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# Antitumor Activity of Methanolic Extract of *Pisonia Aculeata* Leaf

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## Abstract

In order to scientifically appraise some of the anecdotal, folkloric, ethno medical uses of *Pisonia aculeata* Linn. (Nyctaginaceae), the present study was undertaken to examine the antitumor activity of methanolic extract of *Pisonia aculeata* leaves extract on Ehrlich Ascites Carcinoma (EAC) in mice. Tumor was induced in mice by intraperitoneal injection of Ehrlich Ascites Carcinoma cells ($1X10^6$ cells/mouse). Methanolic extract of *Pisonia aculeata* (MPA) was administered to the experimental animals at the doses of 200 & 400 mg/kg/day, p.o. The antitumor effect of the extract was evaluated by using survival time, haematological parameters, increase in body weight, solid tumor volume and peritoneal cell count. Oral administration of MPA increased the survival time and inhibits the weight gain of the tumor bearing mice. After 14 days of inoculation, the extract also reduces the solid tumor volume developed by the EAC cells. The findings of this study indicate that the MPA possesses significant antitumor activity on dose dependent manner.

**Keywords:** *Pisonia aculeata*, ehrlich ascites carcinoma, tumor.

## Introduction

The majority of the world's population in developing countries still relies on herbal medicines to meet their health needs in cases when synthetic medicine could not relieve patients who suffer from hard to cure illnesses like cancer. A number of natural products have been studied for anticancer activity on various experimental models. This has resulted in the availability of nearly 30 effective anticancer drugs[1]. Plant derived compounds, in particular have a special place in anti-cancer therapy, and some of the new chemotherapeutic agents currently available for use in a clinical setting include paclitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precursor for water soluble derivatives [2–4]. Due to lack of effective drugs, cancer is a fatal disease rating the top three cause of death. Many of the chemotherapeutic agents sold for the treatment of cancer are highly expensive, mutagenic, carcinogenic and teratogenic and marrow inhibition limits their applications [5]. Therefore the quest for effective anti-cancer drug is an active research field. Efforts, therefore, are being made to identify naturally occurring anticarcinogens, which would prevent, slow/reverse cancer development [6].

*Pisonia aculeata* Linn. (Nyctaginaceae) is a large scandent shrub, which holds an important place in folklore medicine. It is extensively used by native medical practitioners and tribes for treating swelling, rheumatic pains, jaundice and tumors [7]. Preliminary phytochemical screening of the extract showed the presence of alkaloids, triterpenes, phenolic compounds, flavonoids and glycosides. However no studies to date have been able to demonstrate the pharmacological activities. The present study is focused on

evaluation of the anticancer activity of the methanolic extract of leaves of *Pisonia aculeata* (MPA) against Ehrlich Ascites carcinoma in mice.

## Material And Methods

### Collection and extraction of plant material

Fresh leaves of *Pisonia aculeata* were collected from the surrounding of Tirupati district(517502), AndhraPradesh, India, in the month of April and May 2010 and authenticated by Dr. K. Madhava Chetty, Asst. Professor, Department of Botany, Sri Venkateswara University, Tirupati AndhraPradesh ,India.

The leaves were shade dried and pulverized. The powder was treated with petroleum ether for dewaxing and removal of chlorophyll. Later it was packed (250 g) in soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 ml methanol (70% v/v) as solvent. The methanolic extract was concentrated under vacuum and dried in a desicator (yield 12.5 g, 5 % w/w). Without any purification, aliquot portions of the crude extract were suspended in 5% gum acacia for use on each day of our experiment.The Phytochemical studies were performed as described by Wagner et al, 1984. The extract showed the presence of alkaloids, triterpenes, phenolic compounds, flavonoids and glycosides.

### Animals Used

Swiss albino mice (20-25g) were used throughout the study. They were housed in standard microlon boxes and were given standard laboratory diet and water ad libitum. Each cage contained 6 rats of the same sex with a bedding of husk, and 12-hour light/dark cycles were provided. Environmental conditions were maintained at a temperature of 22°C ± 2°C and a relative humidity of 60% ± 10%.

### Tumor cell lines

Dalton's ascitic lymphoma (DAL) cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur, Kerala. DAL cells were maintained by weekly intraperitoneal (i.p) inoculation of $1\times10^6$ cells / mouse.

