	34.48±0.20
	250	26.03±0.27	26.48±0.22	27±0.21	27.72±0.21	28.66±0.28	29.73±0.32	31.56±0.38	32.84±0.33	34.02±0.30
Ethanol	50	25.98±0.77	26.37±0.78	27.09±0.76	27.78±0.72	28.58±0.69	29.84±0.71	31.26±0.72	32.4±0.67	35.8±0.63
	40	26.29±0.37	26.7±0.35	27.15±0.34	27.73±0.33	28.58±0.36	29.54±0.33	30.94±0.30	32.83±0.22	34.19±0.20
	30	26.66±0.39	26.37±0.39	26.92±0.39	27.53±0.36	28.11±0.36	29.68±0.33	30.69±0.30	31.62±0.33	32.69±0.33
	20	26.66±0.45	27±0.44	27.52±0.45	28.11±0.47	28.68±0.47	29.41±0.47	30.16±0.41	31.02±0.38	31.78±0.34
	10	25.98±0.55	26.31±0.56	25.76±0.57	25.87±0.59	26.42±0.57	27.3±0.55	28.37±0.51	29.49±0.39	30.36±0.37
Aqueous	50	26.25±0.29	26.73±0.29	27.43±0.29	28.02±0.28	28.59±0.29	29.83±0.20	31.59±0.25	33.24±0.24	33.96±0.20
	100	26.08±0.36	26.67±0.39	27.29±0.37	28.19±0.40	29.02±0.44	30.26±0.42	31.45±0.54	32.21±0.59	33.54±0.65
	150	25.84±0.29	26.4±0.30	27.32±0.28	28.53±0.29	29.84±0.30	31.66±0.33	32.9±0.29	34.37±0.37	36.06±0.51
	200	26.35±0.37	26.72±0.37	27.19±0.34	27.77±0.33	28.31±0.33	29.06±0.35	30.44±0.32	33±0.44	34.79±0.39
	250	26.42±0.35	26.78±0.38	27.23±0.36	27.73±0.36	28.24±0.35	29.27±0.33	30.67±0.42	31.93±0.40	33.63±0.30

N=10 for each dose of each extract.

Table 6: Alteration in body weights of Dalton's lymphoma bearing Swiss albino mice after intraperitoneal administration with different extracts of *Schima wallichii*

Anticancer activity

Body weight changes: The transplantation of DLA cells into mice resulted in continuous gain in the body weights until their survival and there was no sign of tumor regression in the negative control group. The DLA mice treated with 50, 100, 150, 200



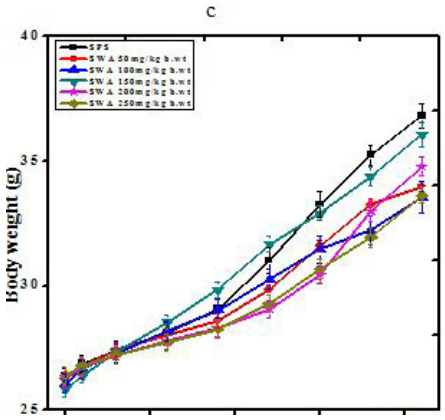


Figure 1: Change in body weight of Dalton's lymphoma bearing Swiss albino mice after treatment with different concentrations of the various extracts of *Schima wallichii*. a: Chloroform extracts; b: Ethanol extract; and c: Aqueous extract. The data are expressed as Mean ±SEM, N=10/dose of each extract

and 250 mg/kg body weight for chloroform and aqueous extracts and 10, 20, 30, 40 and 50 mg/kg body weight of ethanol extract of *Schima wallichii* showed an increase in the body weight with time however, this gain in body weight was lesser when compared to negative control group. This increase in body weight was insignificant up to 21st day of tumor transplantation as compared with day 0 within all the treated groups. The comparison of *Schima wallichii* extract treated groups with negative control revealed a considerable decrease in the body weight due to inhibition of cell propagation (Figure 1).

Survival assay

Dalton's lymphoma transplanted intraperitoneally into mice developed speedily with no signs of regression and all the untreated tumorized mice died within 24 days (Figure 2). The AST and MST for this group were found to be 21.3 and 21 days, respectively (Table 8).

