# STUDY ON THE ANTITUMOR EFFICACY OF SOME SELECT MEDICINAL PLANTS OF ASSAM AGAINST MURINE ASCITES DALTON'S LYMPHOMA

**Bijoy Brahma[1], Surya Bali Prasad*[2], Akalesh Kumar Verma[3], Gabriel Rosangkima[4]**

Cell and Tumor Biology Laboratory, Department of Zoology, North-Eastern Hill University, Shillong-793022, India.

[1,3,] Ph.D. Research Scholar, Cell & Tumor Biology Lab., Department of Zoology, North-Eastern Hill University, Shillong-793022, India.

[4] Post-doctoral Research Fellow, Cell & Tumor Biology Lab., Department of Zoology, North-Eastern Hill University, Shillong-793022, India.

[2]Professor, Cell & Tumor Biology Lab., Department of Zoology, North-Eastern Hill University, Shillong-793022, India.

[*]Corresponding author: Prof. S. B. Prasad, Department of Zoology, North-Eastern HillUniversity, Shillong-793022, India.
E-mail: sbpnehu@hotmail.com;
Fax: +91-364-2550076/2550108; Phone: +91-364-2722318.

**Summary**

In the present study, anticancer activity of some anticancer medicinal plants of Assam state, India, was evaluated. A survey was undertaken in the state of Assam and three plants used in the traditional folk medicine in Assam *viz Terminalia arjuna, Dillenia indica* and *Oroxylum indicum* are selected for screening of anticancer efficacy against Dalton's lymphoma. Crude extracts of the stem bark of the three plants were prepared in different solvents and the efficacies of the extracts were assessed in the Dalton's lymphoma bearing tumor mice. Methanol crude extract of *Oroxylum indicum* was selected for further study for its enhanced efficacy. The efficacy of the methanol crude extract was compared with the standard drug of cisplatin with regard to reduction of tumor volume and tumor body weight. Cell death *in vivo* and *in vitro* was assessed in comparison to the standard drug of cisplatin. The highest increase in the life span ~76.57% ILS was found to be at the dose of 20mg/kg body weight/day for five consecutive days. Increase in tumor volume and body weight due to proliferation of Dalton's lymphoma in the peritoneal cavity came down to near normal level in mice treated with the methanol crude extract of *Oroxylum indicum*. The present study demonstrates that the methanol crude extract of *Oroxylum indicum* is effective against Dalton's lymphoma both *in vitro* and *in vivo*. Further study will be carried out to find out the active components in the crude extract for its efficacy and the possible mechanism of its mode of action.

**Keywords:** Dalton's lymphoma; *Dillenia indica*; *Oroxylum indicum*; *Terminalia arjuna*

## Introduction

The cure for cancer is of paramount importance today. Investigations to understand the disease and research for suitable medications are making the best efforts to combat this disease, but a perfect cure yet remains elusive [1]. An alternative solution to modern system of medicine embodied with severe side effects is the use of medicinal plant preparations to arrest the insidious nature of the disease. Over 60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms [2]. Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their tumoricidal actions against various cancers. The rich and diverse plant source of the North Eastern states of Indiaof medicinal value needs to be well explored and evaluated [3-8]. One of the best approaches in the search for anticancer agents from plants is the selection of plants based on ethnomedical leads.

Based on the questionnaire and personal interview within digenous people of Assam, the three plants i.e. *Dillenia indica*, *Oroxylum indicum* and*Terminalia arjuna* emerged to be regularly used by the traditional healers of Assam and the North Eastern region of India for its medicinal value as a cure for some common ailments. *Terminalia arjuna* (Roxberg) belonging to the family of Combretaceae, also called as Arjun in Hindi is a medium sized tree reaching a height of about 60 to 80 feet. The bark of the tree in the form of decoction with milk and water is found to be a very good stimulant of heart [9]. Besides, it has been reported that the bark paste is locally applied on wounds, ulcer and specially used in promoting the union of fractures [10]. *Oroxylum indicum* (Vent) is a member of the family Bignoniaceae. It is found mainly in the villages and rice fields. The entire plant including root bark, stem bark, leaf, seed, fruit are used for their medicinal values. It is used to treat toothache, rheumatism, wound, splenomegaly, gastralgia, dysentery, cholera, loss of appetite and fever [11-13]. *Dillenia indica* (Linn) is a member of the family Dilleniaceae. It is a medium sized tree. Decoction of leaves and bark is given in diarrhoea, diabetes, stomatitis, dysentery etc. [14,15].

Besides the traditional knowledge on its uses, some scientific investigations have also been done on these three plants with regard to cancer. It has been reported that the extract of the bark of *Terminalia arjuna* inhibited the proliferation of HepG2 cells in a concentration-dependent manner [16]. Some gastroprotective flavonoid constituents like chrysin, baicalein and oroxylin have already been isolated from *Oroxylum indicum* [17]. Chrysin has been found to inhibit expression of hypoxia-inducible factor-1A through reducing hypoxia-inducible factor-1A stability and inhibiting its protein synthesis [18]. Baicalein has been found to induce cell death in human hepatocellur carcinoma cell line [19]. Baicalein induces cancer cell death and proliferation retardation by the inhibition of CDC2 kinase and survivin [20]. The methanolic extract of *Dillenia indica* has been found to have significant anti-leukemic activity in human leukemic celllines U937, HL60 and K562 [15.

However, the detail on evaluation of antitumor activity from these plants, particularly against Dalton's lymphoma has not been explored. Hence, in the present study, based on the ethnomedical claims and some scientific investigations already done, we looked into the antitumor activity of the three different plants against murine ascites Dalton's lymphoma.

