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Antitumor activity of different extracts of *Colocasia gigantea* in Dalton's lymphoma transplanted Swiss Albino mice

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Abstract

The acute toxicity was determined in normal non-tumour bearing mice administered with different doses of various extracts of *Colocasia gigantea* orally or and intraperitoneally. The oral administration of chloroform, ethanol and aqueous extracts of *Colocasia gigantea* were non-toxic up to 2 g/kg body weight. The intraperitoneal administration of different extracts of *Colocasia gigantea* exhibited toxic effect and the LD50 for ethanol extract was found to be 0.2 g/kg b. wt., whereas it was 0.15 g/kg b. wt. for chloroform and aqueous extracts, respectively. The determination of anticancer activity by intraperitoneal administration of 0, 50, 75, 100, 125, 150, 175, 200 or 225 mg/kg b. wt. of ethanol extract into Dalton's lymphoma transplanted mice resulted in a dose dependent rise in tumour regression and increase in the average survival as well as median survival time. The maximum tumour free survivors were observed at 200 mg/kg b. wt. of ethanol extract and further experiments were carried out using this dose. The evaluation of micronuclei showed that the ethanol extract *Colocasia gigantea* increased the frequency of micronucleated mononucleate cells as well as micronucleated binucleate cells in a time dependent manner and their frequencies were maximum at 36 h post-treatment. Similarly, *Colocasia gigantea* ethanol extract increased the apoptosis index in a time dependent manner and the highest apoptosis was observed at 36 h post treatment. The biochemical studies revealed a significant decline in the glutathione concentration, glutathione-s-transferase, superoxide dismutase and catalase activities accompanied by elevated lipid peroxidation. The safety of ethanol extract of 200 mg *Colocasia gigantea* was ascertained by evaluating aspartic acid transaminase, and alanine aminotransferase, creatinine and uric acid at different post treatment times in the liver and kidney of Dalton's lymphoma bearing mice. These parameters did not show any significant alteration and they were within the normal range. The ethanol extract of *Colocasia gigantea* did not show any toxicity orally however, intraperitoneal administration did exert toxic effects and it also induced anticancer activity in tumour cells by increasing tumour free survivors. The cytotoxic effect of ethanol extract may be due to induction of DNA damage in the form of micronuclei and apoptosis and reduction in glutathione, glutathione-s-transferase, superoxide dismutase and catalase accompanied by a rise in the lipid peroxidation.

Key words: *Colocasia gigantea*, Dalton's lymphoma, micronculei, glutathione and apoptosis

INTRODUCTION

The cardiac diseases are the leading cause of death and cancer is the second largest killer disease globally. Despite development of various modern treatment modalities, the mortality rates, especially for solid tumors remains a major cause of concern. It is also projected that mortality rates due to cancer may out number cardiovascular diseases not in too distant a future in the developed world [1]. The chemotherapy is one of the important modalities to treat cancer and the term "chemotherapy" was introduced by Paul Ehrlich in the early nineteen century who defined chemotherapy as the use of chemicals to treat diseases. However, it became associated with cancer treatment in 1943 since then treatment of cancer with chemicals is synonymous with chemotherapy [2]. Several synthetic chemicals find their use in the cancer treatment and role of natural products in cancer treatment was realized when alkaloids isolated from *Catharanthus roseus* were found to be useful in the treatment of hematologic malignancies [3]. Thereafter several other molecules have been isolated from different plants to treat different types of cancers [4]. Podophyllotoxins separated from the ethanol extract of *Podophyllum peltatum* also showed anticancer activity against a wide range of tumors. The etoposide and teniposide, which are derivatives of podophyllotoxins are in frequent clinical use [5]. Camptothecins are natural product derived cancer chemotherapeutic agents that have found their application in clinics to treat different cancers [6]. The taxols are another class of natural products isolated from plants and are in frequent clinical use to treat a wide range of neoplastic disorders [7].

The chemical synthesis of natural products further strengthened their use and may still continue to play a significant role in the treatment of cancer in the years to come [2,8]. The currently used chemotherapy for cancer treatment has several side effects and therefore there is a need for better therapy with lesser side effects [9]. Besides, the high cost as well as lack of effectiveness of the current conventional therapies (chemotherapy and radiation), especially for solid tumors, use of plants for cancer treatment may be alternative medical strategy to treat cancer [10]. The side effects due to most cancer drug/s induced toxicity also act as a driving force to the use of alternative medicine for better cure [11]. Plants are not only safe for long term therapy but also provide nutrition and reduce the side effects of conventional cancer therapy. The high cost and negative impact of conventional therapy, low-cost and safety of plants has been drawing increased attention towards plants and plant derived products for cancer cure [12]. Plants and natural products are still in great demand due to their safety, efficacy and lesser side effects [13] and about 80% individuals in the developing countries still depend on plants to treat different diseases. There are also reports that 25% of modern drugs are obtained from plants and 70% of the drugs introduced in the United States for the past 25 years have their origin in plants [4,14]. Plants contain many phytochemicals which work in a synergistic mode of action in such a way that their uses can complement or damage others or neutralize their possible negative effects. The use of multicompounds is preferred over the use of single drug for the treatment of several diseases including cancer, AIDS, diabetes, etc. due to their beneficial effects [15]. The popularity of use

of herbal medicines by general public is due to the belief that botanicals will provide some measure of benefit over and above modern allopathic medical approaches. They are also considered non-toxic or less toxic than the synthetic molecules.

*Colocasia gigantea (*family: Araceae) commonly called giant taro or elephant's ear, is a large, stemless, tuberous, frost-tender perennial herb, which typically grows up to 4-7' tall and has wide and heart-shaped to arrowhead-shaped, conspicuously-veined, downward-pointing, peltate, dull green to gray green leaves (2-4' long) attached to stout, succulent stems. As the common name suggests, each leaf purportedly resembles the ear of an elephant. It is native to valley forests in China and Southeast Asia. In Fiji, the locals make use of either boiled or baked breadfruit or tubers of taro as slices along with roasted pig [16]. Along with culinary items of taro it is used as medicine to treat constipation and tuberculosis in Hawaii [17]. Pressed juice of petiole of taro is highly cystic and is even said to arrest arterial hemorrhage [18]. Taro is used as medicine in China [19]. Nutritionally, taro is very similar to tannia. It contains starch 17.5% amylose and the rest as amylopectin. Starch grain is very small and the size ranges from 1-4 μm. It is rich in most of the essential amino acids and hence is considered to be a good leafy vegetable. It is reported that 100 g of taro tuber contains 73.1 g moisture, 3 g protein, 0.1 g fat, 1.7 g minerals, 22.1 g carbohydrates, 0.04 g calcium, 0.14 g phosphorus, 2.1 mg iron, 80 IU Vitamin B and trace of Vitamin C [20]. Since not much information is available on *Colocasia gigantea* despite the fact that it is commonly used as a vegetable in India and Southeast Asia as a part of human diet, the present study was undertaken to study the anticancer activity of *Colocasia gigantea* in mice transplanted with Dalton's lymphoma cells.

MATERIALS AND METHODS

Chemicals

Dimethylsulphoxide (DMSO), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), nitrobluetetrazolium (NBT), phenazinemethosulphate (PMS), reduced glutathione (GSH), triton X-100, ethylenediaminetetra-acetic acid (EDTA), sodium pyruvate, thiobarbituric acid (TBA), ethidium bromide, acridine orange, crystal violet, and cytochalasin B were obtained from Sigma Aldrich Chemical Co. (Kolkata, India). Sodium carbonate (Na_2CO_3), trichloroacetic acid (TCA), potassium chloride (KCl), potassium sodium-tartrate, and hydrogen peroxide (H_2O_2), were procured from SD Fine-Chemicals Ltd., Mumbai, India, whereas sodium hydroxide (NaOH), sodium chloride (NaCl), disodium biphosphate (Na_2HPO_4), sulphuric acid (H_2SO_4), hydrochloric acid (HCl), n-butanol, Tris (hydroxymethyl) aminomethane (Tris buffer) and ammonium oxalate were supplied by Merck India Limited, Mumbai. Phenol-chloroform-isoamyl alcohol (PCI), and reduced nicotinamide adenine dinucleotide (NADH) were purchased from HiMedia, Mumbai, India. Doxorubicin, Getwell Pharmaceuticals, Gurgaon, India.

Collection and Preparation of the Extract

The non-infected and matured rhizomes of *Colocasia gigantea* (family- Araceae) were collected from Manipur. The plant was identified by PG Department of Botany, DM college,

Imphal-West, Manipur, India and further authenticated by Botanical Survey of India, Shillong, Meghalaya, India. The non-infected and matured rhizomes were cleaned chopped into thin slices to facilitate drying in shade at room temperature. The dried rhizomes were powdered in an electrical grinder at room temperature. A sample of 100 g of powder was extracted sequentially with chloroform, ethanol and water in a Soxhlet apparatus [21]. The extract was then concentrated to dryness under reduced pressure and stored at -80 until further use. The chloroform, ethanol and water extracts of *Colocasia gigantea* will be called as CGC, CGE and CGA henceforth.

Preparation of Drug and mode of administration

The chloroform (CGC) ethanol (CGE) and aqueous (CGC) extracts of *Colocasia gigantea* were dissolved in appropriate solvent immediately before use, Doxorubicin was dissolved in sterile physiological saline (SPS). Each animal from each group received different treatments according to body weight intraperitoneally.

Animal care handling

The animal care and handling were carried out according to the guidelines issued by the World Health Organization, Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi, India). Swiss albino mice were bred before use in a controlled environment of temperature (24-25ºC), 50% humidity and light and dark (12 h each) cycle. Usually 5-6 animals were housed in a sterile polypropylene cage containing paddy husk (procured locally) as bedding material. Six to eight weeks old male and female Swiss albino mice weighing 28-35 g were procured from the inbred colony for experimentation. The animals were fed with commercially available food pellets and water ad libitum. The Institutional Animal Ethics Committee of Mizoram University, approved the entire study vide letter no. MZUIAEC16-17-01, Aizawl, India.

Acute toxicity determination

The acute toxicity of all extracts was determined both orally and intraperitoneally according to guidelines issued by the Organization for Economic Co-operation and Development (OECD). Albino mice selected by random sampling technique (n=10) of both sexes (5 males and 5 females) were used for each dose of individual extract. The animals were fasted for 18 hours (both food and water were withdrawn) prior to oral or intraperitoneal administration of different extracts of *Colocasia gigantea*. The control group received sterile physiological saline (SPS). The animals were weighed before and after fasting to estimate their weight loss. If mortality was observed in 2-3 animals then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxicity. The LD_{50} for each extracts was calculated using Probit analysis.

Tumor Model

Dalton's lymphoma ascites (DLA) tumor, procured from the Department of Zoology, North-Eastern Hills University, Shillong was propagated in 10-12 weeks old mice by serial

intraperitoneal transplantation of 1 x 10^6 viable tumor cells in 0.25 ml PBS, pH 7.4 under aseptic conditions.

Experimental design

Dalton's lymphoma tumor bearing mice were divided into the following groups:

Negative Control groups. The negative control group were administered SPS alone.

DOX groups: This group of animals was injected with 0.5 mg/kg body weight of doxorubicin, a standard anticancer drug and served as positive control.