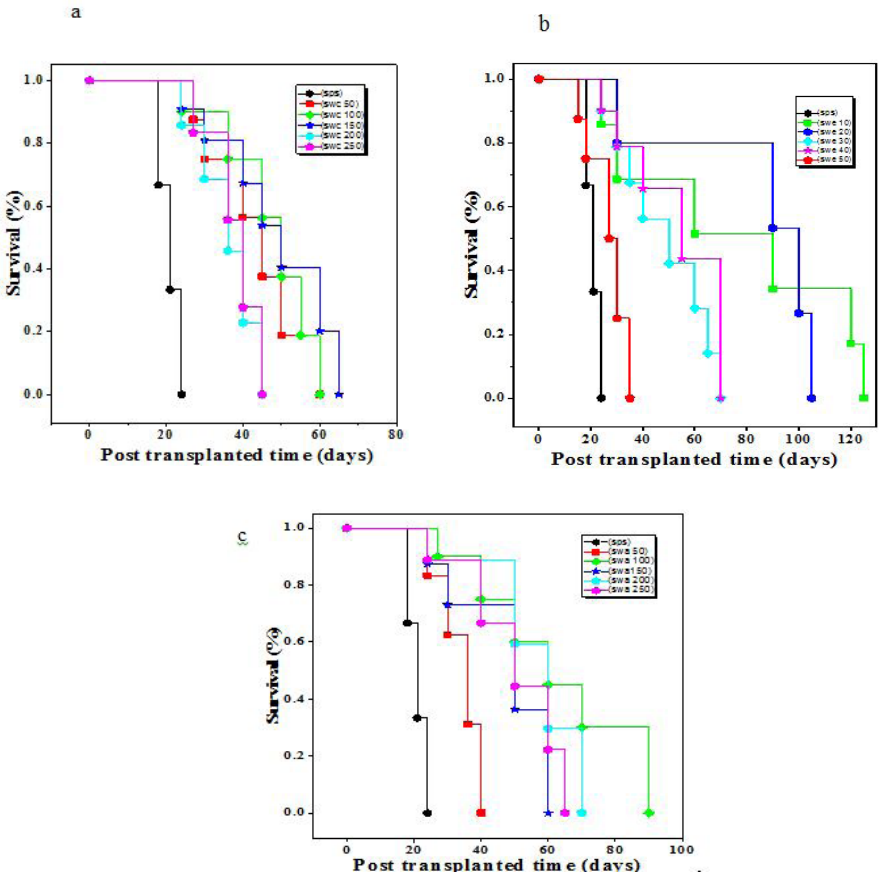


Figure 2: Kaplan Meir's estimate of survival of Dalton's lymphoma ascites bearing mice treated with various extracts of *Schima wallichii* for 9 days consecutively. a: Chloroform extract; b: Ethanol extract and c: Aqueous extract. N=10/dose of each extract.

Post tumor transplantation time (days)	Tumor free survival (%)															
	SPS (Control)	Dose of different extracts (mg/kg body weight)														
		Chloroform					Ethanol					Aqueous				
		50	100	150	200	250	10	20	30	40	50	50	100	150	200	250
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
20	90										80					
25	50		60	90	70		90		80	90	50	80		70	70	70
30	0	70		80	50	70	80	80	70		20	50	90	40		
35			50		30	40			60	60	20	10			60	
40		50		70	10	10			50		0	0	80			60
45		30	30	50	0	0									50	
50		10	20	40					30	50			70	20	40	40
55		0	10											10		30
60			0	20			60		20	40			40	0	20	10
65				0					10						10	
70									0	0					0	
75																
80										0			30			
85													20			
90							50	60								
100								20					0			
120							40	0								

N = 10 for each dose of each extract.

Table 7: Effect of different extracts of *Schima wallichii* on the survival of Dalton's lymphomas ascites bearing mice after intraperitoneal administration

Treatment	Dose (mg/kg body weight)	Survival Time (days)		Increased Life Span (%)	
		MST	AST	IMLS	IALS
SPS(control)	-	21.00±1.3	18.10±2.1	0	0
Chloroform	50	41.44±0.212*	41.47±0.12*	97.35±1.01*	94.68±0.55*
	100	41.39±0.30	38.60±0.03*	97.09±1.42*	81.22±0.16*
	150	47.11±0.32*	48.56±0.10*	124.34±1.52*	127.49±0.48*
	200	34.50±0.20*	32.24±0.06*	64.28 ±0.97*	51.38±0.27*
	250	36.22±0.22*	35.12±0.15*	72.4*±1.058*	64.89±0.70*
Ethanol	10	72.60±0.15*	64.81±0.20*	224.14±7.38*	204.27±0.96*
	20	67.85±0.21*	85.02±0.16*	206.96±6.78*	299.15±0.73*
	30	47.25±0.23*	44.33±0.161*	119.37±3.90*	108.12±0.76*
	40	44.70±0.28*	48.97±0.17*	104.88±4.30*	129.91±0.80*
	50	25.45±0.19*	24.11±0.10*	16.64±2.43*	13.19±0.48*
Aqueous	50	32.25±0.23*	31.8± 0.15*	53.57±1.08*	49.30±0.68*
	100	55.8±0.23*	54.73 ±0.27*	165.71±1.08*	156.95±1.26*
	150	42.8±0.23	38.3 ±0.10*	103.81±1.08*	79.81±0.46*
	200	46.95±0.273*	48.21 ±0.16*	123.57±1.30*	126.34±0.76*
	250	44.05±0.26*	45.74 ±0.32*	109.762±1.25*	114.74±1.52*

*$p < 0.05$, when treatment groups are compared to spontaneous control group.

N = 10 for each dose of each extract.

Median Survival Time (MST); Average Survival time (AST); Increase in Mean Life Span (% IMLS) and increase in Average Life Span (% IALS).