### Effect of MPA on survival time

Animals were inoculated with $1 \times 10^6$ cells/mouse on day '0' and treatment with MPA started 24 h after inoculation, at the doses of 200 & 400 mg/kg/day orally. The control group was treated with same volume of 0.9% sodium chloride solution. All treatments were carried out for 9 days and observation was carried out for 45 days. The animals were subjected for the analysis of median survival time (MST) of DAL group (n=6) and changes in body weight. The antitumor efficacy of MPA (200 & 400 mg/kg/day p.o.) was compared with that of 5-Fluorouracil (20 mg/kg/day i.p. for 9 days). MST was noted with reference to control. Survival times of the treated group (T) were compared with those of the control groups (C) using the following calculation.

$$\text{Increase in life span} = T\text{-}C / C \times 100$$

Where T = number of days treated animals survived and C = number of days control animal survived.

### Effect of MPA on haemotological parameters

In order to detect the influence of MPA on the haemotological status of DAL bearing mice, comparison was made amongst six groups (n= 6) of mice on the $14^{th}$ day after inoculation. The four groups comprised (1) tumor bearing mice, (2-3) tumor bearing mice treated with MPA respectively (200 & 400 mg/kg/day p.o. for first 9 days) and (4) control mice. Blood was drawn from DAL mouse in the conventional way and the white blood cell count, red blood cell count, haemoglobin, protein and differential leukocyte counts were determined. The ascitic fluids were collected on $14^{th}$ day and smeared. The smear was stained with Giemsa stain for cytological studies.

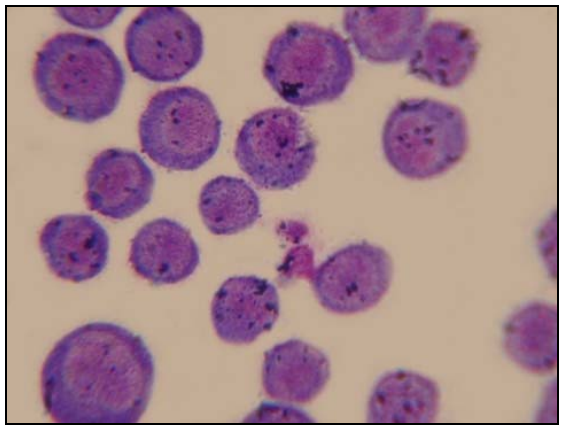
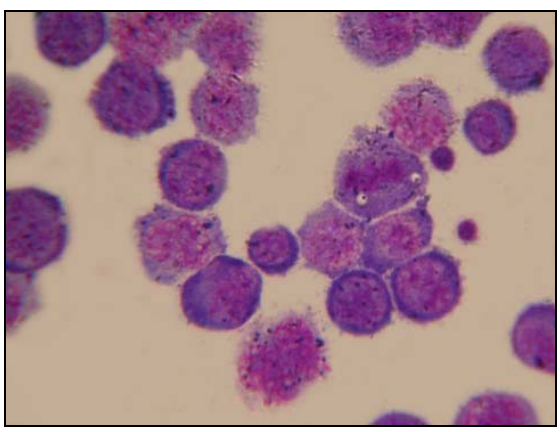


**Fig A.Tumor Control**
**Tumor cells are large in size. It shows with sheet of lymphoma with nucleation.**



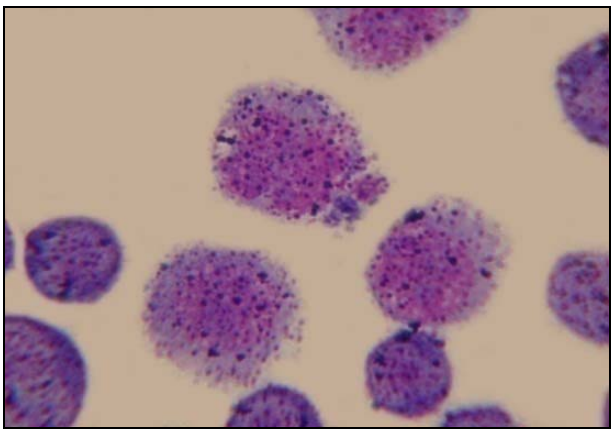
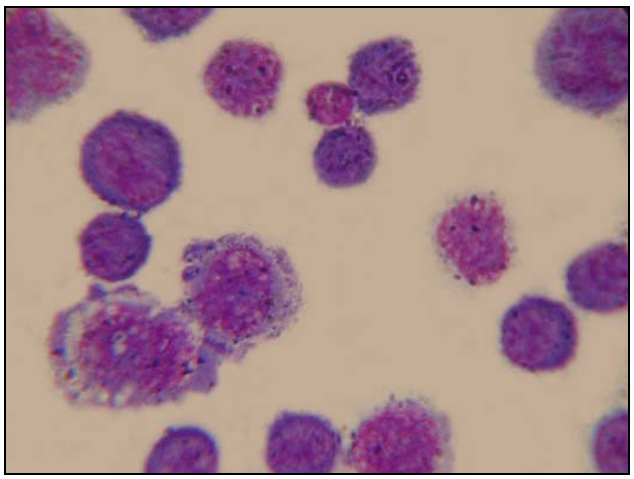