CGE groups: This group of animals received 50, 75, 100, 125, 150, 175, 200 or 225 mg/kg body weight of the ethanol extract of *Colocasia gigantea.*

The tumor bearing animals were given treatment once daily 1 day after tumorization for subsequent 9 days [22]. Each group consisted of ten animals for each dose. The animal survival was monitored daily up to 120 days, since the survival of animals up to 120 days is equivalent to 5 years survival in humans [23]. 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 [22]. The increase in median life span (% IMLS), increase in average life span (% IALS) and the increase in life span (%ILS) 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

ILS (%) = (T/C x 100) - 100

Where, T is the mean survival days of treated mice and C is that of the control mice.

Micronucleus Assay

A separate experiment was performed to study the ability of ethanol extract of *Colocasia gigantea* to induce DNA damage in DLA cells .The grouping and other conditions were similar to that described in the experimental design section, except that the animals were injected with 200 mg/kg b. wt. CGE and the micronuclei were assayed at 12, 24 and 36 h post CGE treatment. The micronuclei were prepared according to the modified method of Fenech and Morley, (1985). Briefly, the DLA cells were aspirated from tumor bearing mice one hour after the last administration of SPS or CGE. The cells were washed twice with sterile PBS and 1X10^6 cells were inoculated into each well of 6 well sterile plates containing 3 ml of MEM and were

allowed to attach for 6 h. Thereafter the cells were treated with 3 µg/ml of cytochalasin-B to block cytokinesis. The cells were left undisturbed and allowed to grow for different times and terminated at 12, 24 and 36 h after the initiation of the cultures. The media containing cytochalasin-B were removed, the cells were washed twice with PBS, dislodged by trypsin EDTA treatment and pelleted by centrifugation at 1000 rpm for 5 min. The cell pellet was disturbed and cells were kept in mild hypotonic (0.75% ammonium oxalate) at 37°C, centrifuged again and the resultant cell pellet was fixed in Carnoy's fixative 3:1 (Methanol:Acetic acid). The cells were pelleted again by centrifugation, and resuspended in a small volume of fixative. The cells were spread on to pre cleaned coded slides so as to avoid observer's bias. The slides containing cells were stained with 0.25% acridine orange (BDH, England, Gurr Cat. No. 34001 9704640E) in Sorensen's buffer (pH 6.8) and subsequently washed in the buffer to remove excess stain. The Sorensen's buffer mounted slides were observed under a DM-2500 fluorescence microscope (Leica Microsystems, Wetzlar, Germany) equipped with 450–490 nm BP filter set with an excitation at 453 nm using a 40 X N Plan objective. A minimum of one thousand binucleate cells with well-preserved cytoplasm was scored for each culture and usually five cultures were used for each group. A minimum of 5000 cells were scored for each culture for the determination of the frequency of micronucleated binucleate cells (MNBNC). The micronucleated cells were scored according to the criteria of Kirsch-Volders *et al.,* (2003) and Fenech *et al.,* (2003).

Apoptosis Assay

A separate experiment was conducted to determine whether *Colocasia gigantea* has the ability to enhance apoptosis in Dalton's lymphoma cells. The grouping and other conditions were essential similar to that described for micronucleus assay. DLA cells were aspirated from tumor bearing mice one hour after the last administration of SPS or CGE. The cells were washed twice with sterile PBS and $1X10^6$ cells were inoculated into each well of 6 well sterile plates containing 3 ml of MEM. The cells were allowed to grow for 12, 24 and 36 h to assess apoptosis. The induction of apoptosis was studied at 12, 14 and 36 h post drug treatment as described earlier (Ribble et al., 2005). 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) stain (Sigma Aldrich Chemical Co. Bangalore, India) and observed under a DM-2500 fluorescent microscope (Leica Microsystems, Wetzlar, Germany). The number of live, and apoptotic cells were counted. A total of 1000 cells were counted for each slide and a total of 5000 cells were counted for each group. The viable cells were recognized by green fluorescent nuclei with organized structure, whereas the early apoptotic cells showed highly condensed or fragmented yellow chromatin in the nuclei. The cells showing orange chromatin, highly condensed and fragmented nuclei were considered as late apoptotic cells. The apoptotic cells also exhibited membrane blebbing as one of the morphological features. Only cells with yellow, condensed, or fragmented nuclei

were counted as apoptotic cells in a blinded, non-biased manner. The apoptotic index was calculated as follows:

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

Biochemical Assays

The alteration in biochemical profile after administration with 200 mg/kg b.wt. CGE was assayed by conducting a separate experiment where grouping and other conditions were essentially similar to that described for apoptosis assay. The animals were sacrificed after nine days of drug treatment at an interval of 2, 4, 6, 8, 12 and 24 hours. Both the treated and untreated Dalton's lymphoma cells were aspirated under sterile conditions, washed with ammonium chloride followed by sterile phosphate buffer saline and pelleted. The cell pellets were weighed and 10% homogenate was prepared in cold sterile PBS (pH 7.4) and used for the estimation of various antioxidant and lipid peroxidation.

Estimation of Glutathione

Glutathione contents were estimated as described earlier [24]. In brief, 1.8 ml of 0.2 M Na_2HPO_4 was mixed with 40 µl of 10 mM DTNB and 160 µl of cell homogenate and allowed to stand for 2 minutes. The absorbance was read against the blank at 412 nm in a UV-VIS Biospectrophotometer (Eppendorf India Limited, Kolkata, India). The blank consisted of distilled water instead of cell homogenate.

Estimation of Glutathione - S – Transferase

Glutathione-s-transferase was determined by the method of Habig *et al.,* (1987). Usually, 0.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.1 ml of 20mM CDNB, and 8.8 ml distilled water were incubated at 37°C for 10 min followed by the addition of 0.5 ml of 20 mM GSH and 0.1 ml of cell homogenate. The absorbance was read at 340 nm with a UV-VIS spectrophotometer at 1 min intervals for 6 minutes. Distilled water was used as a blank.

Catalase Assay

Catalase was assayed according to technique described by Aebi (1984). The 20 µl of cell homogenates was mixed with 1.98 ml of 50 mM phosphate buffer (pH 7.0) in a 3 ml cuvette. The reaction (maintained at 20ºC) was started by adding 1 ml of 30 mM H_2O_2. The decrease in absorbance was monitored at 240 nm for 60 seconds.

Superoxide Dismutase Assay

The activity of SOD was estimated as described by Fried (1975). 100 µl of cell homogenate, 100 µl of 186 µM phenazenemethosulfate, 300 µl of 3.0 mM nitrobluetetrazolium, and 200 µl of 780 µM NADH were incubated for 90 seconds at 30°C. The reaction was terminated by the addition of 1000 µl of acetic acid and then 4 ml n-butanol. The absorbance was read at 560 nm using UV/VIS spectrophotometer. The percent inhibition was calculated by measuring the absorbance of blank without SOD enzyme. The SOD activity was calculated using the formula (Blank-Sample)/Blank X 100.

Lipid Peroxidation Assay

Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids, serve as a convenient index for determining the extent of peroxidation reaction of lipids. MDA has been identified as the product of lipid peroxidation (LOO) that reacts with thiobarbituric acid to give a red species absorbing at 535 nm. LOO assay was carried out following the method of Buege and Aust, 1978. One ml of cell homogenate was added to 2 ml of TCA-TBA-HCl reagent and was mixed thoroughly and heated in a boiling water bath for 15 minutes, cooled immediately at room temperature and centrifuged at 1000 rpm for 10 min. The supernatant was collected and its absorbance was read at 535 nm against blank in a UV-VIS spectrophotometer. The blank contained all the reagents except the cell homogenate substituted with distilled water. The MDA concentration of the sample was calculated using the extinction coefficient of $1.56x10^{6}M^{-1}cm^{-1}$.

Determination of Liver and Kidney function

An experiment was conducted to study the toxic effect of CGE where grouping and other conditions were similar to that of biochemical assay. A 10% homogenate of liver and kidneys was prepared in PBS using a homogenizer (Remi, India). The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated in the liver, whereas uric acid and creatinine were measured in the kidney homogenates with the help of commercial available Respons kits using a Respons 910 autoanalyzer (Diagnostic Systems GmbH¸ Holzheim, Germany).

Statistical Analyses

The statistical analyses were done using Origin Pro 8 SRO v8.0724 (B724), Northampton, MA, USA. The significance for survival analysis was determined by Kaplan Meier test and student's 't' test was applied for biochemical studies followed by Tukey's post -hoc tests for multiple comparisons, wherever necessary. The Wilcoxson's signed rank test was utilized 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).

RESULTS

The results are expressed as the mean ± standard error of the mean (SEM), wherever required in Tables 1-16 and Figures 1-11.

Acute toxicity

Oral administration of the different extracts of *Colocasia gigantea* showed no signs of toxicity up to 2 g/kg b. wt. The acute toxicity assay after the intraperitoneal administration was carried out by up and down method. Based on the animal survival, chloroform and aqueous extracts showed an LD_{50} of 625 mg/kg b. wt. and 710 mg/kg b. wt. respectively, whereas ethanol extract was less toxic as the LD_{50} was823mg/kg b. wt. (Table 4-5).

Table 1: The survival of mice administered intraperitoneally with different doses of chloroform extract of *Colocasia gigantea* rhizomes.

Dose (g/kg b. wt.)	Mortality (%) 0n different days														Total	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
3	100	-	-	-	-	-	-	-	-	-	-	-	-	-	0	Aggressive, dull and all died within first day.
2.5	-	-	30	-	-	20	-	-	-	20	-	-	-	-	30	Aggressive, dull and 70% died before 14 days.
2	-	30	-	-	-	-	10	-	-	-	20	-	-	-	40	Aggressive, dull and 60% died before 14 days.
1.5	-	-	20	-	-	-	20	-	-	10	-	-	-	-	50	Dull, lethargic and 50 % died before 14 days.
1	-	20	-	-	-	10	-	-	-	10	-	-	-	-	60	Dull, lethargic and 60 % died before 14 days.
0.5	-	-	-	10	-	-	-	-	-	-	-	10	-	-	80	Dull and 20 % died before 14 days.
0.2	-	-	-	10	-	-	-	-	-	-	-	-	-	-	90	Active and 10% died before 7 days.
0.15	-	-	-	-	-	-	10	-	-	-	-	-	-	-	90	Active and 10% died on 7 days.
0.1	-	-	-	-	-	-	-	-	-	-	10	-	-	-	90	Active and 10% died before 14 days.
0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Active and all survived.

N=10 for each dose

Table 2: The survival of mice administered intraperitoneally with different doses of ethanol extract of *Colocasia gigantea* rhizomes.

Dose (g/kg b. wt.)	Mortality (%) on different days														Total	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
3	10	-	-	-	-	-	-	-	-	-	-	-	-	-	0	Aggressive, Dull and died within first day.
2.5	-	30	-	20	-	-	20	-	-	-	20	-	-	-	30	Aggressive, dull and 70% died before 14 days.
2	-	-	20	-	-	-	-	-	20	10	-	-	-	-	50	Aggressive, dull and 50% died before 14 days.
1	-	-	10	-	-	-	-	-	20	-	-	-	-	-	60	Dull, lethargic and died before 14 days.
0.5	-	-	-	-	-	-	-	-	-	10	-	-	-	-	80	Dull, lethargic and 40% died before 14 days.
0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	Dull and died before 7 days.
0.15	-	-	-	-	10	-	-	10	-	-	-	-	-	-	90	Dull and died before 10 days.
0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	Active and 10% died before 14 days.
0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Active and all survived.

