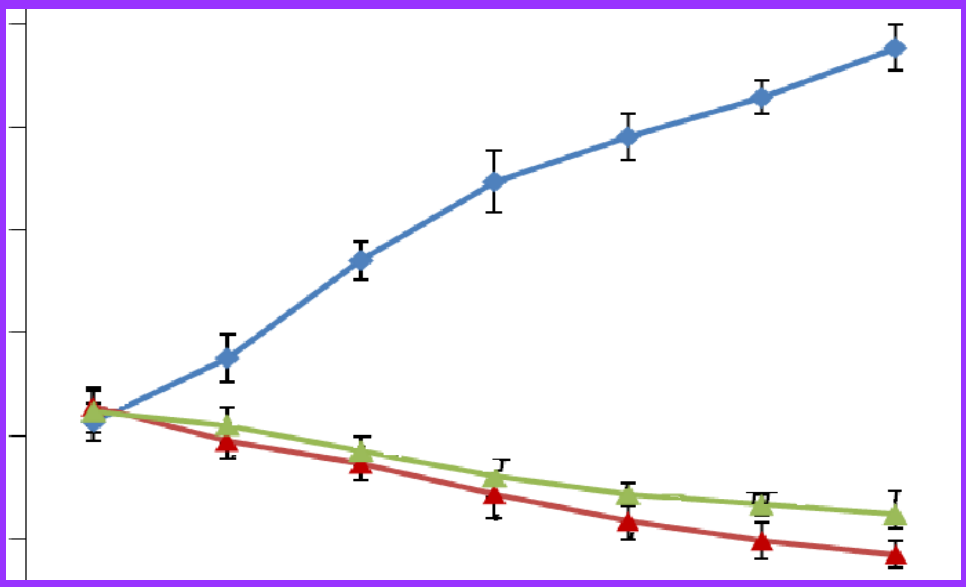

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Research Article

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A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2015; 10: 399-408**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
**ISSN**: 1991-0088

# *In vitro* and *in vivo* evaluation of anti-tumor activity of methanolic extract of *Argyreia nervosa* leaves on Ehrlich ascites carcinoma

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**Article Info**

Received: 21 February 2015
Accepted: 12 April 2015
Available Online: 4 May 2015

DOI: 10.3329/bjp.v10i2.22334

Cite this article:
Sharma B, Dhamija I, Kumar S, Chaudhary H. *In vitro* and *in vivo* evaluation of antitumor activity of methanolic extract of *Argyreia nervosa* leaves on Ehrlich ascites carcinoma. Bangladesh J Pharmacol. 2015; 10: 399 -408.

**Abstract**

The herb of importance like *Argyreia nervosa* has shown wide range of pharmacological activities. Its methanolic extract of *A. nervosa* has been explored against Ehrlich ascites carcinoma (EAC) induced liquid and solid tumor in mice. Liquid and solid tumors were induced by intraperitoneal and subcutaneous transplantation of EAC cells in Balb/C mice. Significant and dose-dependant results are observed when the mice are sacrificed on 15$^{th}$ day for estimation of tumor proliferation, hematological, biochemical and hepatic anti-oxidant parameters. Mean survival time (days) was increased to 36.5 from 20.5 extract treated mice. The extract also showed a decrease ($p<0.001$) in body weight and percentage reduction in tumor volume respectively when it was evaluated in solid tumor induced mice for a period of 30 days. From the result it was concluded that the extract has as a potent anti-tumor activity and that is comparable to 5-fluorouracil.

## Introduction

Many scientific investigations are making best efforts to combat the disease known as cancer/tumor. A tumor, also known as a neoplasm is an abnormal mass of tissue which may be solid or fluid filled, that results when cells divide more than they should or do not die when they should. Pharmacological treatment of tumor can be divided into alkylating agents, antimetabolites, antimitotic, antibiotics, hormones, targeting agents, topoisomerase inhibitors. But all these have side effects like alopecia, myelosuppression, anemia, lymphomas, leukemia, etc. So, plants come out to be resource for new, economic and potentially effective source of drugs for cancer. Herbal plant of botanical name *A. nervosa* or *A. speciosa,* belongs to family- Convolvulaceae. It is a woody climber and is commonly known as Elephant Creeper in English and Samandar-ka-pat, Samundar-sokha, Ghav-patta in Hindi. It has wide range of pharmacological activities (nootropic, aphrodisiac, immunomodulatory, hepatoprotective, anti-oxidant, anti-inflammatory, anti-hyperglycemic, anti-diarrheal, anti-microbial, antiviral, nematicidal, antiulcer, anticonvul-sant, analgesic and central nervous depressant activi-ties) (Galani et al., 2010). The leaves of this plant has been used for several years in Indian traditional medi-cine as an antiphlogistic, emollient, poultices of wounds, ulcers, boils, carbuncles and antitumor (Galani et al., 2010; Joseph et al., 2011; Kirtikar and Basu, 1918).