The results are expressed as percent (%) ± SEM.

Table 8: Effect of various doses of different extracts of *Schima wallichii* on the survival of Dalton's lymphoma ascites bearing mice

The administration of 50, 100, 150, 200 and 250 mg/kg body weight of chloroform extract significantly increased the number of survivors when compared to negative control group ($p<0.05$). The maximum survival of tumorized mice was observed at a dose of 150mg/kg chloroform extract, where 20% of the animals survived up to 60 days post tumor transplantation (Table 8). It has led to an AST of 48.4 days, and MST of 47.5 days and an IMLS of 124.34 % and an IALS of 127.49 %, respectively (Figure 3). Treatment of Dalton's lymphoma bearing mice with 50, 100, 150, 200 and 250 mg/kg body weight of the aqueous extract resulted in a dose dependent rise in the survival of mice up to a dose of 250 mg/kg SWE when compared to SPS control ($p<0.05$) (Figure 3). A maximum number of tumor free survivors were observed at 100 mg/ kg body weight SWA where 40% long term tumor free survivors were recorded up to 60 days and 20% of the animals did survive up to 85 days (Figure 2). The AST of 54.73 days and MST of 55.8 days were recorded for 100 mg/kg with an IMLS of 165.71 % and an IALS of 156.948 %, respectively (Table 8) (Figure 3).

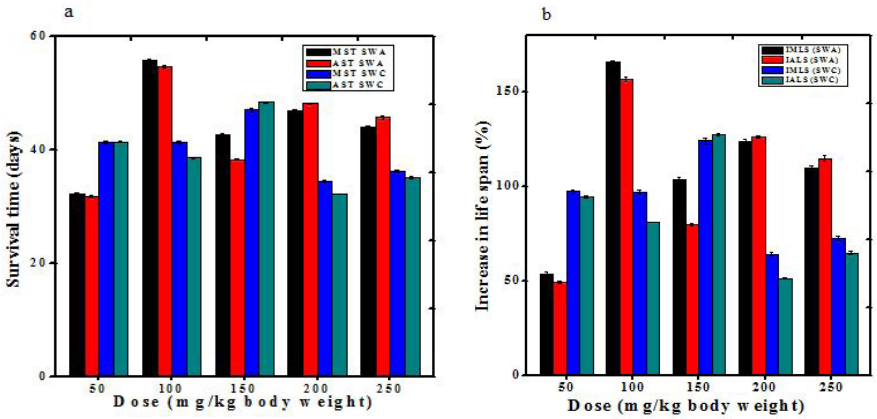


Figure 3: Effect of chloroform and aqueous extracts of *Schima wallichii* on the survival of Dalton's lymphoma ascites bearing mice. a: Median survival time (MST), Average survival time (AST) and b: Increase in mean life span (% IMLS), increase in average life span (% IALS). The results are expressed as Mean ± SEM, N=10/dose of each extract.

The treatment of tumor bearing mice with 10, 20, 30, 40 and 50 mg/kg body weight of the ethanol extract resulted in a rise in the survival and a maximum number of tumor free survivors (40%) were observed at 10 mg/kg body weight where animals survived beyond 120 days with no evidence of disease. The administration of 20 mg/kg body weight of ethanol extract resulted in 60% tumor free survivors up to 90 days however; no survivors could be recorded up to 120 days (Figure 2). The administration of 10 mg/kg body weight SWE resulted in an AST of 64.81 days, and MST of 72.6 days with an IMLS of 224.14 % and an IALS of 204.27 %, respectively (Figure 4). Since 40% animals survived at 10 mg/kg SWE until 120 days or more it was regarded as the best anticancer dose and further investigations were carried out using this dose.

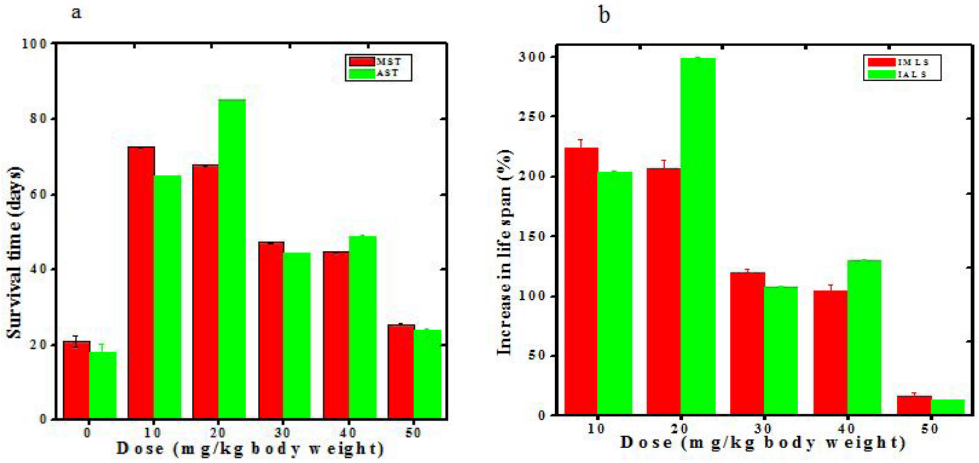


Figure 4: Effect of ethanol extract of *Schima wallichii* on the survival of Dalton's lymphoma ascites bearing mice. a: Median survival time (Red bars), Average survival time (Green bars) and b: Increase in median life span (Red bars) and Increase in average life span (Green bars). The results are expressed as Mean ± SEM, N=10/dose of each extract.