N=10 for each dose

Table 3: The survival of mice administered intraperitoneally with different doses of aqueous extract of *Colocasia gigantea* rhizomes.

Dose (g/kg b. wt.)	Mortality (%) 0n different days														Total	Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
3	100	-	-	-	-	-	-	-	-	-	-	-	-	-	0	Aggressive, dull and all died within 3 hrs.
2.5	100	-	-	-	-	-	-	-	-	-	-	-	-	-	0	Aggressive, dull and all died within first day.
2	20	-	-	-	30	-	-	-	10	-	-	-	10	-	30	Aggressive, dull and 70% died before 14 days.
1.5	-	20	-	-	-	-	10	-	-	-	-	20	-	-	50	Dull, lethargic and 50 % died before 14 days.
1	-	10	-	-	-	-	20	-	-	10	-	-	-	-	60	Dull, lethargic and 60 % died before 14 days.
0.5	-	-	-	-	20	-	-	-	-	-	-	-	-	-	80	Dull and 20 % died before 7 days.
0.2	-	-	-	10	-	-	-	-	-	-	-	-	-	-	90	Active and 10% died before 7 days.
0.15	-	-	-	-	-	-	10	-	-	-	-	-	-	-	90	Active and 10% died on 7 days.
0.1	-	-	-	-	-	-	-	-	-	10	-	-	-	-	90	Active and 10% died before 14 days.
0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	Active and all survived.

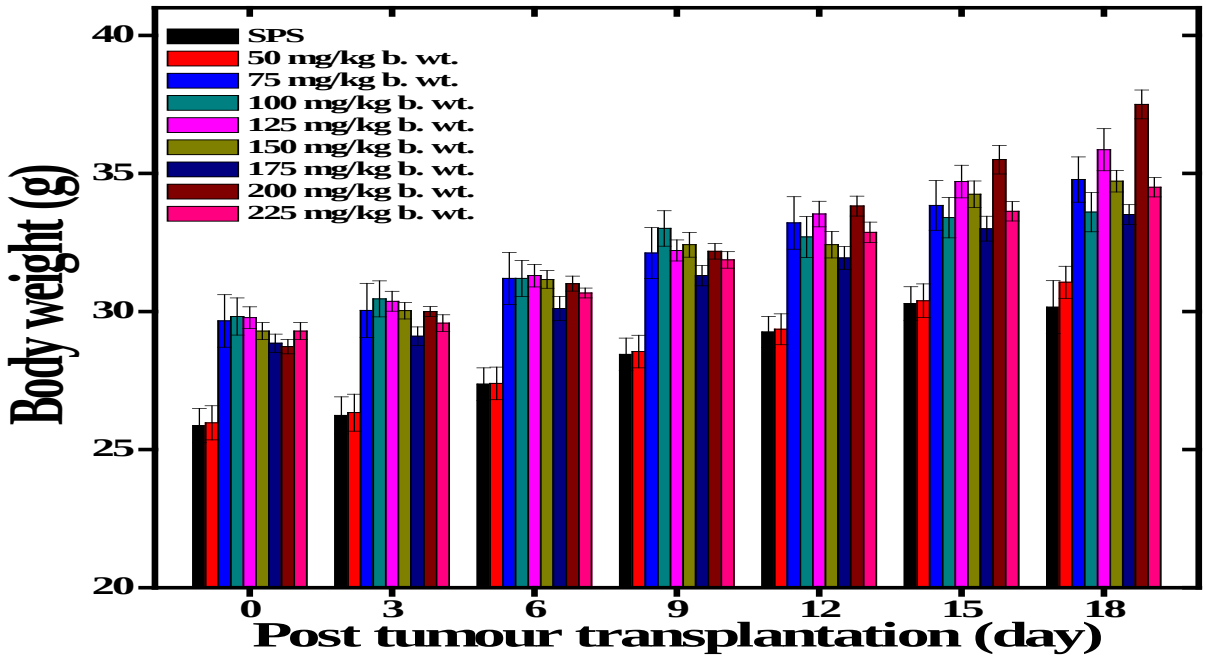

Figure 1: Body weight change of Dalton's lymphoma ascites bearing Swiss Albino mice by treating with ethanol extract of *Colocasia gigantea*. N=10.

Table 4: Acute toxicity of different solvent extracts of *Colocasia gigantea* administered orally in Albino mice.

Extract/ Group	Sex	Dose (g/kg.bwt.)	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	3.2	> 14 days
	F		30	28.9	1.1	> 14 days
			25.8	23.2	2.6	> 14 days
			27	24	3	> 14 days
Chloroform	M	2	29.4	27.5	1.9	> 14 days
			33	30	3	> 14 days
			29.3	27.4	1.9	> 14 days
			31	29.6	1.4	> 14 days
			29.6	27.5	2.1	> 14 days
	F		27	24	3	> 14 days
			29.6	26.3	1.3	> 14 days
			29	27	2	> 14 days
			26.2	25	1.9	> 14 days
			27.6	25.7	1.8	> 14 days
Ethanol	M	2	34.2	31.3	2.9	> 14 days
			32	30.6	1.4	> 14 days
			29.7	28.6	1.1	> 14 days
			27.5	25.8	1.7	> 14 days
			28.7	27.5	1.2	> 14 days
	F		33.1	29	4.1	> 14 days
			30.7	28.7	2	> 14 days
			32.4	30.4	2	> 14 days
			35.4	31	4.4	> 14 days
			32.8	30.8	2	> 14 days
Aqueous	M	2	35.3	33.2	2.1	> 14 days
			33.6	30.6	3	> 14 days
			34.5	32	2.5	> 14 days
			29.7	28.3	1.4	> 14 days
			28.8	26.4	2.4	> 14 days
	F		30.5	28.7	1.8	> 14 days
			31.6	28.8	2.8	> 14 days
			33.6	31.7	1.9	> 14 days
			28.7	28.9	1.8	> 14 days
			29.6	27.7	1.9	> 14 days

N=10 for each dose

Body weight changes

The mice transplanted with DLA cells gained weight continuously due to proliferation of tumor cells until the animal succumbed to death. The tumorized mice did not exhibit signs of tumour regression in the negative control group. The treatment of DLA mice with 50, 75, 100, 125, 150, 175, 200 or 225mg/kg body weight of ethanol extracts of *Colocasia gigantea* exhibited slight elevation in the body weight (Figure 1). The comparison of *Colocasia gigantea*

extract treated groups with negative control revealed a significant reduction in the body weight due to decrease in the cell proliferation (Table 6).

Table 5: Determination of acute toxicity in Swiss albino mice administered various doses of different extracts of *Colocasia gigantea* intraperitoneally.

Extract type	Dose (mg/kg b. wt.)	Survival (%)	LD_{50} (mg/kg)
Chloroform	50	100	625
	100	90	
	150	90	
	200	90	
	500	80	
	1000	60	
	1500	50	
	2000	40	
	2500	30	
	3000	0	
Ethanol	50	100	823
	100	90	
	150	90	
	200	90	
	500	80	
	1000	60	
	2000	50	
	2500	30	
	3000	0	
Aqueous	50	100	710
	100	90	
	150	90	
	200	90	
	500	80	
	1000	60	
	1500	50	
	2000	30	
	2500	0	
	3000	0	

The LD_{50} is determined using Probit analysis. N=10 for each dose

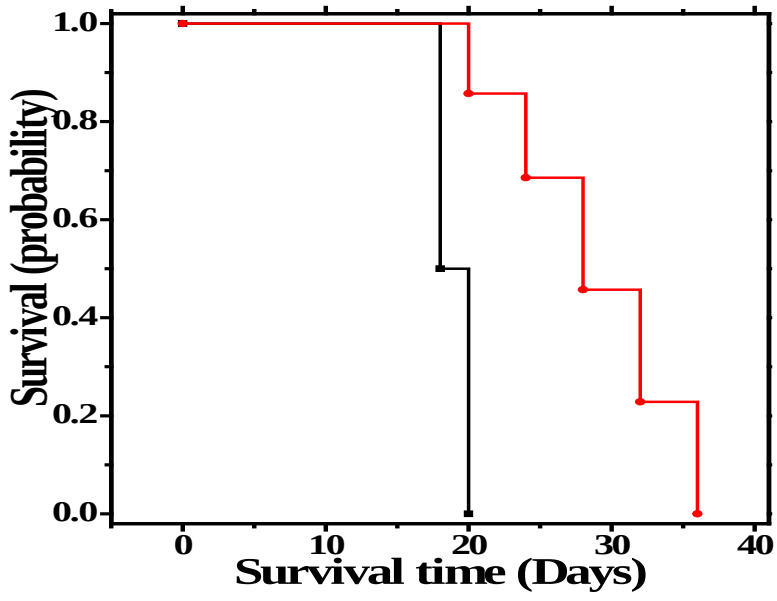

Figure 2(a): The survival of Dalton's lymphoma ascites bearing mice treated for 9 days consecutively with SPS or doxorubicin. Squares: SPS (Control); Circles: Doxorubicin (DOX). N= 10.

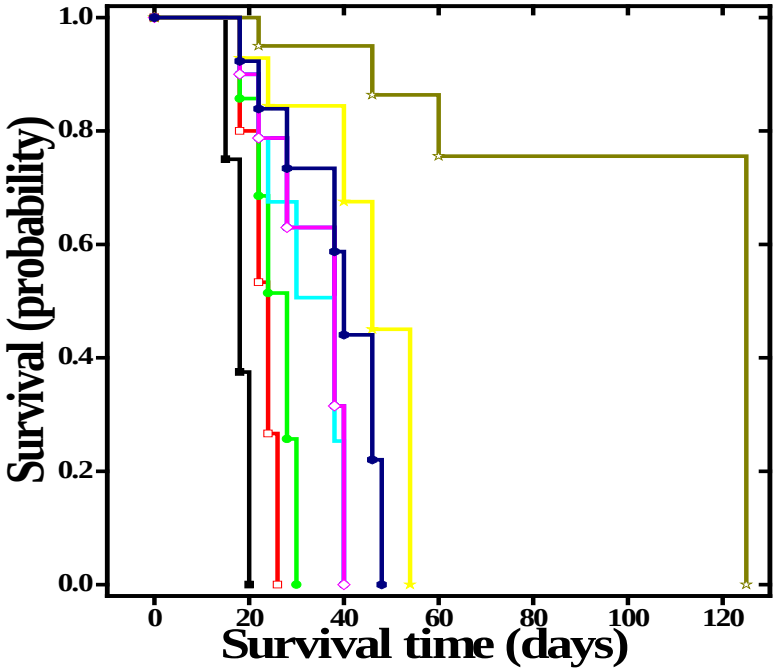

Figure 2(b): Kaplan Meirs' estimate of survival of Dalton's lymphoma ascites bearing mice treated for 9 days consecutively with different doses of ethanol extract of *Colocasia gigantea*. Closed squares: SPS; Open squares: 50 mg/kg b. wt.; Closed circles: 75 mg/kg b. wt.; Open circles: 100 mg/kg b. wt.; Line: 125 mg/kg b. wt.; Open diamonds: 150 mg/kg b. wt.; Closed stars: 175 mg/kg b. wt.; Open stars: 200 mg/kg b. wt.; Closed hexagon: 225 mg/kg b. wt. N=10