## Materials and Methods

***Field survey and selection of plants*:**

During the field survey (September, 2008- December, 2009), the medicinal plants used in traditional healthcare system around Boko town of Kamrup district of Assam state, India was known through literature survey, personal interview and consultation of local herbal practitioners and elders. The traditional healers from different tribes and communities namely Rabha, Garo and Bodo were included during field survey. Herbarium of the plants, *Dillenia indica, Oroxylum indicum* and *Terminalia arjuna*were made and identification was done at the Department of Botany, North-Eastern Hill University, Shillong and accession numbers NEHU 11900, NEHU 11899 and NEHU 11901 respectively was deposited in the department of Botany, North Eastern Hill University, Shillong.Standard methodologies for gathering information on medicinal uses of the plants were followed [21]. The location of the spot where plant collection was done is shown through a geographical map Figure1.

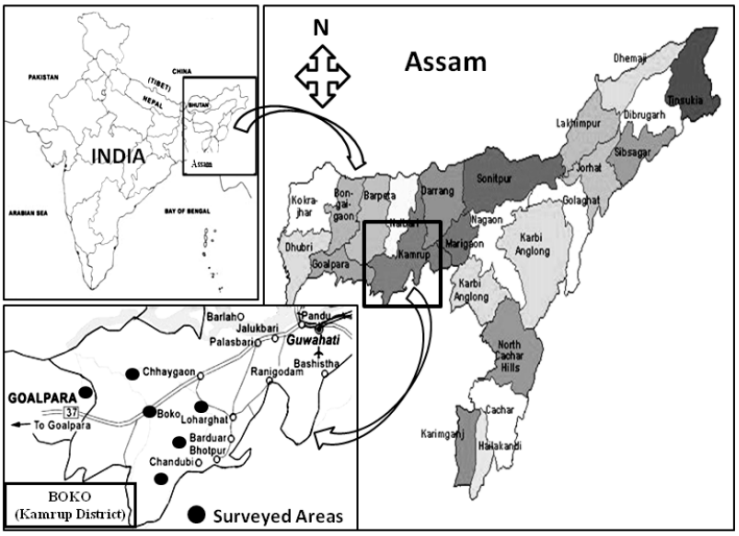


**Figure 1.** Maps showing the surveyed localities of the town of Boko within the state of Assam, which lies in the North-Eastern part of India. Star indicates the location in the map where plants collection was done in the surveyed area.

***Animals and tumor model:***

Inbred Swiss albino mice were maintained under conventional laboratory conditions with free access to commercially available food pellets and water. Mice of both sexes, 10 to12 weeks old and weighing about 25-30g were used for the experiments. Ascites Dalton's lymphoma tumor is maintained *in vivo* by intraperitoneal (i.p.) transplantation of $1\times10^7$ tumor cells per animal (0.25 ml vol., in phosphate buffered saline, pH 7.4 (PBS). Tumor-transplanted hosts usually survived for 18 to 20 days. Animals used in the present study are as per the ethical norms and has been cleared by University institutional ethical committee.

***Preparation of the crude extracts:***

The stem barks of the above selected plants were cleaned from the lichens and other unwanted things, cut into small pieces and shade-dried. The shade-dried stem barks of the plants were powdered and 100g of these powders were extracted with Soxhlet for 72 hours in

petroleum ether, diethyl ether, ethyl acetate, methanol and water. The extracts were dried in vacuum and the percentage yield of different extracts was determined using the following formula:

$$\% \text{ yield} = \frac{weight\ of\ the\ extract\ (\text{mg})}{weight\ of\ the\ whole\ tissue\ powder\ (\text{mg})} \times 100$$

***Antitumor activity study:***

Extracts derived from petroleum ether, diethyl ether and ethyl acetate were dissolved in Tween 20:PBS (1:9), Tween 20:PBS (0.5:9.5) and Tween 20:PBS (0.2:9.8) respectively. Whereas, aqueous and methanol extracts were dissolved in PBS only. The antitumor activity was determined with some slight modification of the method followed by Rosangkima et al., 2008 [22]. The day of tumor transplantation was taken as day zero. The tumor-transplanted animals were divided into six groups with 10 mice in each group. Treatments with different doses (10, 25, 50, 100 and 200mg/kg body weight/day) of the different extracts were given for five consecutive days starting from day 6 of tumor transplantation, and the host survival patterns were recorded. One group served as control which was given only the extract vehicle. The death of animals, if any and change in body weight and tumor volume of animals in different treatment groups were recorded daily. The antitumor efficacy was reported in percentage of average increase in life span (%ILS) calculated using the formula:

$$\%\text{ILS} = \left(\frac{T}{C} \times 100\right) - 100$$

where, T and C are the mean survival days of treated and control groups of mice respectively.

The antitumor activity of the most potent dose against Dalton's ascites lymphoma was compared with the standard reference drug, cisplatin at 4mg/kg body weight [23]. The mice from other set of experiment involving similar treatment schedules were monitored for any change in body weight and then they were dissected and the ascites fluid was collected from the peritoneal cavity. The tumor volume was determined by taking the ascites fluid in a graduated centrifuge tube and expressed in milliliters (ml).