Micronucleus Assay

The frequency of micronuclei bearing mononucleate (MNMNC) and binucleate cells (MNBNC) has been represented separately (Table 9) (Figure 5 and 6). Treatment of Dalton's lymphoma bearing mice with SWE or DOX showed a time dependent rise in the frequency of micronuclei ($p<0.05$) up to 24 h post-drug treatment and a decline thereafter (Figure 5). The frequency of binucleate cells bearing one micronuclei increased with assay time and a maximum number of one micronucleated binucleate cells was scored

at 24 h in 10 mg/kg SWE treated group. The frequency of binucleate cells bearing two micronuclei also revealed a pattern similar to one micronuclei induction which was 10 or more folds higher than the negative control (Table 9) (Figure 6). The positive control doxorubicin also increased the frequency of mononucleate and binucleate cells bearing one and two micronuclei similar to that of SWE treatment, except that the frequencies were higher than that of SWE treatment (Table 9) (Figure 5 and 6).

Cell type	Post assay time (h)	Frequency of micronucleated cell/1000± Standard error of the mean								
		SPS			SWE 10 mg/kg body weight			DOX 0.5 mg/kg body weight		
		One MN	Two MN	Total	One MN	Two MN	Total	One MN	Two MN	Total
Mono nucleate cell	6	5.1±0.28	0.6±0.22	5.7±0.3	31.7±0.63*	0.7±0.26	32.4±0.73*	36.8±1.17*	3.3±0.58*	40.1±0.91*
	12	6.5±0.27	0.6±0.16	7.1±0.31	69.5±0.82*	6.8±1.37*	76.3±1.71*	77.6±1.34*	5.8±0.78*	83.4±1.69*
	24	8.8±0.57	0.9±0.31	9.7±0.45	105.3±1.54*	10.7±0.84*	116.0±2.0*	124.4±1.22*	8.6±0.64*	133±1.32*
	48	8.1±0.23	0.7±0.21	8.8±0.33	91.9±1.58*	10.0±0.70*	101.9±1.66*	119.1±1.43*	8.9±0.61*	128±1.61*
Binucleate cell	6	6.8±0.2	1±0.33	7.8±0.33	33.2±0.51*	0.8±0.25	34.0±0.61*	38.9±1.49*	4.7±0.82*	43.6±1.55*
	12	7.6±0.22	0.5±0.17	8.1±0.23	71.9±0.56*	7±1.26*	78.9±1.56*	84.4±1.36*	7.7±0.58*	92.1±1.39*
	24	10.3±0.3	0.6±0.22	10.9±0.38	109.5±1.96*	12.1±0.75*	121.6±1.91*	132.2±1.12*	12.6±0.96*	144.8±1.59*
	48	8.9±0.43	0.7±0.21	9.6±0.56	99.8±2.10*	9.4±1.00*	109.2±1.93*	121.6±1.17*	8.2±0.66*	129.8±1.39*

*p < 0.05, when treatment groups are compared to spontaneous control group.
N=5 for each assay time for each group.

Table 9: Frequency of micronuclei in the Dalton's lymphoma ascites bearing mice treated with 10mg/kg body weight ethanol extract of *Schima wallichii* (SWE) or 0.5mg/kg body weight doxorubicin (DOX) at different post assay times