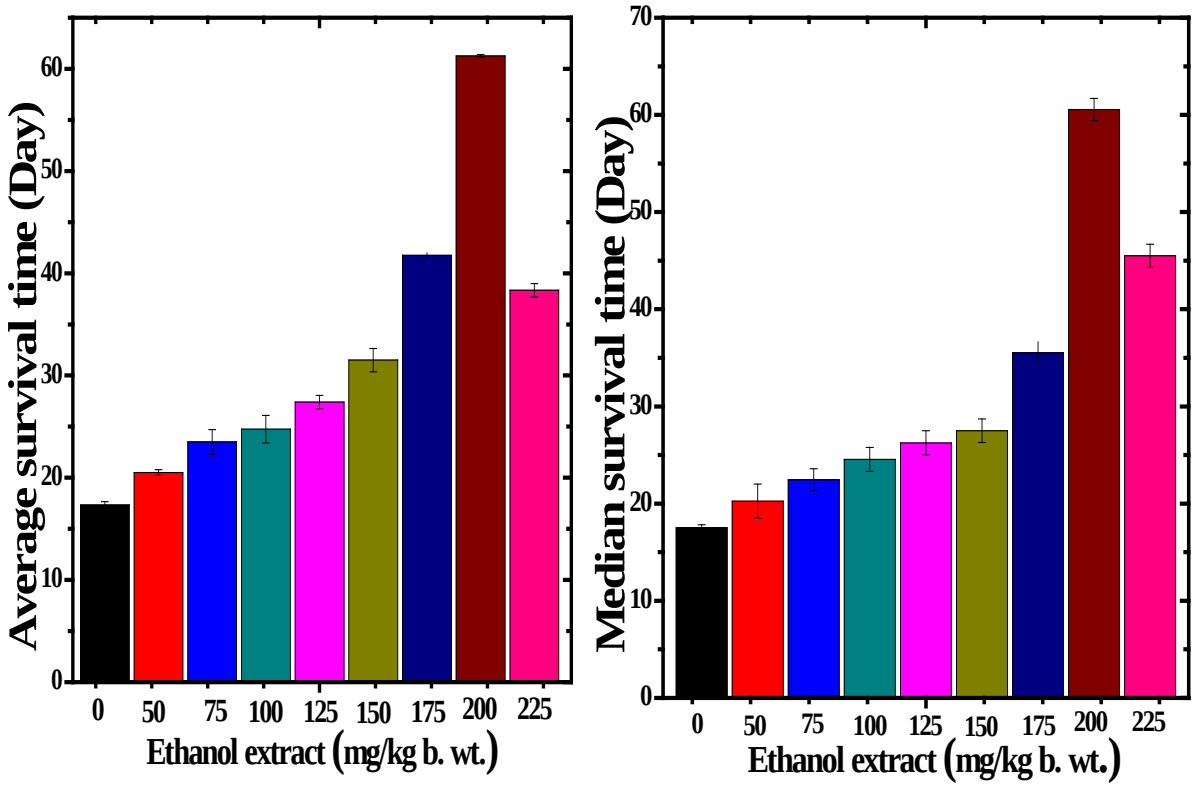

Figure 3: Effect of ethanol extract of *Colocasia gigantea* in Dalton's lymphoma ascites bearing mice on the tumor response assessment based on average survival time (AST) and median survival time (MST). N=10.

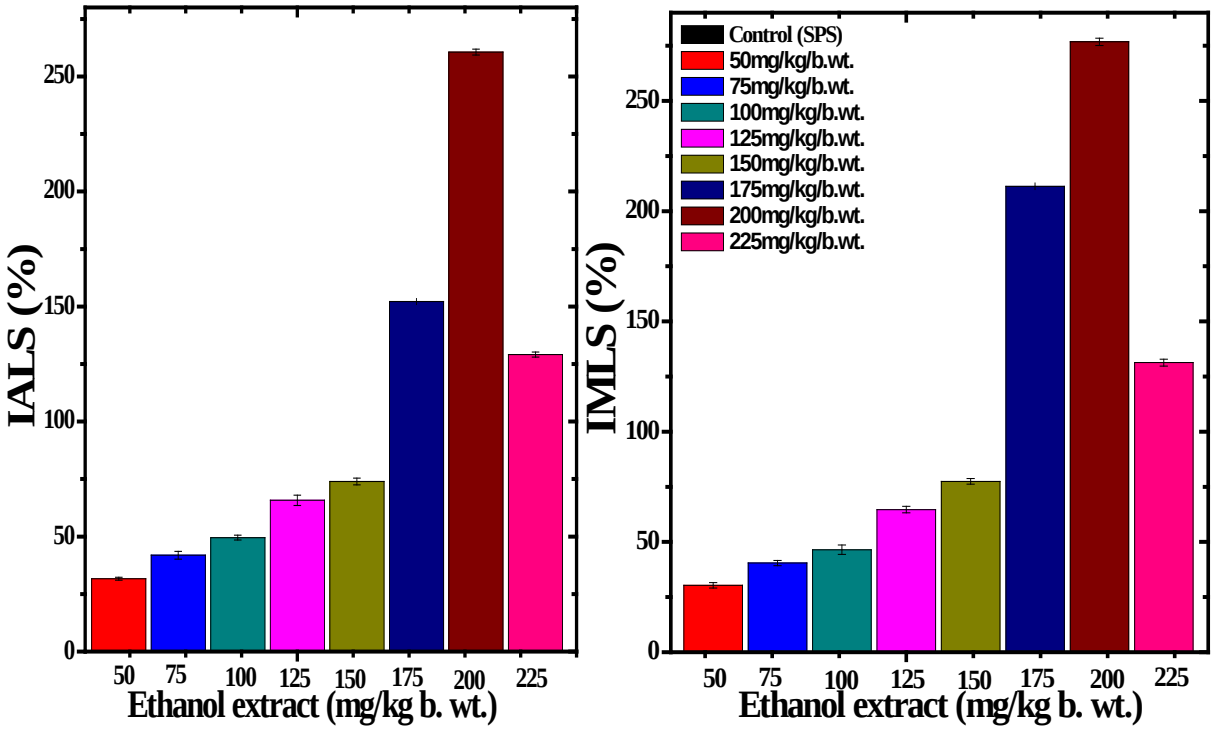

Figure 4: Effect of ethanol extract of *Colocasia gigantea* in Dalton's lymphoma ascites bearing mice on the tumor response assessment based on increase in average life span (% IALS) and increase in mean life span (%IMLS). N=10.

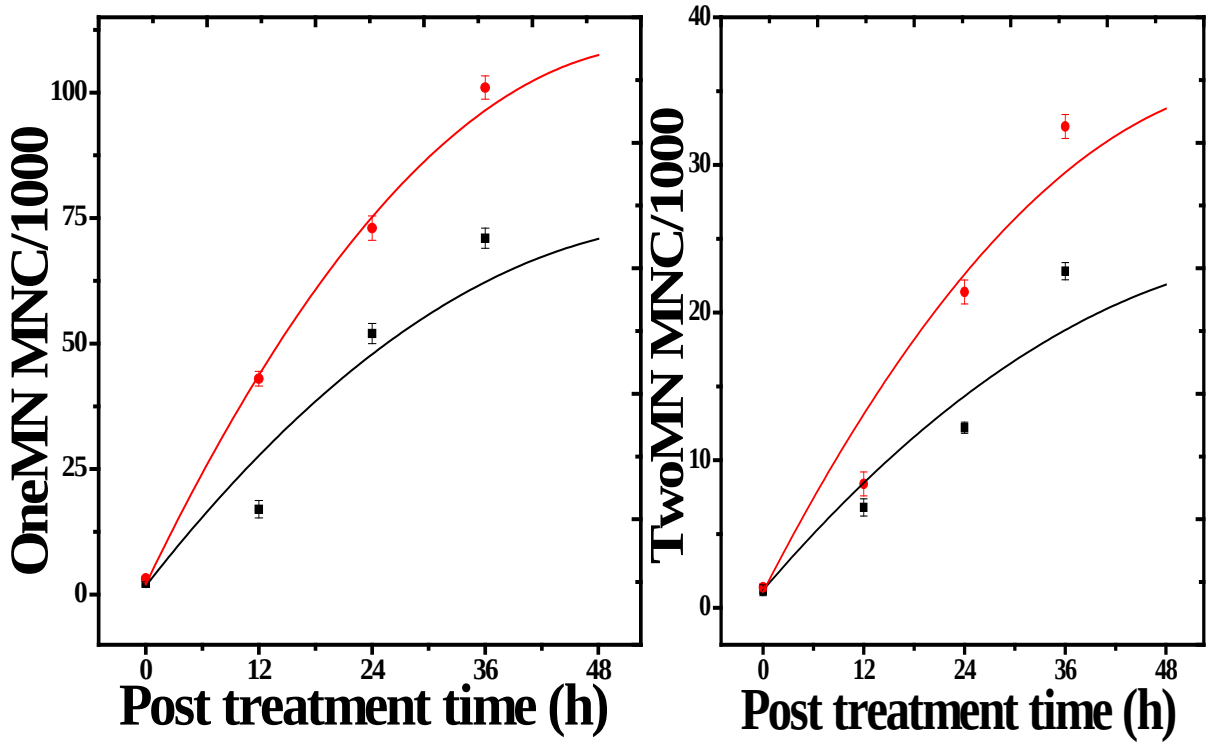

Figure 5(a): Frequency of micronucleated mononucleate cells in Dalton's lymphoma ascites bearing mice treated with 200mg/kg b.wt. ethanol extract of *Colocasia gigantea* (CGE) or 0.5mg/kg b.wt.

doxorubicin (DOX) at different post treatment times. Above left: One micronucleus in mononucleated cells. Above right: Two micronucleus in mononucleated cells. Squares: CGE and Circles: DOX. N=5.