In the present work, methanolic extract of the leaves of plant *A. nervosa* are used to explore and evaluate effect on Ehrlich ascites carcinoma (EAC) *in vitro* and *in vivo*. To the best of our knowledge, no reports have been published previously in this direction of research.

## Materials and Methods

### *Chemicals*

5-Fluorouracil (5-FU), 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was procured from Sigma Aldrich and was obtained from

Rajiv Gandhi Cancer Institute, Rohini, Delhi; trypan blue from Himedia. Rest of chemicals and reagents were of analytical grade. Kits used for biochemical estimation are serum glutamic oxaloacetate transaminase (SGOT) and serum glutamic oxaloacetate transaminase (SGOT) Kits were from Erba. EAC cell line was obtained from Institute of Nuclear Medicine and Allied Sciences (INMAS), Defense Research & Development Organization (DRDO), Delhi and Tc99m-pertechnate was obtained from Board of Radiation & Isotope Technology (BRIT), Board of Radiation and Isotope Technology (BARC), New Delhi (India).

***Preparation of defatted methanolic extract of A. nervosa***

The fresh leaves were collected from Sunder Nursery, Delhi and authenticated by National Bureau of Plant Genetic Resources (NBPGR), Delhi with the reference number NHCP/NBPGR/2013-19. The leaves were then shade dried and pulverized using a grinder and weighed. The powdered leaves were extracted with petroleum ether using Soxhlet apparatus for 18 hours (per soxhlet) until siphon tubes appeared colorless. The ether residue was further extracted with 90% methanol using Soxhlet apparatus for 18 hours (until siphon tube appeared colorless). The above was then filtered, and solvent was removed by evaporating it at 20°C on a heating mantle and %yield was calculated (Badmus et al., 2010).

***Phytochemical screening of extract***

Phytochemical screening i.e. detection of alkaloids, glycosides, saponins, phytosterols, carbohydrates, resins and tannins etc, of the DMEAN was carried out using standard procedures (Khan et al., 1992).

***Animals***

Seventy two healthy female Balb/c mice of 6-8 week old weighing between 20-25 g were issued from Animal House, Division of Cyclotron & Radiation Sciences (DCRS), INMAS, Delhi. The animals were housed in polypropylene cages under controlled temperature of 24 ± 2°C and relative humidity of 55 ± 10% under 12 hours light/dark cycles. They were fed with standard conventional laboratory pellet diet and free access to distilled water *ad libitum* throughout the study. The experimental protocol was approved by Institutional Animal Ethics Committee, DCRS, INMAS, Delhi.

***Cell line and culture media***

The EAC cells were obtained from DCRS, INMAS, Delhi, India and cultured in 96-well plates with growth medium and 10% fetal calf serum. The EAC cells were maintained *in vivo* in Balb/c mice by intraperitoneal transplantation of 2 × $10^6$ cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7–8 of tumor bearing) of the tumor cells. Each animal received 0.1 mL of tumor cell suspension containing 2 × $10^6$ tumor cells intraperitoneally. Cells were routinely grown in the 25 $cm^2$ culture flasks with loosened caps containing minimum essential medium (MEM) supplemented with 10 percent fetal calf serum and 50 µg/mL gentamicin sulfate at 37°C in an atmosphere of 5% $CO_2$ in humidified air in a $CO_2$ incubator (Kumar et al., 2014a).

***In vitro cytotoxicity assay/MTT assay***

Increasing concentrations of extract (1, 5, 7.5, 10, 15, 20 µg/mL) was added to the cells and incubated at 37°C for 14 hours in $CO_2$ incubator with 5%$CO_2$. After incubation %cytotoxicity of the cells was determined using 20 µL of MTT and was estimated using ELISA plate reader. %Growth inhibition is give by following equation

$$\%\ \text{Growth inhibition}/\%\ \text{cytotoxicity} = \frac{(COD - TOD)}{COD} \times 100$$

Where, COD and TOD are controlled and treated optical densities respectively.