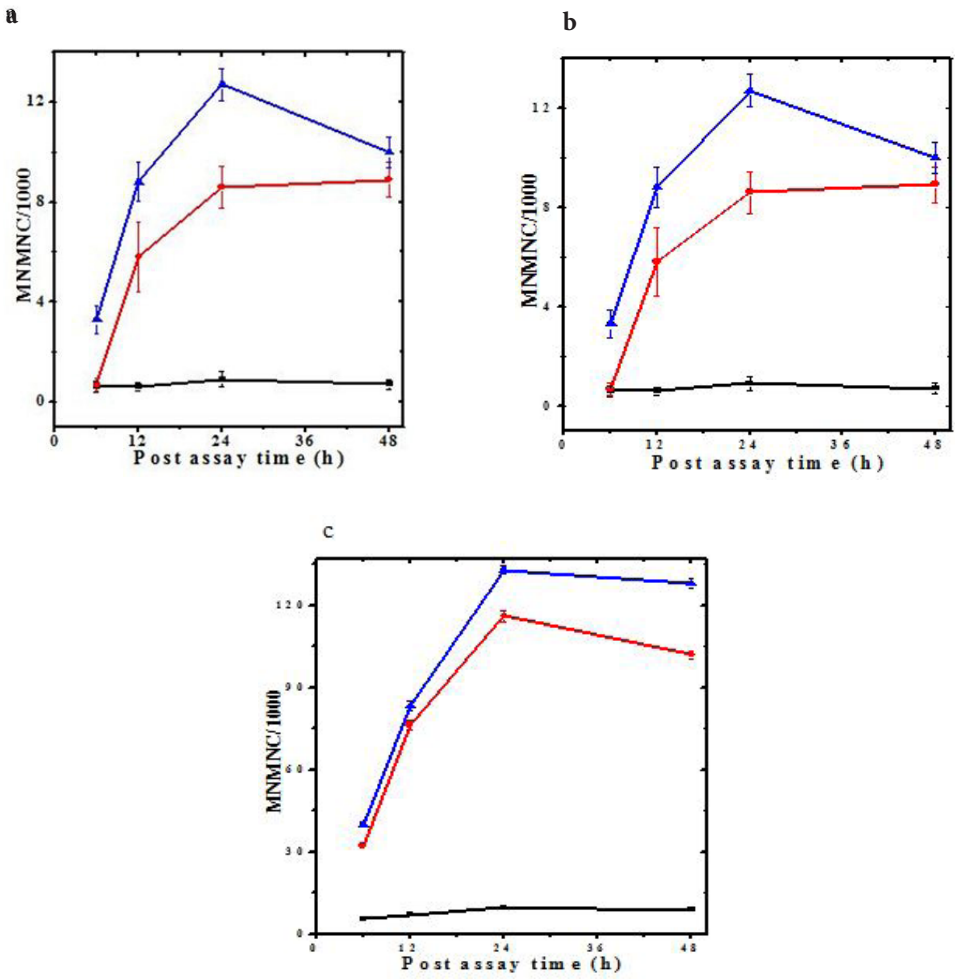


Figure 5: Alteration in the micronuclei formation with assay time in mononucleate Dalton's lymphoma cells treated with 10 mg/kg body weight of ethanol extract of *Schima wallichii* for 9 consecutive days. N = 5 for each assay time. Squares: Sterile physiological saline; Circles: Ethanol extract of *Schima wallichii* and Triangles: Doxorubicin. a: One micronuclei; b: Two micronuclei and c: Total micronuclei

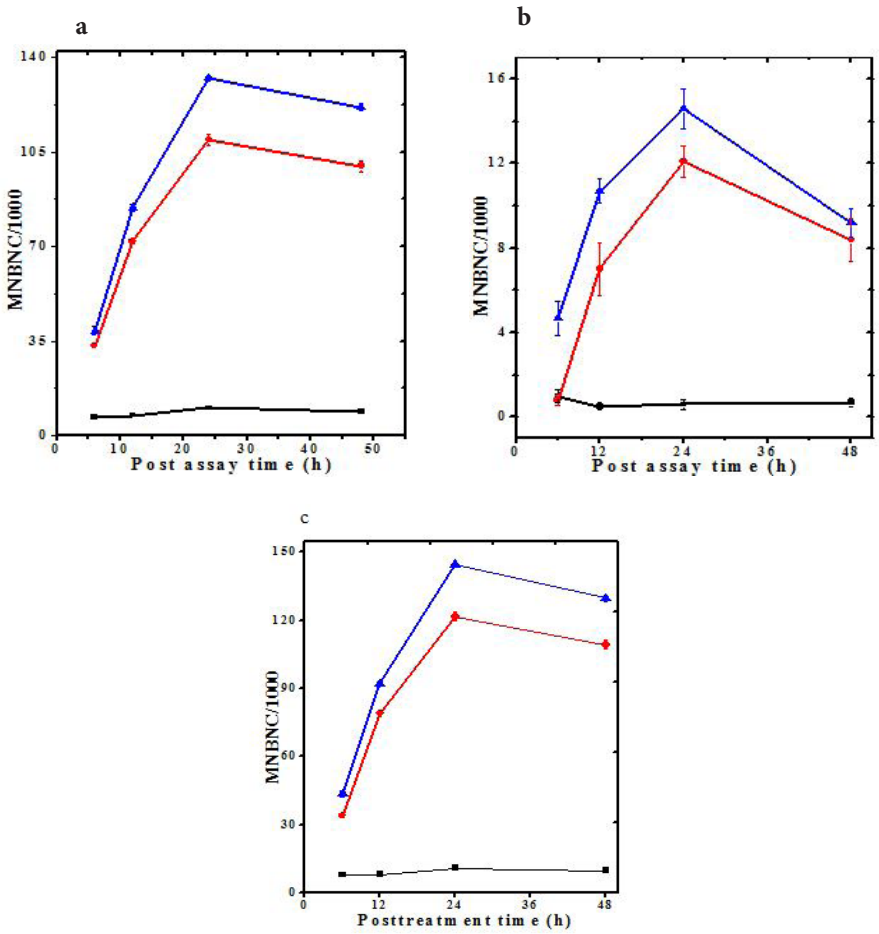


Figure 6: Alteration in the micronuclei formation with assay time in binucleate Dalton's lymphoma cells treated with 10 mg/kg body weight of ethanol extract o *Schima wallichii* for 9 consecutive days. Squares: Sterile physiological saline; Circles: Ethanol extract of *Schima wallichii* and Triangles: Doxorubicin. a: One micronuclei; b: Two micronuclei and c: Total micronuclei in binucleate cells. N=5 for each assay time for each group.