**Fig. B & C-Treated with MPA**
**(200 mg/kg) Shows large lymphoma cell with high n/c ratio. Some of them show cytoplasmic vacuolation.**



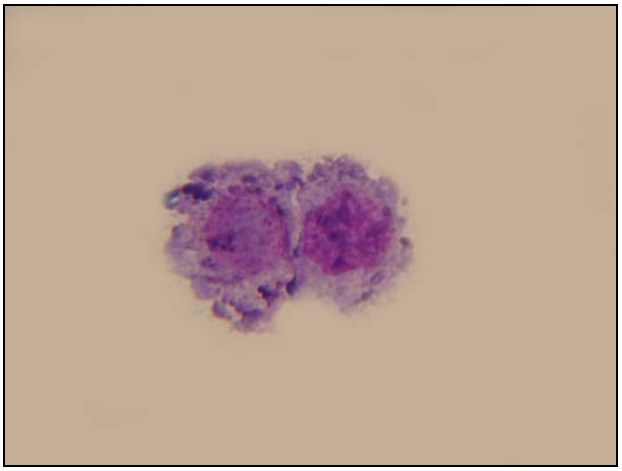


**Fig. D&E-Treated with MPA**
**(400mg/kg) Shows Plasmacytoid feature with varying degree of degeneration, cytoplasmic vacuolation and also showed active mitosis.**

**Fig 1. Cytological Studies –Dalton's Ascites Lymphoma.**

The liver was removed and preserved in tris HCl buffer pH [7.4]. A 10% liver homogenate was used for antioxidant studies such as tissue lipid peroxidation (LPO) superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), glutathione S-Transferase (GST).

**Effect of MPA on normal peritoneal cells**

Five groups of normal mice (n= 5) were used for the study. Two groups received a single administration of MPA at 200 & 400 mg/kg, p.o. The 3rd and 4$^{th}$ groups received the same treatment for two consecutive days. The untreated 5$^{th}$ group was used as control. Cells of peritoneal exudates were collected 24 h after treatment by repeated intraperitoneal wash with normal saline and counted in each of the treatment groups and compared with those of the untreated groups.

**Effect of MPA on solid tumor**

Mice were divided into three groups (n=6). Tumor cells ($1\times10^6$ cells / mice) were injected into the right hind limb of all the animals intramuscularly. Mice of group I were tumor control. Group II to III received MPA respectively (200 & 400mg/kg, p.o.) for 5 alternate days. Tumor mass was measured from 11$^{th}$ day of tumor induction and was repeated every 5$^{th}$ day for a period of 30 days. The volume of tumor mass was calculated using the formula $V = 4/3\ \pi\ r^2$ where r is the mean of $r_1$ and $r_2$ which are two independent radii of the tumor mass.

**Cytotoxicity of MPA by tryphan blue dye exclusion method**

Trypan blue is recommended in dye Exclusion procedures for viable cell counting based on the principle that live (viable) cells actively pump out the dye by efflux mechanism where as dead (non-viable) cells do not. Cytotoxicity was assessed by incubating $1 \times 10^6$ DAL cells in 1 ml phosphate buffer saline with varying concentrations(25-400 μg/ml) of the MPA at $37^0$ C for 3 h in $CO_2$ atmosphere. The viability of the cells was determined by trypan blue exclusion method. In this method dead cells take up the dye where as viable cell exclude the dye. The percentage of dead cells was calculated from which the $IC_{50}$ concentration was determined.