***In vitro MTT Cell Proliferation assay:***

Cell growth inhibition was determined by MTT assay. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is a nonradioactive colorimetric assay [24] to measure cell cytotoxicity, proliferation or viability. Briefly $1\times10^6$ cells in 1ml were seeded on 24 well plates and the cells were treated with different concentrations of methanol extract of *Oroxylum indicum* (20, 40, 60, 80 and 100µg/ml) for 12 hours. At the end of the incubation, medium was removed and MTT (5mg/ml) was added and the cells were further incubated for 4 hours after which the media was removed. Dimethyl sulphoxide (100µl) was added in each well to solubilize the formazan crystals. The absorbance was read at a wavelength of 595nm and the cell viability was calculated as follows:

$$\%\text{ cell viability} = \frac{O.D.\ of\ treated\ cells\ \times 100}{O.D.\ of\ Control\ cells}$$

For graphical presentation of the results, mortality in each treatment was corrected from control mortality using the formula of Schneider-Orelli [25] as follows:

$$\text{Corrected} = \frac{\%\ responded\ in\ treated - \%\ responded\ in\ Control}{100\ - \%\ responded\ \ in\ Control} \text{ X100}$$

***Fluorescence based apoptosis study:***

Fluorescence based *in vivo* apoptosis was determined by using Acridine orange and Ethidium bromide (AO/EtBr) staining method. The viable cell's nucleus stain green due to permeability of only Acridine orange whereas, apoptotic cells appear red due to co-staining of both stains [26]. Dalton's lymphoma (DL) cells were taken out from the peritoneal cavity of the mice previously treated with a single dose of MEOI (20mg/kg body weight) for 24, 48, 72 and 96 hours. The DL cells taken out from the peritoneal cavity were then washed in PBS and made into a suspension of $1\times10^6$ cells per ml. The cells (25µl) were incubated with 1µl of the AO/EtBr staining solution (1 part of 100 µg/ml AO in PBS; 1 part of 100µg/ml EtBr in PBS) and mixed gently. 10µl of the cell suspension was then put on the microscopic slide and covered with a glass coverslip for observation. At least 500 cells were examined in under the fluorescence microscope and photographed.

***Statistical analysis:***

All the experimental data are expressed as the mean ± S.D. Significant differences between control and treated groups were calculated using Student's *t*-test as well as one-way analysis of variance (ANOVA). $P\leq0.05$ was considered as statistically significant.

## Results

***Percentage yield of the extracts:***

The percentage yield of the crude extract ranged from 0.22% to 22.14% (Table 1). The lowest percentage yield (0.22%) was found with the petroleum ether extract of *Terminalia arjuna* while the highest percentage yield (22.14%) was found with the aqueous extract of *Terminalia arjuna*.

**Table 1.** Percentage yield of different solvent extract of the plants

| Extraction solvent | *Oroxylum indicum* (% yield) | *Terminalia arjuna* (% yield) | *Dillenia indica* (% yield) |
|---|---|---|---|
| Aqueous extract | 14.11 | 22.14 | 12.17 |
| Petroleum ether | 0.26 | 0.22 | 0.38 |
| Diethyl ether | 1.62 | 1.71 | 2.23 |
| Ethyl acetate | 1.28 | 2.17 | 2.46 |
| Methanol | 9.72 | 17.06 | 6.12 |

***Antitumor activity of the extracts*:**

The different doses of the extract of three plants prepared using various solvent systems and their effect on the survivability of the hosts in different experimental groups have been shown in Table 2. Comparative survival patterns of tumor-bearing mice treated with the different doses of methanol extract of *Oroxylum indicum* (5 - 50mg/kg body weight) and cisplatin (4mg/kg body weight) is shown in Figure 2. In the antitumor activity study, methanol extract from the stem bark of *Oroxylum indicum* (MEOI) showed the most potent antitumor activity as compared to the other plant extracts against DLA tumor bearing mice with %ILS ~57.18% and ~72.93% at 10 and 25mg/kg body weight/day respectively. Extracts showing %ILS less than 20% were not considered to have antitumor activity. The most effective dose of MEOI was found to be 20mg/kg body weight (%ILS ~76.34%) whereas in case of reference drug cisplatin,

the % ILS value was found to be 82.79% at 4mg/kg body weight which is close to the %ILS of MEOI at 20mg/kg body weight dose (Table 3).

**Table 2.** Antitumor activity of different solvent extract of *Oroxylum indicum, Terminalia arjuna* and *Dillenia indica* stem bark against murine ascites Dalton's lymphoma