Apoptosis Assay

The administration of SWE induced apoptosis in Dalton's lymphoma cells as early as 2 h post drug treatment that continued to rise up to 48 h post assay time. A similar observation has been made in DOX treated group (Figure 7). The induction of apoptosis was significantly higher ($p<0.05$) in both the SWE and DOX treated groups at all the assay times, when compared to negative control (Table 10). The apoptotic cells increased by 6 to 10 folds in SWE treated group when compared with the negative control group (Table 10). The treatment of DLA mice with 10 mg/kg SWE or 0.5 mg/kg DOX resulted in a rise in the necrotic index in a time dependent manner and the maximum necrotic cells were scored at 48 h in both SWE and DOX groups (Table 10) (Figure 8). The rise in necrotic index in DLA cells was significantly ($p<0.05$) higher when compared to concurrent negative control group at all the post drug treatment times (Table 10). The SWE treatment increased the necrotic index by 6-8 folds depending on the assay time (Table 10).

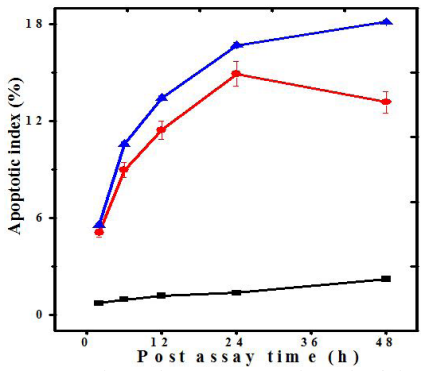


Figure 7: Alteration in the apoptotic index with assay time in the Dalton's lymphoma cells treated with 10 mg/kg body weight of ethanol extract o *Schima wallichii* for 9 consecutive days. Squares: Sterile physiological saline; Circles: Ethanol extract of *Schima wallichii* and Triangles: Doxorubicin. N=5 for each assay time for each group.

Post assay time(h)	Index (Mean ± Standard error of the mean)					
	SPS		SWE 10mg/kg body weight		DOX 0.5mg/kg body weight	
	Apoptotic	Necrotic	Apoptotic	Necrotic	Apoptotic	Necrotic
2	0.72±0.03	0.36±0.03	5.08±0.11*	2.21±0.07*	5.57±0.13*	3.53±0.15*
6	0.95±0.05	0.55±0.03	8.97±0.14*	4.06±0.12*	10.58±0.14*	5.43±0.11*
12	1.17±0.04	0.78±0.03	11.44±0.14*	5.5±0.12*	13.43±0.12*	6.49±0.13*
24	1.36±0.05	0.8±0.03	14.92±0.17*	6.59±0.12*	16.69±0.19*	8.55±0.14*
48	2.22±0.05	0.82±0.03	13.16±0.14*	6.41±0.09*	18.13±0.13*	8.99±0.1*1

*p < 0.05, when treatment groups are compared to spontaneous control group.
N = 5 for each assay time for each group.

Table 10: Induction of apoptosis and necrosis in Dalton's lymphoma ascites bearing mice treated with 10mg/kg body weight ethanol extract of *Schima wallichii* (SWE) or 0.5mg/kg body weight doxorubicin (DOX) at different post assays times

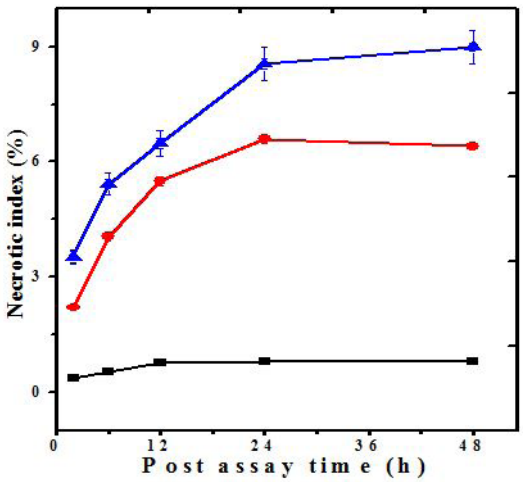


Figure 8: Alteration in the necrotic index with assay time in the Dalton's lymphoma cells treated with 10 mg/kg body weight of ethanol extract of *Schima wallichii* for 9 consecutive days. Squares: Sterile physiological saline; Circles: Ethanol extract of *Schima wallichii* and Triangles: Doxorubicin. N=5 for each assay time for each group.