% Inhibition = No of stained cells / No of stained + no of unstained cell X 10

## Statistical analysis

All the values were expressed as mean ± SEM. The data was statistically analyzed by one-way ANOVA followed by Dunnett's test. The data of haematological, and antioxidant parameters were analyzed using ANOVA followed by Tukey multiple comparison test. P values < 0.05 were considered significant.

# Results and Discussion

The methanolic extract was prepared by using soxhlet apparatus and the yield was found to be 12.5 g, 5 % w/w. Preliminary phytochemical analysis of MPA showed the positive test for alkaloids, triterpenes, phenolic compounds, flavonoids and glycosides.

**Antitumor studies**

**Mean Survival Time (MST)**

Any potential anticancer drug is expected to increase the mean survival time and thus increasing life expectancy[8]. Mice transplanted with DAL in our studies have MST of 16 days, which was increased to 23 & 29 days by MPA respectively (200 and 400 mg/kg). These results are almost comparable to that of 5-FU, the standard drug for which the MST was 32 days (Table 1).

**Changes in Body Weight**

There is a tendency for increase in body weight in tumor bearing mice, which is the result of increased formation and collection of ascitic fluid. Potential anticancer drugs decrease this increased body weight by decreasing the formation of ascites and this effect is due to the cytotoxicity against malignant cells, which induce ascites. Tumor bearing mice showed an

increase in body weight to the extent of 14.92 g. The increase in body weight was considerably less on treatment with MPA (200 & 400 mg/kg). The effect is almost 50% of that produced by 5-FU the standard drug tested. In DAL bearing mice, the body weight of control animal was 14.92 g. The increased body weight was reduced significantly (P<0.01) to 8.76 and 7.05g respectively on treatment with MPA (200 & 400 mg/kg) (Table 1).

**Table 1 : Effect of MPA treatment on the survival and Average Body weight Changes of Tumor Bearing Mice.**

| Treatment | MST (d) | Life Span (%) | Average increase in body wt (g) |
|---|---|---|---|
| **Tumor control (Saline 2 ml/kg, p.o.)** | 16 ± 1. 15 | - | 14.92 ± 1.27 |
| **5-FU (20 mg/kg, i.p.)** | 32 ± 1.02[a] | 100 | 5.45 ± 0.86 [a] |
| **MPA (200 mg/kg p.o)** | 23 ± 1.18 [a] | 43.75 | 8.76 ± 0.53 [a] |
| **MPA (400 mg/kg p.o)** | 29 ± 1.56 [a] | 81.25 | 7.05 ± 0.95 [a] |

**n = 6 animals; Days of drug treatment = 9**
**[a]P< 0.01 Vs Tumor control**
**Data were analyzed by using one way ANOVA followed by Dunnett test.**

**Table 2 : Effect of MPA (200 & 400 mg/kg p.o.) on Haemotological Parameters.**

| Treatment | Hb (g%) | RBC (million/mm$^3$) | WBC ($10^3$cells/mm$^3$) | Proteins (g %) | Differential Count % | | |
|---|---|---|---|---|---|---|---|
| | | | | | Lymphocytes | Neutrophils | Monocytes |
| **Normal mice** | 14.7 ± 0.58 | 4.5 ± 0.17 | 8.2 ± 0.54 | 7.8 ± 0.26 | 72 ± 1.5 | 26 ± 1.2 | 2 ± 0 |
| **Tumor bearing mice (14 days)** | 8.3 ± 0.67[a] | 2.6 ± 0.08 [a] | 20.8 ± 1.67 [a] | 12.7 ± 1.15 [a] | 23 ± 1.8 [a] | 76 ± 1.4 [a] | 1 ± 0 |
| **MPA (200 mg/kg,p.o)** | 10.7 ± 1.06 [b] | 3.4 ± 0.32[b,f] | 15.7 ± 0.86 [a,f] | 11.2 ± 0.14[b] | 64 ± 1.6 [c,d] | 34 ± 1.7 [b,d] | 2 ± 0 |
| **400 mg/kg, p.o** | 12.6 ± 0.53 [e] | 4.2 ± 0.16 [d] | 11.9 ± 1.06[d] | 8.9 ± 0.16 [e] | 69 ± 2.4[d] | 30 ± 1.6 [d] | 1 ± 0 |

**n = 6; Days of drug treatment = 9; Values are expressed as mean ± SEM.**
**[a]P < 0.001; [b]P < 0.01; [c]P< 0.05 Vs Normal mice**
**[d]P < 0.001; [e]P< 0.01; [f]P< 0.05 Vs Tumor mice.**
**Data were analysed by one way ANOVA followed by Tukey multiple comparison test**