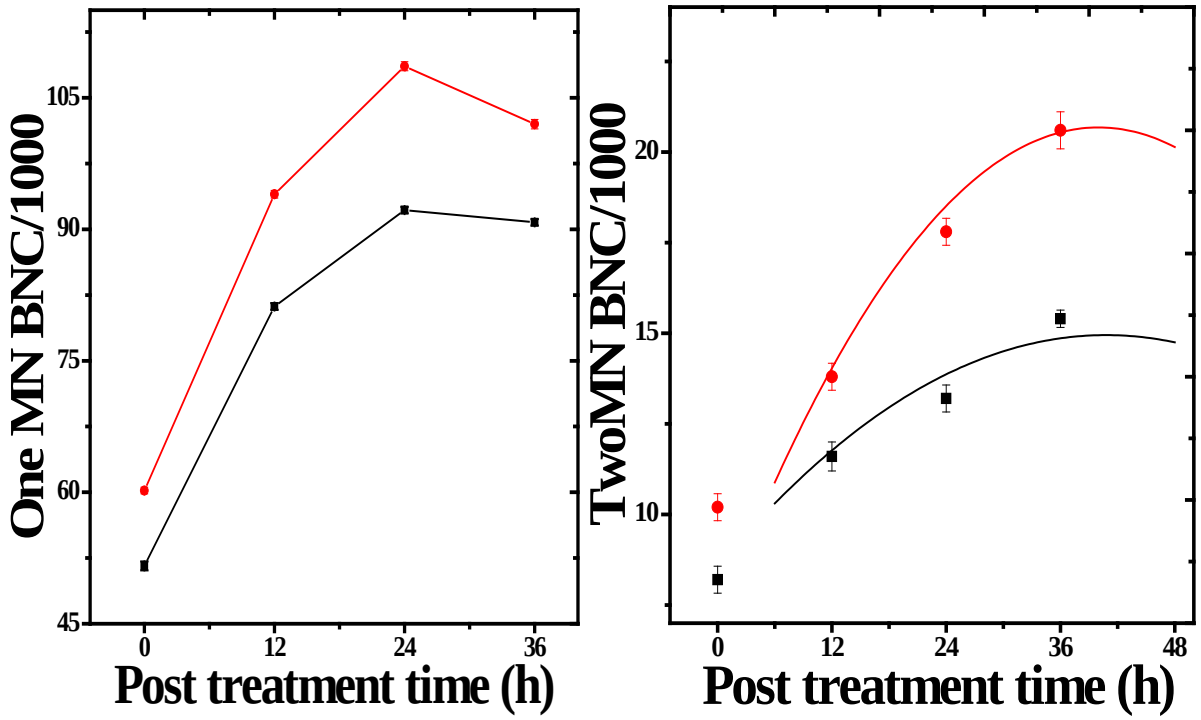

Figure 5(b): Frequency of micronucleated binucleate cells in Dalton's lymphoma ascites bearing mice treated with 200 mg/kg b. wt. ethanol extract of *Colocasia gigantea* (CGE) or 0.5mg/kg b. wt. doxorubicin (DOX) at different post treatment times.Above left: One micronucleus in binucleated cells. Above right: Two micronucleus in binucleated cells. Squares: CGE and Circles: DOX. N=5.

Anticancer activity

Dalton's lymphoma transplanted mice developed tumour rapidly with no signs of regression and all the untreated tumorized mice died within 18-20 days (Table 7). The AST and MST for this group were found to be 17.33 and 17.5 days, respectively (Table 5; Figure 4).

The treatment of tumorized mice with 50, 75, 100, 125, 150, 175, 200 or 225 mg/kg body weight CGE caused a significant ($p<0.001$) rise in the number of survivors when compared to spontaneous control group (Table 7; Figure 2). A 30% rise in survival was observed in animals treated with 50 mg/kg CGE by 20 days. Time of survival increased with increasing dose up to 175 mg/kg where 20% animals survived up to 48 days and all animals succumbed to death after 54 days (Table 7). A further increase in CGE dose elevated the survival of animals up to 50% until 60 days (Table 7). The AST of 44 and MST of 60.5 days were reported for 200 mg/kg CGE leading to an IMLS of 211.27% and an IALS of 152.16%, respectively (Table 8; Figure 3).

Micronucleus Assay

The frequency of micronuclei bearing mononucleate (MNMNC) and binucleate cells (MNBNC) with one and two micronuclei has been represented separately (Table 9, Figure 4(a) & 4 (b)). Treatment of Dalton's lymphoma bearing mice with CGE or DOX showed a time dependent rise in the frequency of micronuclei ($p<0.001$) in a dose dependent manner up to 36 h post-drug treatment in both the mononucleate and binucleate cells (Figure 4). The CGE treatment

not only induced mononucleated and binucleated cells bearing one micronuclei but also the cells bearing two micronuclei (Figure 4).

Table 6: Change in body weight of Dalton's lymphoma bearing Swiss albino mice.

Dose (mg/ kg. b.wt)	Body weight (g)±SEM												
	Post tumour transplanted time (day)												
	0	3	6	9	12	15	18	21	24	28	30	33	36
0	25.97± 0.62	26.34± 0.67	27.4± 0.59	28.55± 0.59	29.36± 0.56	30.39± 0.61	30.26± 0.96						
50	25.97± 0.62	26.34± 0.67	27.4± 0.59	28.55± 0.59	29.36± 0.56	30.39± 0.61	31.06± 0.58	31.42± 0.59	31± .62				
75	29.66± 0.95	30.04± 0.98	31.2± 0.94	32.12± 0.92	33.21± 0.95	33.84± 0.90	34.78± 0.82	35.25± 0.91	34.37± 0.55	34.5± 0.51	29.66± 0.95	30.04± 0.98	31.2±0. 94
100	29.82± 0.67	30.46± 0.65	31.2± 0.65	33.01± 0.64	32.7± 0.74	33.4± 0.73	33.6± 0.71	33.52± 0.64	33.97± 0.64	34.77± 0.70	34.2±0. 51	35.15± 0.31	35.7±0 ±.67
125	29.78± 0.39	30.37± 0.36	31.3± 0.41	32.21± 0.38	33.53± 0.46	34.71± 0.59	35.86± 0.76	37.01± 0.89	36.42± 0.82	37.52± 0.90	37.2±1. 09	29.78± 0.39	30.37± 0.36
150	29.29± 0.31	30.03± 0.30	31.16± 0.32	32.42± 0.45	32.42± 0.48	34.25± 0.48	34.72± 0.39	35.67± 0.40	36.62± 0.39	38.1±0. 47	39.36± 0.51	40.36± 0.61	40.7±0. 51
175	28.85± 0.33	29.11± 0.33	30.11± 0.44	31.3± 0.37	31.94± 0.41	33±0.4 5	33.51± 0.36	34.37± 0.38	34.98± 0.24	35.82± 0.23	36.75± 0.32	37.9±0. 42	38.91± 0.51
200	28.73± 0.26	30± 0.18	31.01± 0.27	32.18± 0.28	33.82± 0.36	35.5± 0.52	37.5± 0.52	39.2±0. 59	41.2±0. 29	42.2±0. 25	44.05± 0.02	45±0.2 6	45.6±0. 18
225	29.29± 0.31	29.58± 0.30	30.67± 0.18	31.87± 0.30	32.87± 0.37	33.63± 0.35	34.5± 0.35	35.48± 0.26	36.66± 0.31	37.22± 0.27	38.14± 0.32	39.02± 0.43	39.67± 0.60

N=10 for each dose

Table 7: Effect of ethanol extract of *Colocasia gigantea* on Survival of Dalton's lymphomas ascites bearing mice treated with various doses administered intraperitoneally.

Post tumor transplant time (day)	SPS (Control)	Survival (%)							
		Ethanol Extract (mg/kg b. wt.)							
		50	75	100	125	150	175	200	225
0	100	100	100	100	100	100	100	100	100
18	30	90	90	60	90	50	80	100	90
20	0	90	90	60	90	50	80	100	90
22	0	70	80	40	80	40	80	90	60
24	0	10	40	40	40	40	70	90	60
26	0	0	40	40	40	40	70	90	60
28	0	0	20	20	40	30	70	80	50
30	0	0	0	20	20	30	70	80	50
32	0	0	0	20	2 0	30	70	80	50
38	0	0	0	0	10	2 0	70	70	40
40	0	0	0	0	0	0	40	70	20
44	0	0	0	0	0	0	40	60	20
46	0	0	0	0	0	0	20	60	10
48	0	0	0	0	0	0	20	60	0
54	0	0	0	0	0	0	0	60	0
60	0	0	0	0	0	0	0	50	0
120	0	0	0	0	0	0	0	50	0

N=10.

Apoptosis Assay

The administration of CGE or DOX induced apoptosis in Dalton's lymphoma cells as early as 12 h post drug treatment in a time dependent manner (Figure 5). The number of apoptotic cells in CGE treated DLA cells significantly ($p<0.001$) increased when compared to concurrent control group at all the post CGE treatment times and maximum number of apoptotic cells were reported at 36 h post-treatment (Table 10). This increase in apoptotic index was 14 folds higher for the all the post CGE treatment times (Table 10).

Biochemical Assays

Glutathione content

The treatment of DLA mice with 200 mg/kg b. wt. CGE led to a significant decrease in the glutathione contents since 2 h post treatment and it continued to decline up to 24 h post treatment, where the reduction in GSH concentrations was highest (Figure 6). The difference in this alleviation in GSH contents between 8, 12 and 24 h was non-significant (Table 11). The CGE treatment reduced the GSH contents comparable to DOX treatment (Table 11).

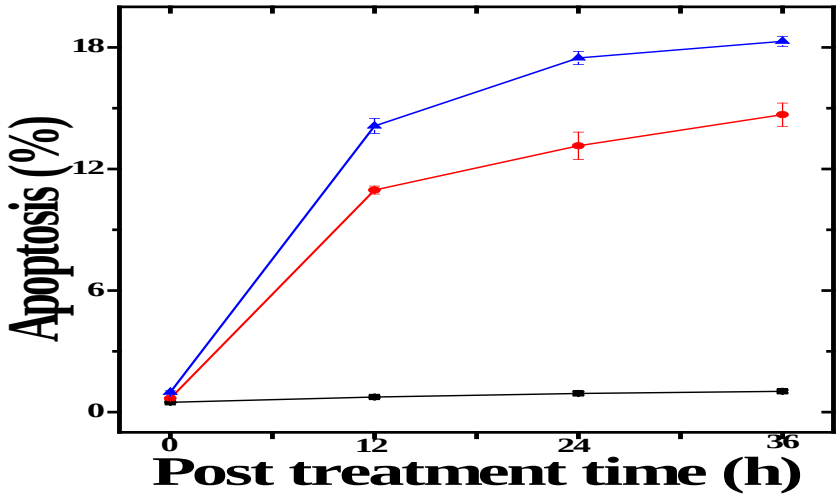

Figure 6: Apoptotic index in Dalton's lymphomas ascites bearing mice treated with 200 mg/kg b. wt. ethanol extract of *Colocasia gigantea* (CGE) or 0.5mg/kg b. wt. doxorubicin (DOX) at different post treatment times. N=5. Squares:SPS; Circle: CGE and Triangles:DOX.

Glutathione - S – Transferase (GST)

The GST activity declined significantly ($p<0.001$) in the DLA mice treated with 200 mg/kg b. wt. CGE (Table 12). The GST activity showed a time dependent decline and the maximum decline was found at 24 h post CGE treatment (Figure 7). The DOX treatment also showed a similar decline in GST activity (Figure 7).

Catalase (CAT) activity

Administration of CGE and DOX led to a gradual and time dependent decline in the catalase activity until 24 h post treatment (Figure 8), where it was 1.4 fold lower than the SPS

treatment (Table 13). The decline in the GST activity was significant ($p<0.001$) when compared to negative SPS treatment (Table 13).

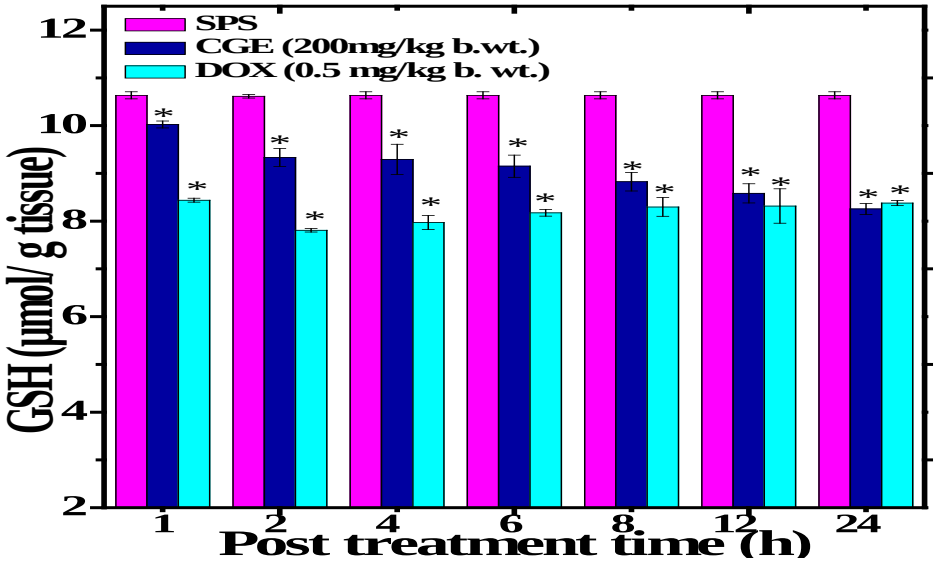

Figure 7: Alteration in the Glutathione contents in mice bearing Dalton's lymphoma ascites treated with 200 mg/kg b. wt. *Colocasia gigantea* extract (CGE) or doxorubicin (DOX). *p<0.001 when treatment are compared with control (SPS) group. No symbol= no significance. N=10.