Discussion

The realization of cancer as a disease in human stimulated several investigation and there has been a constant endeavor to fight against the disease by evolving various modalities. The chemotherapy has emerged as one of the most important and promising modalities of cancer treatment however, it also affects normal cells of different organs leading to many adverse side effects including second malignancies in the survivors [4,5]. The cancer mortality is approximately 63% globally despite availability of state of the art treatment strategies, which indicates the need of alternative strategies to contain or reduce the cancer related mortalities. The history of use of plants and natural products for healthcare is as old as the human civilization [39,40]. The plants contain several molecules and use of plants for cancer treatment may prove most useful as they may attack cancer cells through multiple mechanisms [41]. The plant-derived anticancer agents may be effective inhibitors of cancer cells with less toxic adverse side effects. Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products and the plant kingdom has been the most significant source of these drugs [9,10]. In addition, the emergence of resistance to cancer chemotherapy has stimulated researchers to turn to natural products of plant or marine origin. Many herbs have been evaluated in clinical studies and are currently being investigated to understand their tumoricidal properties against various cancers [42]. The advantage of natural products is that they are natural in origin and hence most biocompatible with minimum side effects in comparison to chemical synthetic products [43]. Therefore, the present study was undertaken to assess the ability of *Schima wallichii* to exterminate the Dalton's lymphoma cells transplanted in mice.

The oral acute toxicity studies have shown that 4 g/kg body weight of chloroform and aqueous extracts and 2g/kg body weight for ethanol extract of *S. wallichii* were non-toxic in the normal mice, whereas the intraperitoneal administration led to acute toxicity and the LD_{50} was 500 mg/kg body weight for chloroform and aqueous extracts, respectively and 100 mg/kg body weight for ethanol extract. The acute toxicity studies revealed that the ethanol extract had highest toxicity when administered intraperitoneally and the toxicity level of ethanol extract has been five times higher as compared to chloroform and aqueous extracts. There are no reports regarding the acute toxicity of *S. wallichi.* However other plants like *Alstonia scholaris* and *Nigella sativa* were found to exhibit toxic effect beyond 1000 mg/kg after intraperitoneal administration [44,45]. The *Colocasia gigantea* extracts were non-toxic

up to 2 g/kg body weight in mice however, the intraperitoneal administration has been found to be more toxic [46]. Similarly, administration of *Helicia nilagirica* intraperitoneally exerted toxic effects in acute toxicity studies in mice [47]. Oral administration of *Pericampylus glaucusor* and *S. alata* did not show any toxicity up to 4 and 3 g/kg body weight in mice [48,49].

Evaluation of antineoplastic activity of *S. wallichii* in Dalton's lymphoma transplanted in the peritoneum of Swiss albino mice showed that the mice without any treatment developed tumor speedily and all the untreated control mice died within 24 days after tumor inoculation with an AST and MST of 21.3 and 21 days, respectively. A similar effect has been observed in earlier studies [46,47]. The tumorized mice receiving different extract of *S. wallichii* significantly enhanced the life span of tumorized mice due to regression of tumors, which increased the life span up to 60, 90 and 120 days for chloroform, aqueous and ethanol extracts, respectively. The most potent extract proved to be the ethanol extracts where 40% of the tumor free survivors were observed beyond 120 days indicating its efficacy in killing the Dalton's lymphoma cells. The studies on the anticancer activity of *S. wallichii in vivo* are unavailable. However, it has been found to be cytotoxic to cultured HeLa cells [28]. The recent studies from this laboratory have shown that ethanol extract of *Colocasia gigantea* and aqueous extract of *Helicia nilagirica* were effective in killing the Dalton's lymphoma tumor cells in tumorized mice [46,47]. The extracts of *Alstonia scholaris, Aphnamixis polystachya, Ervatamia heyneana, Hygrophila spinosa, Podyphyllum hexandrum, Rubia cordifolia, Tinospora cordifolia and Tylophora indica* have been found to increase the tumor free survivors earlier [50-58].