**Table 3. Effect of MPA (200 & 400 mg/kg p.o.) on LPO, Antioxidants and GST Levels in DAL-Induced Tumor Bearing Mice.**

| Treatment | Dose (mg/kg) | LPO | SOD | Catalase | GPx | GST |
|---|---|---|---|---|---|---|
| **Normal (saline)** | 2 ml/kg | 7.13 ± 0.54 | 4.15 ± 0.12 | 45 ± 0.78 | 8.05 ± 1.04 | 1.36 ± 0.07 |
| **Tumor control** | - | 20.65 ± 1.73 [a] | 1.32 ± 0.35[a] | 18 ± 0.53 [a] | 3.64 ± 0.65 [c] | 3.82 ± 0.26 [a] |
| **MPA** | 200 | 15.12 ± 0.67 [a,e] | 2.35 ± 0.14 [a,e] | 26 ± 0.64 [a,d] | 4.93 ± 0.37 [b] | 2.16 ± 0.13 [b,d] |
| | 400 | 11.08 ± 0.82 [d] | 3.46 ± 0.17 [d] | 37 ± 0.96 [a,d] | 6.87 ± 0.52 [f] | 3.29 ± 0.18 [a] |

**N = 6; values are expressed as mean ± SEM.**
**[a]P < 0.001; [b]P < 0.05; [c]P < 0.01 Vs. Normal**
**[d]P < 0.001; [e]P < 0.01; [f]P< 0.05 Vs. Tumor Control**

**Data were analysed by one way ANOVA followed by Tukey multiple comparison test**
**LPO - μ moles of MDA/ min/mg protein**
**SOD - units/min/mg protein**
**CAT - μ mole of $H_2O_2$ consumed/ min/mg protein**
**GPx - μ moles of GSH oxidised/min /mg protein**
**GST - μ MOLES OF CDNB CONJUGATION FORMED/MIN /MG PROTEIN**

**Haematological Parameters**

In malignancy there is always an alteration of various haematological parameters which increase in a few and decrease in others. There is a decrease in Hb, RBC and lymphocytes in malignancy accompanied by an increase in WBC especially neutrophils, and protein (Table 2). These changes are due to iron deficiency or due to haemolytic of myelopathic conditions induced by malignancy. MPA have very well reverted the above haematological parameters altered by the transplantable tumor of DAL. MPA may have direct tumoricidal DAL effect and thereby maintain normal haematological profile.

**Effect of MPA on Lipid Peroxidation**

The levels of lipid peroxidation in liver tissue were significantly increased (Table 3), by 20.65 ± 1.73, in DAL control group as compared to the normal group (P<0.001). After administration of MPA at different doses (200 and 400 mg/kg) to DAL bearing mice the levels of lipid peroxidation were reduced by 15.12 ± 0.67 and 11.08 ± 0.87 respectively as compared to DAL control group (P < 0.001).

**Effect of MPA on Antioxidants**

The levels of superoxide dismutase (SOD) in the liver of DAL bearing mice decreased (P<0.001) in comparison with normal group. After administration of MPA at the dose of 200 and 400 mg/kg increased levels of SOD significantly when compared to that of DAL control group (P<0.001). The catalase (CAT) level in DAL control group decreased (P<0.001) compared with normal group. Treatment with MPA at the dose of 200 and 400 mg/kg significantly increased CAT levels respectively on dose dependent manner when compared to that of DAL (P<0.001; P<0.01)

Inoculation with DAL drastically decreased the GST and GPx content in DAL control group as compared with normal group (P < 0.001). Administration of MPA at doses of 200 and 400 mg/kg to the DAL bearing mice increased GST and GPx levels respectively as compared with DAL control group (P<0.001; P<0.05) (Table 3).

**Peritoneal Cell Count**

The tendency for cancer cells is to decrease the peritoneal cell count whereas it is increased in normal animals or those treated with anticancer drugs. The implication of this is as discussed later. MPA (200 & 400 mg/kg) increase peritoneal cell count in normal mice treated with these MPA extract for one day and two days (Table 4). Increase in peritoneal cell count induced by MPA is an indirect indication of their anticancer property.