| | | *Oroxylum indicum* | | *Terminalia arjuna* | | *Dillenia indica* | |
|---|---|---|---|---|---|---|---|
| Extract solvent | Dose (mg/kg/day) | Life span (days) (mean ± SD) | ILS (%) | Life span (days) (mean ± SD) | ILS (%) | Life span (days) (mean ± SD) | ILS (%) |
| Water | Control | 19.16±0.71 | - | 19.50±0.63 | - | 18.16±0.25 | - |
| | 10 | 20.33±0.86 | 6.10 | 19.33±0.75 | –0.87 | 18.16±0.38 | 0 |
| | 25 | 18.50±0.43 | –3.44 | 18.33±0.48 | –6.00 | 16.16±0.46 | –11.01 |
| | 50 | 15.66±0.36 | –18.26 | 15.50±0.66 | –20.51 | 16.16±0.64 | –11.01 |
| | 100 | 14.66±0.48 | –23.48 | 14.33±0.81 | –26.51 | 14.66±0.77 | –19.27 |
| | 200 | 13.16±0.99 | –31.62 | 13.33±1.16 | –31.64 | 14.16±0.94 | –22.02 |
| Methanol | Control | 18.66±0.39 | - | 17.66±0.38 | - | 18.33±0.24 | - |
| | 10 | 29.33±0.71 | 57.18* | 21.50±0.62 | 21.74* | 27.33±0.61 | 49.09* |
| | 25 | 32.16±0.25 | 72.93* | 18.83±0.47 | 6.625 | 25.50±0.41 | 39.11* |
| | 50 | 27.00±0.49 | 44.69* | 17.33±0.39 | -1.86 | 20.83±0.47 | 13.63 |
| | 100 | 23.80±0.22 | 27.72* | 12.50±0.11 | -29.21 | 18.00±0.83 | -1.80 |
| | 200 | 20.66±0.91 | 10.75 | 11.16±0.78 | -36.80 | 14.66±0.66 | -20.02 |
| Ethyl acetate | Control | 19.25±0.45 | - | 17.96±0.77 | - | 19.21±0.27 | - |
| | 10 | 24.26±0.41 | 26.02* | 21.90±0.28 | 21.93* | 23.24±0.91 | 20.97* |
| | 25 | 28.16±0.32 | 46.28* | 22.54±0.65 | 25.50* | 25.61±0.36 | 33.31* |
| | 50 | 23.40±0.51 | 21.55* | 18.43±0.27 | 2.61 | 22.31±0.57 | 16.13 |
| | 100 | 20.97±0.27 | 8.93 | 16.20±0.61 | –9.79 | 18.99±0.96 | –1.14 |
| | 200 | 18.23±0.78 | –5.29 | 14.61±0.55 | –18.65 | 16.27±0.44 | –15.30 |
| Diethyl ether | Control | 19.51±0.97 | - | 18.71±0.20 | - | 18.27±0.31 | - |
| | 10 | 21.01±0.62 | 7.68 | 21.61±0.61 | 15.49 | 20.61±0.42 | 12.80 |
| | 25 | 24.36±0.41 | 24.85* | 22.52±0.53 | 20.36* | 21.94±0.56 | 17.26 |
| | 50 | 20.11±0.53 | 3.07 | 20.34±0.84 | 8.71 | 20.05±0.71 | 9.74 |
| | 100 | 17.26±0.68 | –11.53 | 18.21±0.81 | –2.67 | 16.26±0.57 | –11.00 |
| | 200 | 15.38±0.75 | –21.16 | 16.03±0.58 | –14.32 | 15.25±0.54 | –16.52 |
| Petroleum ether | Control | 18.23±0.81 | - | 18.69±0.34 | - | 19.11±0.28 | - |
| | 10 | 18.33±0.76 | 0.54 | 18.58±0.61 | –0.58 | 19.28±0.48 | –0.88 |
| | 25 | 18.90±0.75 | 3.67 | 18.23±0.49 | –2.46 | 17.67±0.57 | –7.53 |
| | 50 | 16.66±0.43 | –8.61 | 17.80±0.58 | –4.76 | 15.27±0.81 | –20.09 |
| | 100 | 15.22±0.52 | –16.51 | 15.23±0.56 | –18.51 | 14.26±0.58 | –25.37 |
| | 200 | 13.65±0.67 | –25.12 | 14.65±0.67 | –21.61 | 14.18±0.63 | –25.79 |

Plant extract treatment was given intraperitoneally (i.p.) for 5 consecutive days starting from day 5 of tumor growth. ILS = Increase in life span. Results are mean±S.D, n = 6. *ILS (%) ≥ 20% is generally regarded as effective against cancer.

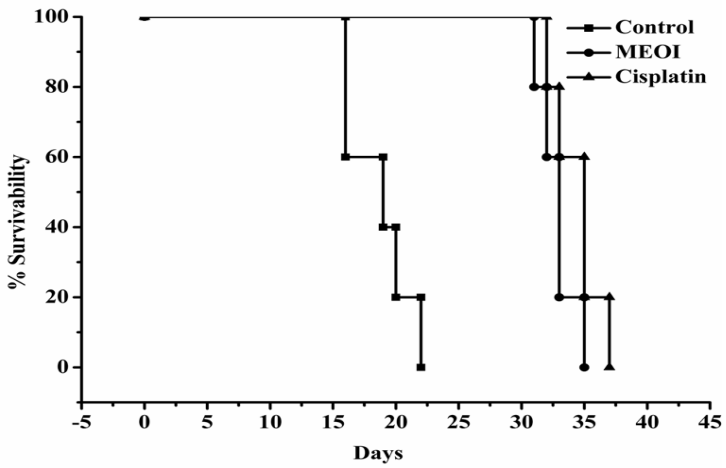


**Figure 2.** Graph showing the survival pattern of tumor-bearing mice after treatment with the most potent dose of MEOI (20mg/kg body weight) and standard reference drug, cisplatin (4mg/kg body weight). Results are mean of 5 independent experimental sets.

**Table 3.** Antitumor activity of different doses of MEOI and cisplatin (4mg/kg body weight)

| Treatment | Dose (mg/kg/day) | Survival days (Mean ± S.D) | ILS (%) |
|---|---|---|---|
| *Oroxylum indicum* (MEOI) | Control | 18.60±0.81 | - |
| | 5 | 23.33±0.65 | 26.10 * |
| | 10 | 28.16±0.55 | 52.25 * |
| | 15 | 30.67±0.71 | 65.76 * |
| | 20 | 32.80±0.43 | 76.34 * |
| | 25 | 30.50±0.56 | 64.86 * |
| | 30 | 28.50±0.77 | 54.05 * |
| | 50 | 24.16±0.91 | 30.63 * |
| Cisplatin (CDDP) | 4 | 34.00±0.59 | 82.79 * |

Results are mean±S.D. 0.25 ml volume of the extract solution was administered daily for 5 consecutive days starting from the 5$^{th}$ day of tumor transplantation. Control animals received the same volume of the extract vehicle. *ILS (%) ≥ 20%.

For further study, 20mg/kg body weight dose of MEOI was used for the treatment. Decrease tumor volume was observed in the mice treated with MEOI and cisplatin by ~72.27% and ~99.91% respectively on the 32$^{nd}$ day as compared to 18$^{th}$ day of tumor-bearing control group (Figure 3). Tumor growth reflects the change in body weight and there was a constant increase in the body weight of tumor-bearing control mice reaching 42.26g on the 18$^{th}$ day of tumor growth. In MEOI and cisplatin-treated groups of mice, the average body weight of the mice was reduced to ~22.72% and ~26.77% respectively on the 32$^{nd}$ day as compared to 18$^{th}$ day of control group (Figure 4).