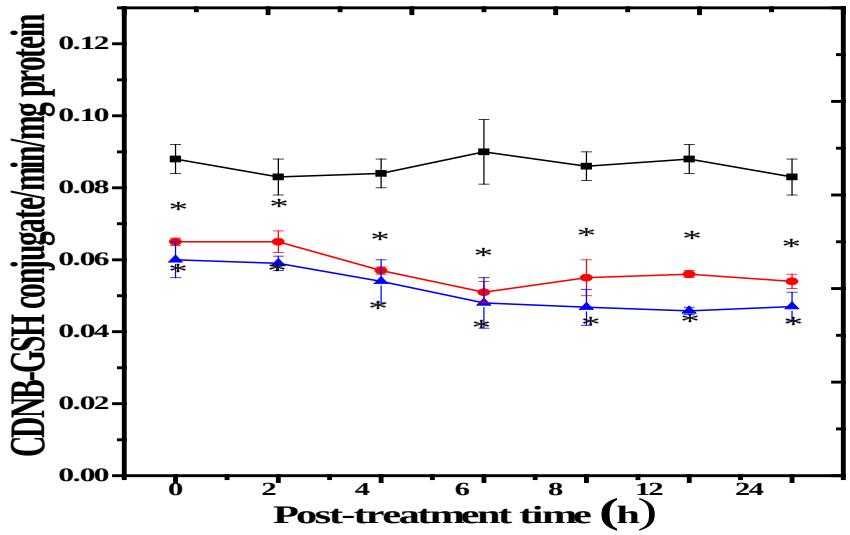

Figure 8: Alterations in the glutathione-s-transferase (GST) activity in Dalton's lymphoma ascites bearing mice treated with 200 mg/ kg b. wt. of *Colocasia gigantea* (CGE) or doxorubicin (DOX). *p<0.001 when treatment are compared with control (SPS) group. No symbol= no significance. Squares:SPS; Circle: CGE and Triangles:DOX. N=10.

Superoxide dismutase (SOD) activity

The SOD activity decreased in a time dependent manner in the DLA mice treated with CGE or DOX treatment until 6 h post treatment where a greatest reduction in SOD activity was observed after CGE administration (Figure 9). This decline was approximately 2.3 and 3 fold for CGE and DOX treatment, respectively when compared to negative SPS control at 6 h post

treatment (Table 14). The SOD activity increased with time after 6 h but did not reach to negative control level even at 24 h post treatment where it was 2 fold lower (Table 14).

Table 8: Effect of ethanol extract of *Colocasia gigantea* on Dalton's lymphoma ascites bearing mice and the tumor response assessment based on median survival time (MST) and average survival time (AST).Increase in mean life span (% IMLS) and increase in average life span (% IALS).

Treatment	Dose (mg/kg.b.wt.)	MST	AST	IMLS (%)	IALS (%)
Control (SPS)	0	17.5±0.33	17.33±0.32	0.05±0.00	0.05±0.00
Ethanol	50	20.25±1.75*	20.5±0.28*	30.3±1.24#	31.63 ±0.65#
	75	22.45±1.13*	23.5±1.21*	40.42±1.18#	41.88±1.68#
	100	24.55±1.24*	24.75±1.35*	46.48±2.11#	49.53±1.055#
	125	26.25±1.25*	27.4±0.65*	64.67 ±1.46#	65.76±2.24#
	150	27.5±1.21*	28.5±1.14*	66.47±1.24#	68.94 ±1.44#
	175	35.5±1.15*	36.75±0.25*	131.33±1.46#	129.12±1.27#
	200	60.55±1.15*	44±0.12*	211.27±1.69#	152.16 ±1.29#
	225	45.5±1.18*	58.22±0.65*	276.79 ±1.58#	260.59 ±1.09#

N=10, **p<0.001, #p<0.0001 when treatment are compared to control group.*

Table 9: Frequency of micronuclei in Dalton's lymphoma ascites bearing mice treated with 200mg/kg b.wt. ethanol extract of *Colocasia gigantea* (CGE) or 0.5 mg/kg b. wt. doxorubicin (DOX) at different post treatment times.

Cell type	Post treatment time (h)	Micronucleated cells/1000±SEM								
		SPS			CGE 200 mg/kg b. wt.			DOX 0.5 mg/kg b. wt.		
		One MN	Two MN	Total	One MN	Two MN	Total	One MN	Two MN	Total
Mononucleated cell	0	1.8±0.37	0.1±0.37	2.6±0.51	2.2±0.37	1.2±0.37	1.7±0.34	3.2±0.20	1.4±0.24	2.3±0.12
	12	4.2±0.37	1.4±0.51	5.6±1.51	17±1.73	6.8±o.58	22.8±0.37	43±1.45	8.4±0.81	45.6±0,75
	24	5.4±0.51	1.6±0.40	7±0.70	52±2.0	12.2±0.37	58.2±1.02	73±2.42	21.4±0.81	88.8±1.24
	36	6.4±0.24	1.8±0.49	8.2±0.37	71±2.0	22.8±0.58	91±0.95	101±2.31	32.6±0.81	119.8±0.91
		One BN	**Two BN**	**Total**	**One BN**	**Two BN**	**Total**	**One BN**	**Two BN**	**Total**
Binucleated cell	0	4.2±0.37	0.8±0.37	5±0.89	51.6±0.51*	8.2±0.37*	59.8±0.86	60.2±0.37*	10.2±0.37*	71.2±0.66
	12	5.2±0.37	1.2±0.58	6.4±0.92	81.2±0.37*	11.6±0.4*	92.8±0.73	94±0.44*	13.8±0.37*	107.8±0.8
	24	5.6±0.51	1.4±0.51	7±1.00	92.2±0.37*	13.2±0.37*	105.4±0.75	108.6±0.51*	17.8±0.37*	126.4±0.87
	36	5.8±0.58	1.8±0.49	7.6±1.08	90.8±0.37*	15.4±0.24*	106.2±0.58	102±0.54*	20.6±0.51*	122.6±1.03

****p<0.001when treatment are compared with concurrent control (SPS) group.***
No symbol= no significance. N=5 for each time

Table 10: Apoptotic index in Dalton's lymphoma ascites bearing mice after treatment with 200 mg/kg b. wt. ethanol extract of *Colocasia gigantea* (CGE) or 0.5 mg/kg b. wt. doxorubicin (DOX) at different post treatment times.

Post treatment time (h)	Apoptosis (% ± SEM)		
	SPS	CGE	DOX
0	0.48±0.02	0.68±0.03*	0.98±0.07#
12	0.74±0.05	10.96±0.19#	14.12±0.36#
24	0.92±0.08	13.14±0.67#	17.48±0.31#
36	1.02±0.07	14.68±0.57#	18.8±0.24#

p<0.05, #p<0.001 when treatment are compared with concurrent control (SPS) group.

No symbol= no significance. N=5 for each time

Table 11: The effect of 200 mg/kg b. wt. ethanol extract of *Colocasia gigantea* (CGE) or Doxorubicin (DOX) on the glutathione contents in mice bearing Dalton's lymphoma ascites at different post treatment times.

Post treatment time (h)	Glutathione (GSH) (µM/mg protein), Mean ± SEM		
	SPS	CGE	DOX
0	10.63±0.07	10.02±0.07*	8.43±0.04*
2	10.61±0.03	9.33±018*	7.81±0.03*
4	10.63±0.07	9.29±0.31*	7.97±0.14*
6	10.63±0.07	9.15±0.23*	8.17±0.07*
8	10.61±0.03	8.82±0.19*	8.30±0.19*
12	10.63±0.07	8.58±0.2*	8.32±0.36*
24	10.63±0.07	8.26±0.11*	8.38±0.05*

p<0.001when treatment are compared with concurrent control (SPS) group.

No symbol= no significance. N=10 for each time

Table 12: The effect of 200 mg/kg b. wt. ethanol extract of *Colocasia gigantea* (CGE) or doxorubicin (DOX) on the glutathione-s-transferase activity in mice bearing Dalton's lymphoma ascites at different post treatment times.

Post treatment time (h)	Glutathione-S-Transferase (U/mg protein), Mean ± SEM		
	SPS	CGE	DOX
0	**0.088±0.004**	**0.065±0.001***	**0.06±0.005***
2	**0.083±0.005**	**0.065±0.003***	**0.059±0.002***
4	**0.084±0.004**	**0.057±0.001***	**0.054±0.006***
6	**0.09±0.009**	**0.051±0.003***	**0.048±0.007***
8	**0.086±0.004**	**0.055±0.005***	**0.0468±0.005***
12	**0.088±0.004**	**0.056±0.001***	**0.0458±0.001***
24	**0.083±0.005**	**0.054±0.002***	**0.047±0.004***

p<0.001 when treatment are compared with concurrent control (SPS) group.

No symbol= no significance. N=10 for each time

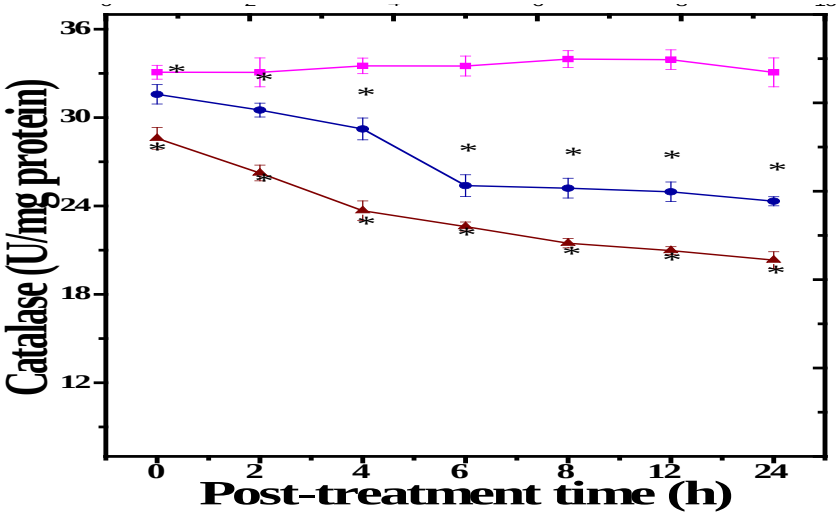

Figure 9: Alterations in the catalase (CAT) activity of Dalton's lymphoma ascites bearing mice treated with 200 mg/ kg b. wt. of *Colocasia gigantea* (CGE) or doxorubicin (DOX). *p<0.001 when treatment are compared with control (SPS) group. No symbol= no significant. Squares:SPS; Circle: CGE and Triangles:DOX. N=10.