**Table 4. Effect of MPA (200 & 400 mg/kg p.o.) treatment on enhancement of peritoneal cell count in normal mice.**

| Experiment | Number of peritoneal cells ($1 \times 10^6$) / mouse | |
|---|---|---|
| | 200 mg/kg | 400 mg/kg |
| Control | 4.1 ± 0.12 | 4.1 ± 0.31 |
| Treated once | 5.6 ± 0.23 | 6.5 ± 0.25[a] |
| Treated twice on two consecutive days | 7.2 ± 1.10 [a] | 7.9 ± 0.68 [a] |

**N = 5/group; Values are expressed as mean ± SEM.**
**[a]P < 0.01 Vs Control.**
**Data were analyzed by using one way ANOVA followed by Dunnett test.**

**Table 5 : Effect of MPA (200 & 400 mg/kg p.o.) on solid tumor volume.**

| Treatment | Dose (mg/kg) | Solid tumor volume (ml) | | | |
|---|---|---|---|---|---|
| | | 15th day | 20th day | 25th day | 30th day |
| Tumor control | - | 3.15 ± 0.12 | 4.08 ± 0.27 | 4.98 ± 0.23 | 5.96 ± 0.32 |
| MPA | 200 | 2.56 ± 0.36 | 3.41 ± 0.15[b] | 3.90 ± 0.12[a] | 4.62 ± 0.11[a] |
| | 400 | 2.23 ± 0.15[b] | 3.30 ± 0.11[b] | 3.67 ± 0.19 [a] | 4.25 ± 0.41[a] |

**N = 6 animals; Values are expressed as mean ± SEM.**
**[a]P<0.01; [b]P<0.05 Vs Tumor Control.**
**Data were analyzed by using one way ANOVA followed by Dunnett test.**

**Solid tumor volume and short-term cytotoxicity**

Estimation of solid tumor volume is a direct method of evaluation of anticancer activity. It is indeed a suitable method, which does not involve sacrificing the animal. In the study, the tumor mass was directly measured after implantation intramuscularly. The solid tumor volume was increased by 5.96 ± 0.32 in DAL bearing mice, treatment with MPA decreased significantly ($P<0.01$, $P<0.05$), the tumor volume to 4.62 ± 0.11 and 4.25 ± 0.24 ml respectively on dose dependent manner at the end of 30 days (Table 5). The antitumor effect of MPA is very well evident from these observations.

**Table 6 : Cytotoxicity Effect of MPA on DAL by Tryphan Blue Dye Exclusion assay.**

| Concentrations (µg/ml) | % cell viability | % cell death | IC 50 (µg/ml) |
|---|---|---|---|
| 25 | 81.4 | 18.6 | 135 |
| 50 | 68.6 | 31.4 | |
| 100 | 57.5 | 42.5 | |
| 200 | 40.3 | 59.7 | |
| 400 | 23.8 | 76.2 | |

**Average of 3 determinations, 3 replicates**
**$IC_{50}$, Drug concentration inhibiting 50% cellular growth following 3 h of drug exposure.**

Short term cytotoxicity studies by trypan blue exclusion method is a very simple method which can be carried out within a short time of 3 hrs. It is a precise method, which takes in to account the viable and also the dead cells in addition to estimation of $IC_{50}$ concentration. The short term in vitro cytotoxicity study showed the $IC_{50}$ of MPA to be 135 µg /ml. MPA produced concentration dependent inhibition of cell growth on DAL.

## Discussion

The reliable criteria for evaluating an anticancer drug are prolongation of lifespan of the animal [8] and decrease in WBC count of blood [9]. Our results show an increase in life span accompanied by a reduction in WBC count in MPA treated mice. These results clearly demonstrate the antitumor effect of MPA against DAL and this fact is further supported by an increase in peritoneal cell count. Treatment with MPA in DAL being mice caused a significant ($P<0.01$) decrease in the body weight. This may be due to the cytotoxic effect of these extract. These effects are comparable to that of 5-FU, the standard drug.

Increase in peritoneal cell count is a proof and an indirect evidence for anticancer activity and this method has been used by other workers for anticancer studies [10,11]. This effect is presumed to be due to enhancement and activation of macrophages or through modification of cytokine product in peritoneal cavity. In the same line, the anticancer activity of MPA is evident in our studies by increase in peritoneal cell count induced in normal mice. The common problems encountered in cancer chemotherapy are myelosuppression and anaemia [12,13]. Anaemia occurring in tumor bearing mice is mainly due to reduction in RBC or haemoglobin production, and this may occur either due to iron deficiency or due to haemolytic or other myelopathic conditions [14]. Treatment with MPA brought back the haemoglobin content, RBC and WBC counts to near normal. This indicates that MPA have a protective effect on the haemopoietic system. Further, analysis of haemotological parameters showed minimum toxic effect in mice treated with MPA. In DAL bearing mice, haematological parameters were reversed to normal by MPA administration (9 days).