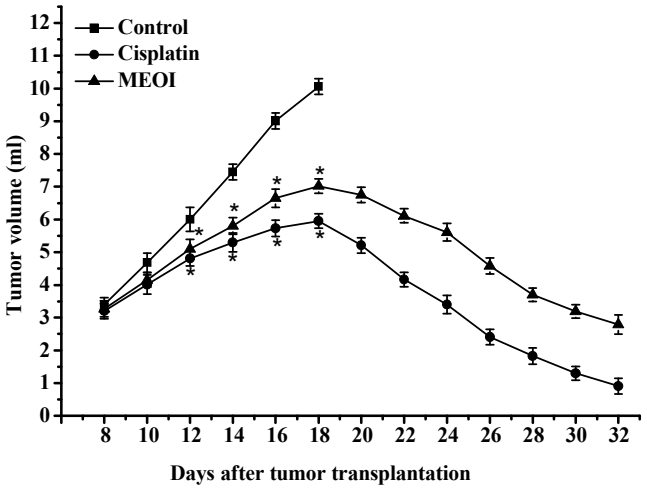


**Figure 3.** Graph showing changes in the tumor volume in the mice in cisplatin (4mg/kg body weight) and MEOI (20mg/kg body weight) treated groups. Results are mean ± S.D., Student's *t*-test, n=6, as compared to the corresponding control values. *P ≤ 0.05.

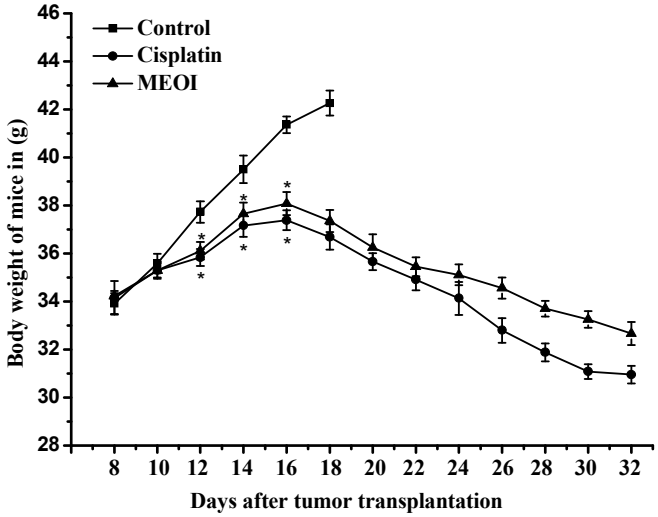


**Figure 4.** Graph showing changes in the body weight of the mice in cisplatin (4mg/kg body weight) and MEOI (20mg/kg body weight) treated groups. Results are mean ± S.D., Student's *t*-test, n=6, as compared to the corresponding control values. *P ≤ 0.05.

***MTT Cell Proliferation assay:***

MTT assay was performed to assess the viability of the cells when incubated with the MEOI (Figure 5). It was observed that the viability of the Dalton's lymphoma cells decreased in a dose-dependent manner. The values of $IC_{50}$ of cisplatin and MEOI were 44.4µg/ml and 63.2µg/ml respectively. The regression values and correlation of regression of the cisplatin were $y = 12.34x + 23.32$ and $R^2 = 0.95967$ respectively while that of MEOI were $y = 13.3x + 8.5$ and $R^2 = 0.96121$ respectively.

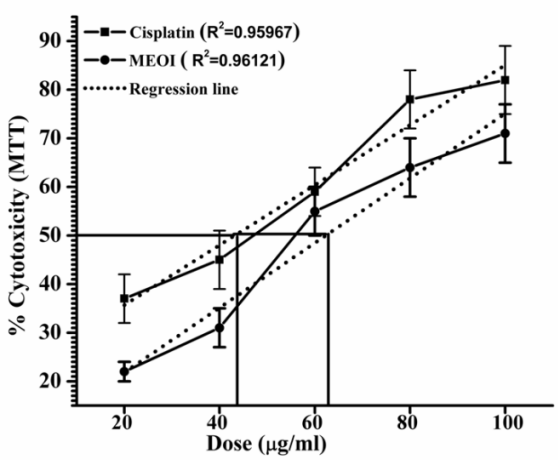


**Figure 5.** Graph showing cytotoxicity of MEOI and cisplatin against Dalton's lymphoma cells *in vitro*. $IC_{50}$ values were calculated using regression line. Results are mean ± S.D., n=5. *P ≤ 0.05..

***Fluorescence based viability and apoptosis:***

Control DL cells appeared rounded in shape with green fluorescence suggesting no sign of apoptosis (Figure 6a). After 24 hours of MEOI treatment (20mg/Kg body weight) nuclear constriction and early apoptotic features were very much prominent (Figure 6b) while during 48 hours of treatment, reduction in cell volume and loss of cell membrane integrity and appearance of membrane blebbing were observed (Figure 6c). During 72 hours of incubation, nuclear fragmentation was observed with many late apoptotic cells and few early apoptotic cells (Figure 6d). During 96 hours of treatment, changes in cellular morphology, including chromatin condensation, membrane blebbing, fragmented nuclei, large size cytoplasmic and membrane vacuoles were seen with complete loss of membrane integrity (Figure 6e). The vital dye acridine orange stained both live and dead cells, whereas ethidium bromide stained only those cells that have lost their membrane integrity. The percentage of cells undergoing apoptosis was 17.2% during 24 hours of treatment and increased to 62.4% after 96 hours of treatment. However, with cisplatin treatment the percentage of cells undergoing apoptosis was 33.4% and 64.2% during 24 hours and 96 hours of treatment respectively (Figure 7).