The triggering of DNA damage is one of the important aspects to induce cytotoxicity in tumor cells. The analysis of micronuclei provides an indirect way to study the DNA damage. The micronuclei arise as a result of DNA double strand breaks, DNA exchanges, faulty or suppressed DNA repair, mis-segregation of chromosomes and spindle defects leading to cell death [59-64]. The formation of DNA DSBs and micronuclei is often the consequence of simultaneous excision repair of damages, wrong base incorporation and failure of the appropriate gap-filling event that leads to DSB, which are converted into micronuclei after a cell, undergoes division [65]. This may happen only if the level of DSBs exceeds the repair capacity of dividing cells, which is mainly due to either the mis repair of DSBs by the dysfunctional homologous recombination [66]. The ability of ethanol extract of *S. wallichii* to induce DNA damage was studied by micronucleus assay, where administration of 10 mg/kg body weight of *S. wallichii* in tumor bearing mice resulted in a significant increase in the micronuclei frequency in the mononucleate as well as binucleate DLA cells indicating that ethanol extract of *S. wallichii* efficiently induced DNA damage. Treatment of Dalton's lymphoma bearing mice with SWE showed a time dependent elevation in the frequency of micronuclei up to 24 h post treatment and a decline thereafter. A similar effect has been observed earlier [64,67]. Recently, treatment of DLA mice with the ethanol extract of *Colocasia gigantea* and aqueous extract of *Helicia nilagirica* increased the frequency of micronucleated cells and highest frequency of micronucleated cells was recorded at 24 h [46,47]. The other plant extract from *Tinospora cordifolia* and *Aphnamixis polystchya* have been reported to kill tumor cell by inducing DNA damage in the form of micronuclei [55,64]. The peak frequency of micronuclei at 24 h may be due to the fact that DLA cells take 24 h to undergo division after SWE treatment and cells bearing micronuclei are at this time are first division cells and thereafter the reduction in micronuclei may be due to the division of micronucleated and other cells that will reduce the micronuclei frequency due to dilution and increased cell population. The SWE induced not only one micronuclei but also cells with two micronuclei indicating that it induced complex multiply site of DNA damage that would have repressed the DNA damage repair leading to tumor cell death and increase in tumor free survivors. Likewise, ethanol extract of *Colocasia gigentea* and aqueous extract of *Helicia nilagirica* have been reported to induce cells bearing two micronuclei earlier [46,47]. A similar effect has been observed in 9L tumor tumorized rats after cisplatinum therapy and 20 Gy irradiation, which induced two and three micronuclei bearing cells in addition to cell bearing one micronuclei [68]. A number of studies have indicated that the cells expressing micronuclei are dying cells and correlation between cell killing and micronuclei has been established [60,63,64].

The remission of tumor and increase in tumor free survivors by SWE may be due to the induction of apoptosis, which will be able to remove the tumor cells efficiently, Therefore, we were interested in knowing whether the SWE induced DNA damage caused cell death by apoptosis? The treatment of tumor bearing mice with SWE induced apoptosis in a time dependent manner leading to increased tumor free survivors in the present study. A similar effect has been observed in the DLA cells *in vivo* treated with ethanol extract of *Colocasia gigantea* or aqueous extract of *Helicia nilagirica* [46,47]. The infliction of DNA damage in the tumor cells by SWE had triggered a cascade of biochemical and molecular events that triggered apoptosis, which was characterized by chromosome condensation, DNA fragmentation, membrane blebbing and formation of apoptotic bodies and cell death [69,70]. The SWE triggered DNA damage leading to the activation of p53, which would have initiated a cascade of events leading removal of the damaged cells by inducing apoptosis. This triggering of apoptosis may due to suppression of NF-κB that would have stimulated Bax, which in turn would have activated caspase 3 and 8 killing the tumor cells. The SWE has been reported to induce apoptosis by activation of caspase 3 and 8 activities in HeLa cells in our earlier study [28]. The various plant extracts have been reported to induce apoptotic mode of cell death in different cultured cell lines earlier [71-73].

The exact mechanism of action by which SWE triggered tumor cell kill in the present study is not clearly known. However, SWE may have used multiple putative mechanisms to induce cell death in the tumor bearing mice. First and foremost important action seems to be the induction of DNA damage in the tumor cells, which is corroborated by increased frequency of micronuclei and apoptosis. The reduction in GSH, GST, catalase and SOD seems to be another mechanism that may have initiated a cascade of events leading to cell kill in the present study. The SWE has been reported to decline GSH, GST, catalase and SOD in HeLa cells earlier [28]. The increased lipid peroxidation may have initiated non-apoptotic form of cell death thus increasing the tumor free

survivors. The SWE may have also utilized molecular pathways by suppressing the transcriptional activation of NF-κB, COX-II, and Nrf2 which are overexpressed in the tumor cells and give them survival advantage [74-76]. The suppression of these cytokines would have triggered apoptosis by activation of p53, cMyc and Bax followed by alleviated expression of Bcl_{xL}, survivin, IAP, c-FLIP and IKK that would have induced caspase 8 and 3 actvity [28,77,78]. The SWE has been known to activate caspase 8 and 3 in HeLa cells earlier [28].

Conclusions

The different extracts of *Schima wallichii* were found to be non-toxic up to 2 g when administered orally however, the intraperitoneal administration resulted in a LD_{50} of 500 mg/kg body weight for chloroform and aqueous extracts, whereas it was only 100 mg/kg body weight for ethanol extract. The intraperitoneal administration of chloroform, aqueous and ethanol extracts led to increase in the tumor free survivors for 60, 90 and beyond 120 days, respectively. The ethanol extract was most potent and its mechanism of action seems to be due to increased micronuclei frequency, apoptosis and lipid peroxidation. The attrition in the GSH concentration and activities of GST, catalase and SOD may have also contributed in their own way to kill tumor cells. The inhibition of NF-κB, COX-II, and Nrf2 may have triggered events that led to increased cell death by SWE. The activation of caspase 8 and 3 may be due to transcription activation of p53, cMyc and Bax and reduced expression of BclxL, survivin, IAP, c-FLIP, and IKK, which may have contributed to bring out cell death by apoptosis.

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