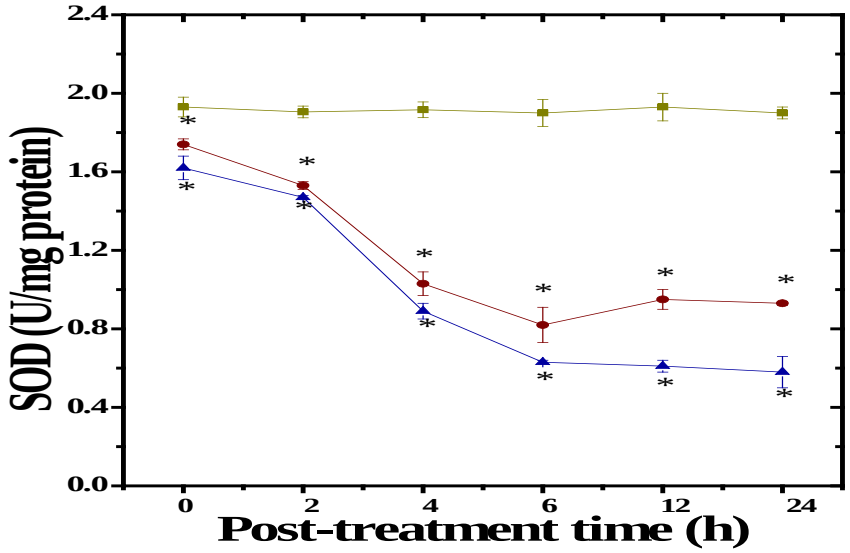

Figure 10: Alterations in the Superoxide dismutase (SOD) activity of Dalton's lymphoma ascites bearing mice treated with 200 mg/ kg body weight of *Colocasia gigantea* (CGE) or doxorubicin (DOX). *p<0.001 when treatment are compared with control (SPS) group. No symbol= no significant. Squares:SPS; Circle: CGE and Triangles:DOX. N=10

Lipid peroxidation (LOO)

Treatment of DLA mice with 200 mg/kg b. wt. CGE led to 3 fold elevation in the lipid peroxidation as early as 1 h post –treatment (Table 15) when compared to SPS group. Increase in assay time resulted in a further rise in LOO and 3.6 fold elevation was recorded at 6 h post treatment in the CGE group (Table 15). The LOO increased in a time dependent manner up to 6 h post treatment in both CGE and DOX treated group and started to increase gradually until 24 h post treatment (Figure 10) where LOO was still higher than the SPS treatment (Table 15).

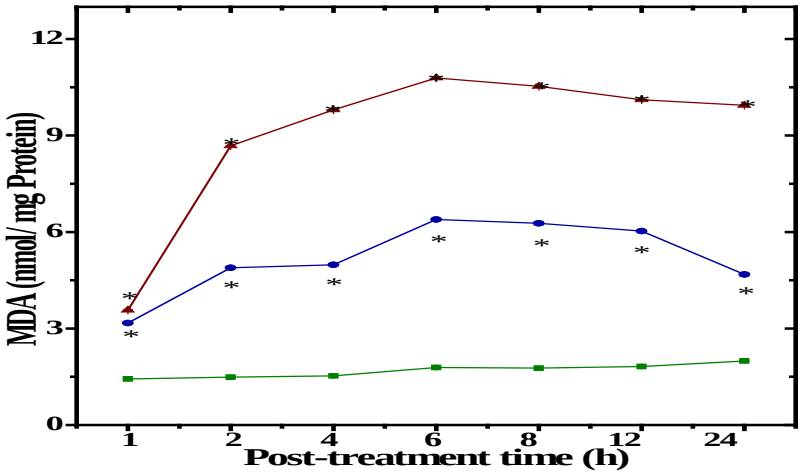

Figure 11: Alterations in the lipid peroxidation (LOO) in the Dalton's lymphoma ascites bearing mice treated with 200 mg/ kg b. wt. of *Colocasia gigantea* (CGE) or doxorubicin (DOX). *p<0.001 when treatment are compared with control (SPS) group. No symbol= no significant. Squares:SPS; Circle: CGE and Triangles:DOX. N=10

Table 13: The effect of 200 mg/kg b. wt. ethanol extract of *Colocasia gigantea* (CGE) or doxorubicin (DOX) on the catalase activity in mice bearing Dalton's lymphoma ascites at different post treatment times.

Post treatment (h)	Catalase (CAT) (U/mg protein), Mean ± SEM		
	SPS	CGE	DOX
0	33.08 ± 0.47	31.58 ± 0.66	28.59 ± 0.74*
2	33.07 ± 0.98	30.51 ± 0.47	26.24 ± 0.53*
4	33.51 ± 0.53	29.23 ± 0.74*	23.68 ± 0.66*
6	33.50 ± 0.68	25.38 ± 0.74*	22.60 ± 0.31*
8	33.93 ± 0.57	25.21± 0.67*	21.47 ± 0.31*
12	33.93 ± 0.67	24.96 ± 0.67*	20.97± 0.27*
24	33.07 ± 0.98	24.32 ± 0.31*	20.32± 0.57*

p<0.001 when treatment are compared with concurrent control (SPS) group.
No symbol= no significance. N=10 for each time

Table 14: The effect of 200 mg/kg b. wt. ethanol extract of *Colocasia gigantea* (CGE) or doxorubicin (DOX) on the superoxide dismutase activity in mice bearing Dalton's lymphoma ascites at different post treatment times.

Post treatment time (h)	Superoxide dismutase (SOD) (U/mg protein), Mean ± SEM		
	SPS	CGE	DOX
0	1.93 ± 0.05	1.74 ± 0.028 *	1.62 ± 0.06*
2	1.905 ± 0.03	1.53 ± 0.02*	1.47 ± 0.01*
4	1.916 ± 0.04	1.03 ± 0.06*	0.89 ± 0.04*
6	1.90 ± 0.069	0.82 ± 0.09*	0.63 ± 0.01*
12	1.93 ± 0.07	0.95 ± 0.05*	0.61 ± 0.03*
24	1.90 ± 0.03	0.93 ± 0.01*	0.58 ± 0.02*

p<0.001when treatment are compared with concurrent control (SPS) group.

No symbol= no significance. N=10 for each time

Table 15: Alterations in the Lipid peroxidation in mice bearing Dalton's lymphoma treated with *Colocasia gigantea* extract (CGE) and Doxorubicin (DOX).

Post treatment time (h)	Lipid Peroxidation (nmol/mg Protein), Mean±SEM		
	SPS	CGE	DOX
0	1.43 ± 0.012*	3.17 ± 0.005*	3.57 ± 0.017*
2	1.49 ± 0.01*	4.89 ± 0.02*	8.68 ± 0.015*
4	1.53 ± 0.03*	4.98 ± 0.01*	9.80 ± 0.028*
6	1.79 ± 0.02*	6.39 ± 0.02*	10.79 ± 0.03*
8	1.77±0.03*	6.27±0.05*	10.53±0.03*
12	1.82 ± 0.01*	6.03 ± 0.01*	10.11 ± 0.02*
24	1.99 ± 0.02*	4.68 ± 0.05*	09.94 ± 0.02*

p<0.0001when treatment are compared with concurrent control (SPS) group.

No symbol= no significance.

N=10 for each time

Liver and Kidney function tests

The intraperitoneal administration of CGE (200 mg/kg b. wt.) for consecutive 9 days did not significantly alter aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in the liver and creatinine and uric acid in the kidney of mice (Table 16 and Figure 11). Therefore, the treatment of 200 mg/kg body weight of CGE did not cause undesirable effect on the liver.

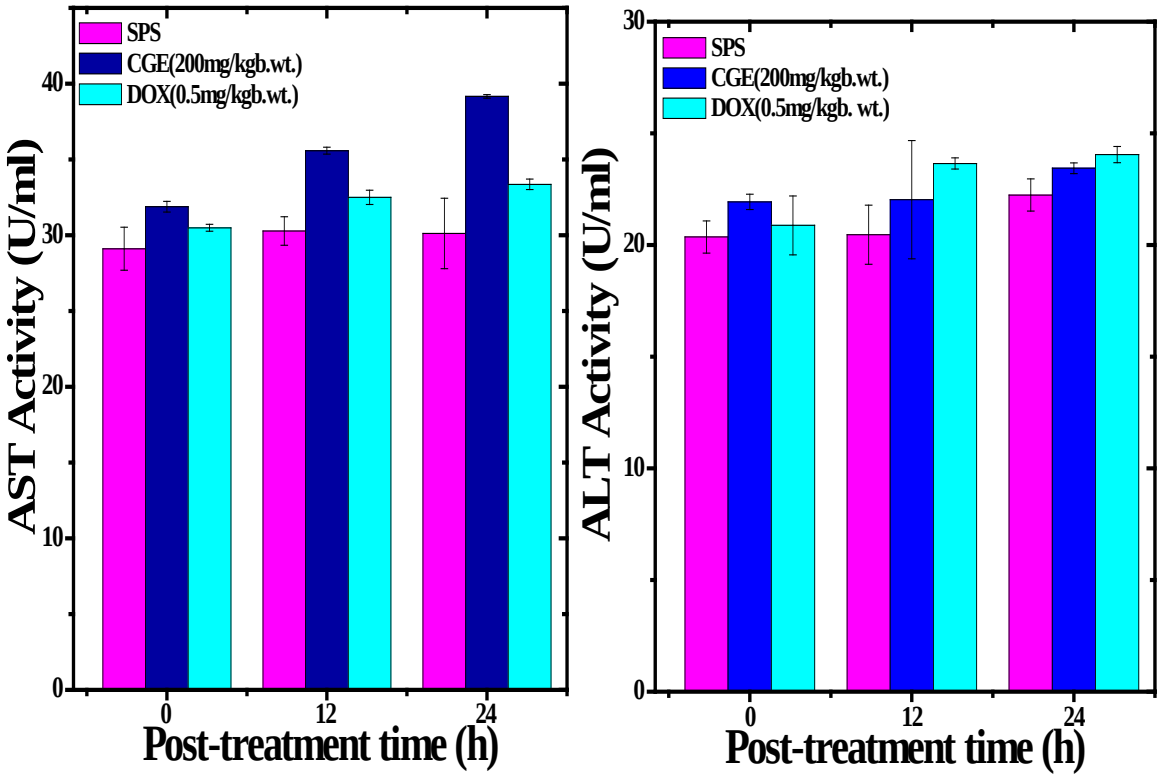

Figure 12(a): The alteration in the liver function by 200 mg/kg b. wt. *Colocasia gigantea* (CGE) or 0.5 mg/kg b. wt. doxorubicin DOX in Dalton's lymphoma ascites bearing mice. N=10