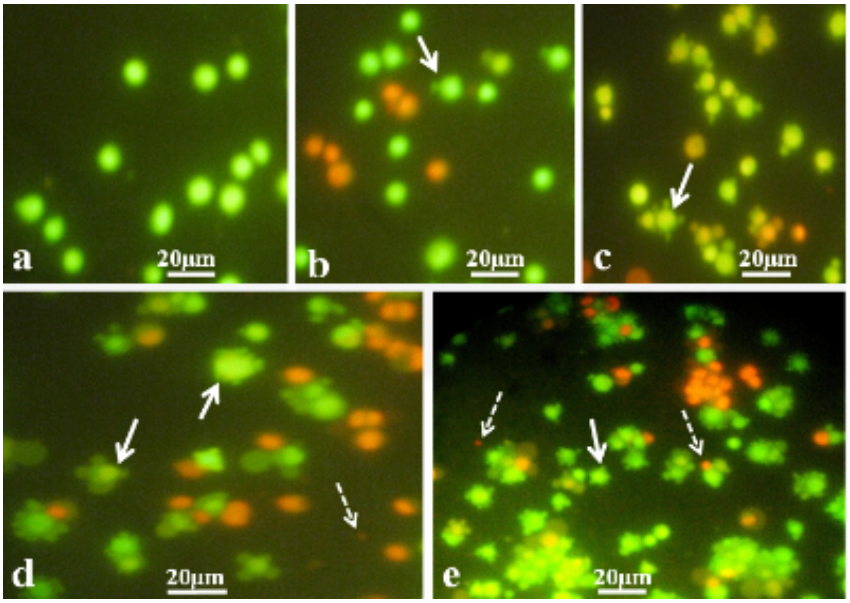


**Figure 6.** Acridine orange and ethidium bromide (AO/EtBr) double staining of DL cells collected from control group of mice (a) and MEOI-treated groups during 24 (b), 48 (c), 72 (d) and 96hr (e) of treatment.

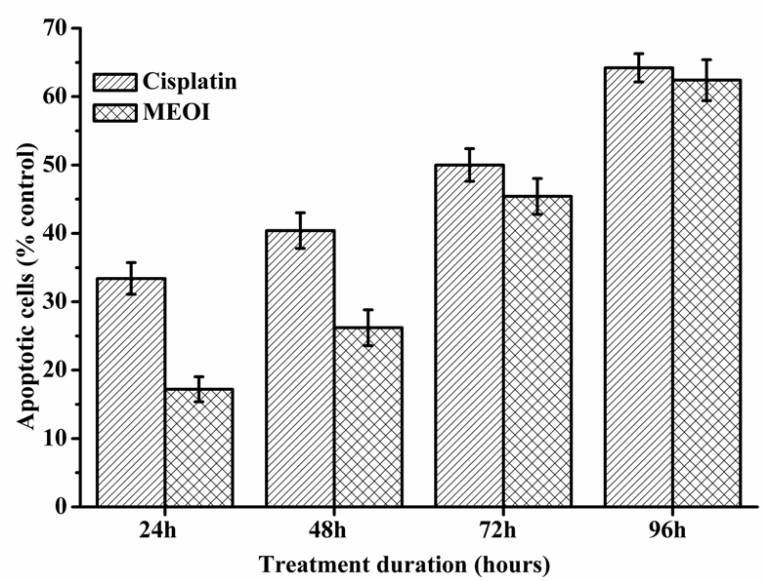


**Figure 7.** Histogram showing the percent apoptotic cells in MEOI (20mg/kg body weight) and cisplatin (4mg/Kg body weight) treated groups. Results are mean ± S.D., n = 5.

## Discussion

Several studies have been conducted on plants and herbs under a multitude of ethnobotanical grounds. Plants have also served as a good source of anticancer agents [2, 27]. Like all other indigenous tribal communities, tribes of Assam namely, Bodo, Garo, and Rabha, have a very close association with nature and have an indigenous knowledge for treatment of different ailments. A large number of plants possessing anticancer properties have been

documented from this region [28, 5, 29]. Plant-derived anticancer agents are now becoming good sources of anti-cancer lead compounds and have been found to possess less side-effects and are generally found to bespecifically cytotoxic to proliferating cancer cells only [30]. Stem bark of *Dillenia indica, Oroxylum indicum* and *Terminalia arjuna* are regularly used in the traditional system of medicine by the tribal people [6-7]. The present investigation was carried out to evaluate the antitumor therapeutic efficacy of these plants against murine ascites Dalton's lymphoma.

Ascites Dalton's lymphoma has been regularly used in the study of antitumor/anti-cancer activity of anti-cancer drugs, plant and animal products [31, 32, 22]. The peritoneal cavity of the Dalton's lymphoma-bearing mice accumulate ascites fluid which serve as the nutritional source of the DL cells therefore a rise in the ascites fluid is observed with the increase in the proliferation of the DL tumor cells. The selected three plants were therefore tested for their antitumor capability measured in terms ofincrease in the life span of the DL tumor-bearing mice. Among the extracts of the stem bark of these plants prepared in different solvents, it was observed that the methanol extract of *Oroxylum indicum* (MEOI) prolonged the life span of the tumor-bearing mice more than the other two plants(Table 2-6). Therefore, the MEOI was undertaken for further study to examine its effect during antitumor efficacy.