Cytological studies of ascitic fluid on the 14$^{th}$ day in DAL bearing mice revealed that the tumor cells are large in size showed binucleation. In MPA 200 mg/kg treated animals bearing DAL, the cells showed high n/c ratio, plasmocytoid feature and cytoplasmic vacuolation. In MPA 400 mg/kg treated animals bearing DAL, the cells, showed plasmacytoid feature with varying degree of degeneration, cytoplasmic vacuolation and also showed active mitosis. All these

cytological studies indicate the cytotoxic effect of MPA.

Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids, and can induce lipid peroxidation in vivo[15]. Increased lipid peroxidation causes degeneration of tissue. Lipid peroxide formed in the primary site is be transferred through the circulation and provokes damage by propagating the process of lipid peroxidation.[16] Malondialdehyde, the end product of lipid peroxidation has been reported to be higher in carcinomatous tissue than in non diseased organs [17]. Glutathione, a potent inhibitor of neoplastic process, plays an important role as an endogenous antioxidant system that is found particularly in high concentrations in liver and is known to have key functions in the protective process .

The free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells and their function is to provide a defence against the potentially damaging reactivates of superoxide and hydrogen peroxide[18] reported a decrease in SOD activity in DAL bearing mice which might be due to loss of Mn SOD activity in DAL cells and loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and CAT activities as a result of tumor growth was also reported[14]. Similar findings were observed in the present investigation with DAL bearing mice. Plant derived extracts containing antioxidant principles should cytotoxicity towards tumor cells[19] and antitumor activity in experimental animals[20]. The lowering of lipid peroxidation, and increases in levels of SOD, catalase GST and GPx in MPA treated groups indicates its potential as an inhibitor of DAL induced intracellular oxidative stress. Antitumor activity of these antioxidants is either through induction of apoptosis [21] or by inhibition of neovascularization [22]. The involvement of free radicals in tumors is well documented [23,24].

In DAL -bearing mice, there was a regular and rapid increase in ascitic fluid volume. Ascitic fluid is the direct nutritional source for tumor growth; it meets the nutritional requirements of tumor cells [25]. MPA treatment decreased the volume of solid tumor and the viable cancer cell count, and increased the lifespan. It may be concluded that MPA decreases the nutritional fluid volume and thereby arrests tumor growth and increases the lifespan. There was a reduction in solid tumor volume in mice treated with MPA ($P<0.001$). The present study reveals that the extract was cytotoxic towards DAL.

Plants have played an important role as a source of effective anticancer agents, and it is significant that 60% of currently used anticancer agents are derived from natural sources, including plants, marine organisms, and microorganisms.[26,27] Plant-based medicine has definitely found a role in cancer treatment (chemotherapy), and the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively.[28] In particular, there is growing interest in the pharmacological evaluation of various plants used in, Indian traditional system of medicine.

Plant-derived natural products such as flavonoids, terpenoids, and steroids, etc. have received considerable attention in recent years due to their diverse pharmacological properties, including antioxidant and antitumor activity [29,30]. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infection and degenerative diseases.

Moreover, they have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation [31]. and angiogenesis [32]. All these observations clearly indicate a significant antitumor and cytotoxic effect of the extract of the leaves of MPA.

## Conclusion

The antitumor effect of the extract was evaluated by using survival time, haematological parameters, increase in body weight, solid tumor volume and peritoneal cell count. It was found that the oral administration of MPA increased the survival time and inhibits the weight gain of the tumor bearing mice. After 14 days of inoculation, the extract also reduces the solid tumor volume developed by the EAC cells. The findings of this study indicate that the MPA possesses significant antitumor activity on dose dependent manner.

## Authors Contributions

SPG has done the entire study, includes collection of leaves, extraction, selection and animal studies, cell line studies and analysis of the data.

BR has guided in designing the present study, selecting the animal models, and getting cell lines and help in doing the analysis of the data.

TS, Dean, has helped in designing the study and given valuable suggestions.

## Acknowledgement

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