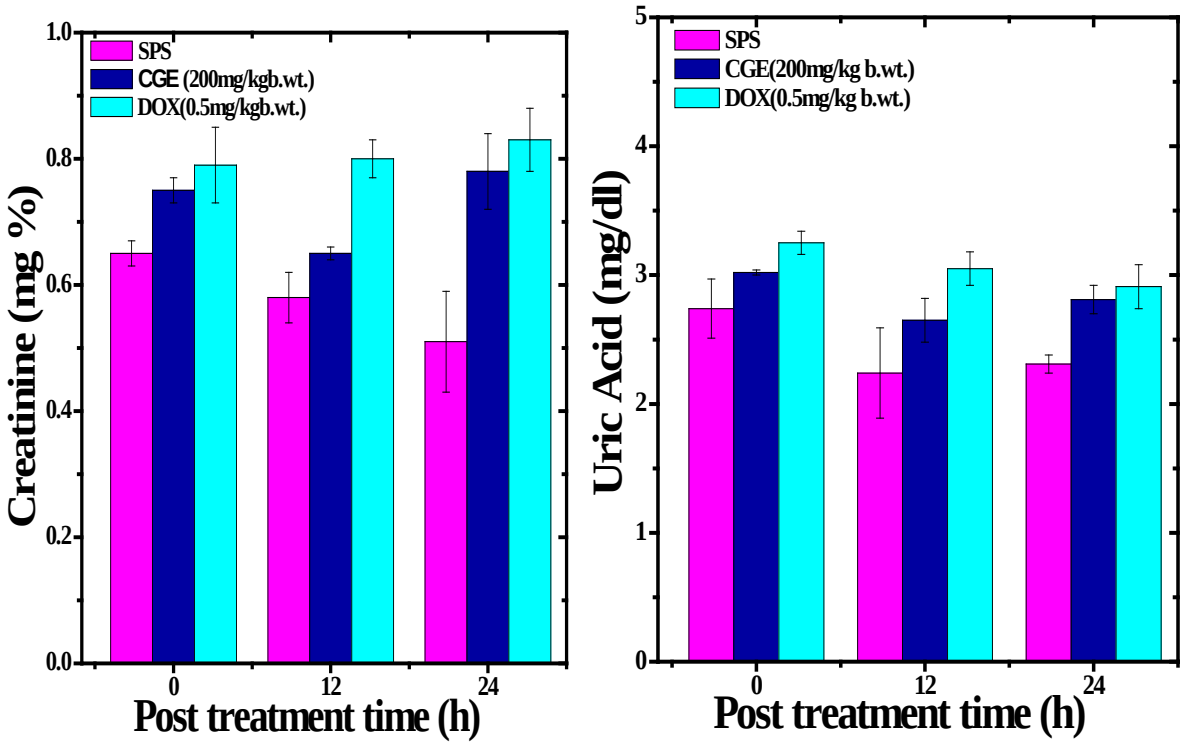

Figure 12(b): The alteration in the kidney function by 200 mg/kg b.wt. *Colocasia gigantea* (CGE) or 0.5 mg/kg b. wt. doxorubicin DOX in Dalton's lymphoma ascites bearing mice. N=10.

Table 16: Effect of *Colocasia gigantea* extract (CGE) and Doxorubicin (DOX) on the liver and kidney function of Dalton's lymphoma bearing mice.

Treatment	Dose (mg/kg.b.wt.)	Post treatment time (h)	Liver function test		Kidney function test	
			AST (U/ml)	ALT (U/ml)	Creatinine (mg%)	Uric acid (mg/dl)
Control (SPS)	0	0	29.11±1.42	18.36±0.72	0.75±0.02	2.74±0.23
		12	29.28±0.94	19.46±1.32	0.58±0.04	2.24±0.35
		24	29.12±2.32	22.24±0.72	0.51±0.08	2.31±0.07
CGE	200	0	33.88±0.35	23.93±0.34	0.75±0.02	3.02±0.02
		12	37.58±0.23	21.03±2.65	0.60±0.01	2.65±0.17
		24	38.16±0.12*	23.44±0.24	0.85±0.06	2.81±0.11
DOX	0.5	0	31.49±0.23	21.88±1.32	0.79±0.06	3.65±0.09
		12	30.50±0.47	24.65±0.25	0.86±0.03*	3.76±0.13*
		24	35.36±0.35*	23.81±0.36	0.87±0.05*	3.81±0.17*

p<0.05 when treatment group are compared with control group.
No symbol= no significance. The results are the Mean ± SEM. N=10 for each time

DISCUSSION

Chemotherapy is one of the most preferred modality to treat cancer, especially when patients have metastasis. Despite increased survival and cure rates, chemotherapy increases toxicity in the normal tissues and also rapidly dividing tissues leading to morbidity and mortality [25,26]. The definite therapy to reduce the toxic effects of chemotherapy is not yet available and efforts to reduce adverse toxic side effects without compromising their efficacy to cure

tumors shall continue. Herbal medicines have been practiced in the world since the advent of human history and their scientific evaluation may help to develop new pharmacophores that can be used as modern therapeutic agents to cure cancer. The use of herbal medicines as adjuvant may be reduce the toxic side effects of chemotherapy and increase its efficacy on neoplastic cells at the same time protecting the normal tissue from chemotherapy-induced toxic side effects. The herbal drug may enhance the immune surveillance of normal tissues, which are affected adversely during neoplastic transformation. The inclusion of herbal medicine in chemotherapy may improve the therapeutic index by killing neoplastic cells and reducing the toxicity to normal tissues [27]. The natural products may play an important role by killing neoplastic cells and not allowing the normal cells to transform into the malignant phenotype. 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 [28]. Therefore, the present study was undertaken to evaluate the ability of *Colocasia gigantea* to kill the Dalton's lymphoma cells transplanted in mice.

Different extracts of *Colocasia gigantea* administered with a single oral dose showed no signs of any toxicity up to 2 g/kg b. wt. in Swiss albino mice hence it can be considered safe orally. However, the intraperitoneal mode of administration revealed significant toxicity, where the chloroform, ethanol and aqueous extracts showed LD_{50} of 625 mg/kg b. wt., 823 mg/kg b. wt. and 710 mg/kg b. wt., respectively. In an earlier study the LD_{50} for interaperitoneal administration was found to be lower than oral admiration for leaf extract of *Blighia unijugata* [29].

Assessment of antitumour activity on Dalton's lymphoma transplanted intraperitoneally nto mice indicated that DLA cells grew rapidly and all the tumorized mice died within 18-20 days with an average survival time (AST) and median survival time (MST) of 17.33 and 17.5 days respectively. Treatment of DLA mice with different doses of CGE led to a rise in the survival of mice in a dose dependent manner and a maximum number of survivors were observed at a dose of 200 mg/kg b. wt. with a 50% tumor free survivors beyond 120 days. The increase in tumor free survivors have been reported for the stem bark extract of *Alstonia scholaris, Aphnamixis polystachya, Ervatamia heyncana, Hygrophila spinosa, Podyphyllum hexandrum, Rubia cordifolia, Tinospora cordifolia* and *Tylophora indica* earlier [30-36].

The infliction of DNA damage is one of the important events to kill tumor cells and many chemotherapeutic agent induce DNA damage to kill neoplastic cells [37]. The ability of ethanol extract of *Colocasia gigantea* to trigger the DNA damage was tested in the tumorized mice and it was found that CGE induced DNA damage as evidenced by the increase in the formation of micronuclei in mononucleated as well as binucleated cells effectively. Treatment of Dalton's lymphoma bearing mice with CGE showed a time dependent elevation in the frequency of micronuclei up to 36 h post treatment. A similar effect has been observed earlier [38-40]. The CGE induced not only one micronuclei but also cells with two micronuclei indicating that it induced complex DNA damage in the form of multiply damaged sites that

would have repressed the DNA damage repair leading to higher cell death. A number of studies have indicated that the cells expressing micronuclei are dying cells and correlation between cell killing and micronuclei has been reported [38-41]. The micronuclei assay provides an indirect measure of DNA damage since the micronuclei arise due to defective cell division, mis-segregation of chromosomes, DNA exchanges and faulty or suppressed DNA repair leading to cell death [39-45]. 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 [46]. This may happen only if the level of DSBs exceeds the repair capacity of dividing cells, which is mainly due to either the misrepair of DSBs by the dysfunctional homologous recombination [47].

The apoptosis induction is a silent form of cell death and many chemotherapeutic agents induce apoptosis to shrink the tumor [48,49]. One of the important cause of cell death by CGE seems to be induction of apoptosis. Treatment of DLA mice with CGE triggered apoptosis in a time dependent manner leading to increased tumor free survivors in the present study. The infliction of DNA damage in the cells by CGE may have triggered a cascade of biochemical and molecular events inducing apoptosis, which was characterized by chromosome condensation, DNA fragmentation, membrane blebbing and formation of apoptotic bodies and cell death [50,51].

The cancer cells are always at higher oxidative stress and a further increase in oxidative stress will stimulate cells to undergo DNA damage and thus it is able to kill tumor cells effectively. The chemotherapeutic agents are known to kill tumor cells by modulating oxidative stress [52,53]. The treatment of tumor bearing mice has reduced the glutathione concentration as increased GSH is involved in resistance to apoptosis and also to chemotherapy, whereas reduced glutathione sensitizes cancer cells to death [54-58]. Glutathione is an important biomolecules synthesized by cells and it plays crucial role in the detoxification, cell differentiation, proliferation and apoptosis however, reduced GSH levels cause oxidative stress and aids in cell death [56,59-61. The cell killing effect of CGE may be due to its ability to reduce glutathione concentration in the tumor cells. Similarly, treatment of Dalton's lymphoma with CGE had a negative effect on the activities of GST, catalase and SOD, which are also involved in resistance to chemotherapy [62-64]. This depletion in their activities may have made tumors cells susceptible to the cytotoxic effect of CGE causing increased tumor free survival in the present study.

The lipid peroxidation is involved in increased oxidative stress and cell death when chemotherapeutic agents come into the contact of cancer cells. Since lipid are integral part of cell membrane and their peroxidation damages the cell membrane thus killing the cells effectively [65,66]. The increase of lipid peroxidation in DLA cells by CGE may have killed the tumor cells by damaging their membrane and inducing damage of proteins and nucleic acids [66].

The mechanism of tumor cell kill by CGE is not well understood. However, employment of multiple putative pathways to kill tumor cells seems to be operational in the present study. The increase in lipid peroxidation may have changed cell membranes and also the important macromolecules like DNA and proteins that in turn may have killed the tumor cells and increased the tumor free survivors in the present study. The CGE has actually increased the DNA damage in both mono and binucleated tumor cells and also induced apoptosis which supports the above contention. The reduction in GSH, GST, catalase and SOD by CGE would have increased the oxidative stress in the DLA cells bringing effective cell killing in the present study. At molecular levels negative modulation of Nrf2, COX-II and NF-κB by CGE may have favored the tumor cell killing as the activation of these genes is involved in failure of tumor therapy [67-69]. Since CGE induced apoptosis it may have stimulated apoptotic cascade by upregulating p53, caspases, Bax and other proteins in the present study. Some other unknown mechanisms may also have contributed to the cytotoxic effect of CGE.

CONCLUSIONS

The CGE killed tumor cells and increased the tumor free survival, which may be due to its ability to induce DNA damage and it increased micronuclei and apoptosis. The apoptosis may have been triggered by the activation of p53, Bax and p21 and caspases. It may have also suppressed the transcriptional activation of NF-κB, COX-II and Nrf2. The elevation in lipid peroxidation and depletion in GSH, GST, catalase and SOD may have played a major role in inducing DNA damage and stimulating apoptotic and non-apoptotic pathways that finally killed the DLA cells and increased the tumor free survivors in CGE treated mice beyond 120 days.

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