In MEOI, 20mg/kg body weight/day dose was found to give a good %ILS value (76.34%). Cisplatin, one of the most effective cancer chemotherapeutic agents was used here as a reference drug for comparison at the dose of 4mg/kg body weight/day showed a% ILS value of about ~82.79%.In the DL tumor-bearing mice, secretion of the ascites fluid increases as a nutritional requirement for the DL cells; however, the MEOI decreased the volume of ascites fluid which would help decrease in the rate of the proliferation of the DL cells. The increase in the secretion of the ascites fluid and the increased rate of proliferation of the DL cells leads to the increase in the body weight of the mice [33]. Therefore, in the regular monitoring for any possible variation in the body weight it was found that the group of mice treated with the MEOI there was a significant decrease in the body weight as compared to control mice. It may be concluded that MEOI increases the life span of DL tumor-bearing mice by decreasing the tumor volume and arresting the tumor growth. The decrease in the tumor volume and body weight in the treated group of mice was near to the values observed with the standard drug of cisplatin. *In vitro* cytotoxicity assays can be used to predict human toxicity and for the general screening of chemicals [34]. *In vitro* cytotoxicity assay with the MTT test revealed an $IC_{50}$ value at 63.2µg/ml for MEOI while for cisplatin under the same conditions it was 44.4µg/ml. This may suggest that as compared to cisplatin, MEOI is required in bigger dose to cause cell death. This may be because the crude extract is a mixture of compounds and hence some compounds might be acting antagonistically to each other.

It was previously reported that plant extracts containing antioxidant principles showed cytotoxicity towards tumor cells and inhibit tumor cell proliferation both *in vivo* and *in vitro* conditions [35]. Antitumor activity of different plant extract is either through induction of apoptosis, necrosis, autophagy or by inhibition of neovascularisation [36]. Therefore, in our recent study MEOI extract was tested for apoptosis study to understand the nature of cell death. Apoptosis is a distinct mode of cell death that is responsible for deletion of cells in normal tissues; it also occurs in specific pathologic contexts. Morphologically, it involves rapid condensation and budding of the cell with the formation of membrane-enclosed apoptotic bodies containing well-preserved organelles, which are phagocytosed and digested by nearby resident cells [37-38]. However, in the development of cancer, the mechanism of controlled proliferation

and apoptosis in the tumor cells is disturbed [39]. Some anti-cancer drugs and especially some anti-cancer agents from plants have been found to cause apoptosis in the tumor cells [40]. The assay based on AO/EtBr fluorescence staining is a good reliable analysis for the confirmation of apoptotic features compared to other methods [41]. Treatment with the MEOI caused in morphological and ultrastructural features of DL cells which included chromatin condensation, membrane blebbing, large size cytoplasmic and membrane vacuoles, loss of membrane integrity as well as nuclear fragmentation.

From the results of the present study, it may be concluded that methanol extract of *Oroxylum indicum* have the potential to prolong the life span of DL tumor-bearing mouse. It may also be suggested that methanol extract of *Oroxylum indicum* possess good cytotoxicity against DL cells. However, to understand the mechanism of its action, further detailed study is required.

**Acknowledgements:**

We acknowledge the University Grants Commission, New Delhi (India) for providing Research fellowship in science for CSIR-UGC NET JRF to Bijoy Brahma and Post-doctoral Research Fellowship to G. Rosangkima. The author is thankful to Mr. Lukumoni Rabha who helped as an interpreter and guide during the field survey for medicinal plants. The author is also thankful to all traditional healers from Kamrup and Goalpara districts of Assam (India) who helped and shared the required information during field survey and collection with regard to medicinal plants.

## References

1. Grizzi F, Chiriva-Internati M. Cancer:looking for simplicity and finding complexity. Cancer Cell International 2006;6:1-7.
2. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. Journal of Ethnopharmacology 2005;100:72-79.
3. Das NJ, Saikia SP, Sarkar S, et al. Medicinal Plants of North Kamrup district of Assam used in primary healthcare system. Indian Journal of Traditional Knowledge 2006;5:489-493.
4. Das S, Khan ML, Rabha A, et al. Ethnomedicinal plants of Manas National Park, Assam, Northeast India. Indian Journal of Traditional Knowledge 2009;8:514-517.
5. Mao AA, Hynniewta TM, Sanjappa M. Plant wealth of Northeast India with reference to ethnobotany. Indian Journal of Traditional Knowledge 2009;8:96-103.
6. Purkayastha J, Nath SC. Biological activities of ethnomedicinal claims of some plant species of Assam. Indian Journal of Traditional Knowledge 2006;5:229-236.
7. Saikia B. Ethnomedicinal plants from Gohpur of Sonitpur district, Assam. Indian Journal of Traditional Knowledge 2006;5:529-530.
8. Sajem AL, Gosai K. Ethnobotanical investigations among the Lusai tribes of North Cachar Hills district of Assam, Northeast India. Indian Journal of Traditional Knowledge 2010;9:108-113.
9. Halder S, Bharal N, Mediratta PK, et al. Anti-inflammatory, immunomodulatory, and antinociceptive activity of *Terminalia arjuna* Roxb bark powder in mice and rats. Indian Journal of Experimental Biology 2009;47:577-583.

10. Jain S, Yadav PP, Gill V, et al. *Terminalia arjuna* a sacred medicinal plant: phytochemical and pharmacological profile. Phytochemical Review 2009;8:491-502.
11. Gokhale M, Bansal YK. An avowal of importance of endangered tree *Oroxylum indicum* (Linn.) Vent. Natural Product Radiance 2005;5:112-114.
12. Gupta RC, Sharma V, Sharma N, et al. *In vitro* Antioxidant Activity from Leaves of *Oroxylum indicum* (L.) Vent-A North Indian Highly Threatened and Vulnerable Medicinal Plant. Journal of Pharmacy Research 2008;1:65-72.
13. Sastry AVS, Sastry VG, Mallikarjun P, et al. Chemical and pharmacological evaluation of aqueous extract of root bark of "*Oroxylum indicum"* Vent. International Journal of Pharmacy and Technology 2011;3:1796-1806.
14. Alam MB, Hossain MS, Haque ME. Evaluation of antimicrobial and toxicity of different fractions of *Dillenia indica* (Linn) bark extract. J Global Pharma Technology 2010;2:37-42.
15. 15. Kumar D, Mallick S, Vedasiromoni JR, et al. Anti-leukemic activity of *Dillenia indica* L. fruit extract and quantification of betulinic acid by HPLC. Phytomedicine 2010;17:431–435.
16. Sivalokanathan S, Vijayababu MR, Balasubramanian MP. Effects of *Terminalia arjuna* bark extract on apoptosis of human hepatoma cell line HepG2. World Journal of Gastroenterology 2006;12:1018-1024.
17. Babu TH, Manjulatha K, Kumar GS, et al. Gastroprotective flavonoid constituents from *Oroxylum indicum* (Vent). Bioorganic & Medicinal Chemistry Letters 2010;20:117-120.
18. Fu B, Xue J, Li Z, et al. Chrysin inhibits expression of hypoxia-inducible factor-1A through reducing hypoxia-inducible factor-1A stability and inhibiting its protein synthesis. Molecular Cancer Therapeutics 2007;6:220-226.
19. Matsujaki Y, Kurokawa N, Terai S, et al. Cell death induced by baicalein in human hepatocellular carcinoma celllines. Japanese Journal of Cancer Research 1996;87:170-177.
20. Chao J, Su W, Liu H. Baicalein induces cancer cell death and proliferation retardation by the inhibition of CDC2 kinase and survivin associated with opposite role of p38 mitogen-activated protein kinase and AKT. Molecular Cancer Therapy 2007;6:3039-3048.
21. Jain SK, Rao RR. A Handbook of Field and Herbarium Techniques, Today and Tomorrow Publishers, New Delhi, India.
22. Rosangkima G, Rongpi T, Prasad SB. Role of glutathione and glutathione related enzymes in the antitumor activity of *Dillenia pentagyna* in Daltons Lymphoma bearing mice. International Journal of Cancer Research 2008;4:92-102.
23. Ajith TA, Janardhanan KK. Cytotoxic and antitumor activities of a polypore macrofungus, Phellinusrimosus (Berk) Pilat. J Ethnopharmacology 2003;84:157-62.
24. Campling BG, Pym J, Galbraith PR, et al. Use of the mtt assay for rapid determination of chemosensitivity of human leukemic blast cells. Leukemia Research 1988;12:823-831.
25. Oghiakhe S, Holliday NJ. Evaluation of insecticides for control of overwintering *Hylurgopinus rufipes* (Coleoptera: Curculionidae). Journal of Economic Entomology 2011;104:889-894.
26. Spector DL, Goldman RD, Leinwand LA. Cells: A Laboratory manual. Cold Spring Harbor Laboratory Press, USA.
*27.* Wang H, Lee K. Plant derived anticancer agents and their analogs currently in clinical use or in clinical trials. Botanical Bulletin of Academia Sinica 1997;38:225-235.

28. Jaiswal V. Culture and ethnobotany of Jaintia tribal community of Meghalaya, Northeast India-A mini review. Indian Journal of Traditional Knowledge 2010;9:38-44.
29. Shoeb M. Anticancer agents from medicinal plants. Bangladesh Journal of Pharmacology 2006;1:35-41.
30. Gordaliza M. Natural products as leads to anticancer drugs. Clinical Translational Oncology 2007;9:767-776.
31. Khynriam D, Prasad SB. Changes in endogenous tissue glutathione level in relation to murine ascites tumor growth and the anticancer activity of cisplatin. Brazilian Journal of Medical and Biological Research 2003;36:53-63.
32. Prasad SB, Verma AK, Rosangkima G, et al. Antitumor activity of *Mylabris cichorii* extracts against murine ascites Dalton's Lymphoma. Journal of Pharmacy Research 2010;3:3006-3009.
33. Prasad SB, Giri A. Antitumor effect of cisplatin against murine ascites Dalton's lymphoma. Indian Journal of Experimental Biology 1994;32:155-162.
*34.* Vlietinck AJ, Apers S. Biological Screening Methods in the Search for Pharmacologically Active Natural Products. In: Tringali C, eds. Bioactive Compounds from Natural Sources, Isolation, Characterization and Biological Properties, Taylor & Francis, London, 2001:1-29.
35. Jiau-Jian L, Larry WO. Over expression of manganese-containing superoxide dismutase confers resistance to the cytotoxicity of tumor necrosis factor and/or hyperthermia. Cancer Research 1977;57:1991-1998.
36. Putul M, Sunit C, Pritha B. Neovascularisation offers a new perspective to glutamine-related therapy. Indian Journal of Experimental Biology 2000;38:88-90.
37. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis-A Link between Cancer Genetics and Chemotherapy. Cell 2002;108:153-164.
38. Kerr JFR, Winterford CM, Harmon BV. Apoptosis-Its Significance in Cancer and Cancer Therapy. Cancer 1994;73:2013-2026.
39. Lowe SW, Lin AW. Apoptosis in Cancer. Carcinogenisis 2000;21:485-495.
40. Gerl R, Vaux DL. Apoptosis in the development and treatment of cancer. Carcinogenesis 2005;26:263-270.
41. Bonilla-Porras AR, Jimenez-Del-Rio M, Velez-Pardo C. Vitamin K3 and vitamin C alone or in combination induced apoptosis in leukemia cells by a similar oxidative stress signalling mechanism. Cancer Cell International 2011;11:19-30.