***In vivo cytotoxic activity of extract in EAC inoculated mice (liquid tumor model)***

54 Balb/c mice were divided into five groups (n = 12 except Group I where n = 6) and given food and water *ad libitum*. Group I served as normal control and was given 10% v/v DMSO (4 mL/kg i.p.). Rest of the groups received EAC cells (2 × $10^6$ cells/mouse i.p.). This was considered as day '0'. Group II served as EAC control i.e. no treatment. Group III and Group IV received extract at a dose of 200 and 400 mg/kg i.p. after 24 hours of EAC transplantation, for fourteen consecutive days, respectively. Group V received standard drug 5-FU (20 mg/kg i.p) for fourteen consecutive days. After last dose 6 mice from each group (except Group I) were kept on fasting for 18 hours and then blood was collected by cardiac puncture for biochemical and hematological estimation and the rest 6 mice from each group was kept to calculate % increase in life span (% ILS) and Mean survival time (MST). The antitumor activity of extract was measured in liquid tumor induced animals with respect to the following parameters.

***%Cell viability***

On 15$^{th}$ day, after an overnight fasting, the viability and non viability of the cell were checked by trypan blue assay as per the method given in the literature (Jung-Hynes et al., 2011). These viable cells were counted and %cell viability was calculated as given below

$$\%\ \text{Cell viability} = \frac{\text{Total viable cells (unstained)}}{\text{Total cells (stained + unstained)}} \times 100$$

***Mean survival time (MST) and percentage increase in life span (% ILS)***

The effect of the extract on percentage increase in life span was calculated on the basis of mortality of the experimental mice (Bala et al., 2010; Kumar et al., 2014a).

$$\% \text{ ILS} = \left[\frac{\text{MST}_T}{\text{MST}_C} - 1\right] \times 100$$

Where, $MST_T$ is mean survival time (days) of the extract treated group and $MST_C$ is mean survival time of EAC control group whereas MST was calculated by dividing total number of days animals survived to total number of animals in the experiment (Dhamija et al., 2013).

***Hematological parameters and biochemical estimations***

On 15th day, after an overnight fasting, six animals from each group were anesthetized and blood was collected by cardiac puncture. The blood was collected in EDTA coated vials and was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count, and white blood cell (WBC) count by standard procedures.

Serum was immediately separated from blood after centrifugation, blood at 5,000 rpm for 15-20 min. Serum separated was used for the estimation of biochemical parameters like SGOT, SGPT using Erba Kits (Haldar et al., 2010).

***Anti-oxidant parameters and total protein count***

The liver was excised, rinsed in ice-cold normal saline followed by cold 0.15 M Tris HCl (pH 7.4), dried and weighed. A 10% w/v homogenate was prepared in 1.15% KCl. Homogenate was utilized for estimation of glutathione reductase (Ramesh et al., 2012), nitric oxide (Hendra et al., 2011) and total protein count (Kumar et al., 2013a; Kumar et al., 2013b).

***In vivo cytotoxic activity of DMEAN in EAC inoculated mice (solid tumor model)***

Each animal was given 0.1 mL tumor cell suspension containing $2 \times 10^6$ tumor cells subcutaneously in right hindlimb (thigh). 18 Balb/c mice were divided into five groups (n = 6) and given food and water *ad libitum*. All the animals in each groups received EAC cells ($2 \times 10^6$ cells/mouse i.p.) This was considered as day '0'. Group -VI served as EAC control and was given 10% v/v DMSO. 24 hours after EAC transplantation, Group VII and Group VIII received extract (400 mg/kg i.p) and standard drug 5-FU (20 mg/kg i.p) for fourteen consecutive days, respectively. The anti-tumor activity of extract was measured in solid tumor induced animals with respect to the following parameters.

***Body weight***

Body weight of all the mice was measured using digital balance on the day of transplantation i.e. day 0, and then every 5th day for a period of 30 days (Sreelatha et al., 2011).

***%Reduction in tumor volume***

Tumor mass was measured using digital vernier caliper from the 11th day of transplantation. The measurement was carried out every 5th day for a period of 30 days (Banerjee et al., 2001; Kumar et al., 2014b) and % reduction in tumor volume (R) was calculated using following formula

$$\% R = \left(\frac{V_c - V_t}{V_c}\right) \times 100$$

Where,

$$r = \frac{(r_1 + r_2)}{2} \quad V = \frac{4}{3}\pi r^3$$

$V_c$ and $V_t$ are tumor volume of EAC control group and treated group respectively. Volume was determined by general formula of sphere as

where, $r_1$ and $r_2$ are radius from two perpendicular axis of tumor.

***Statistical analysis***

All data are expressed as mean ± S.E.M. (n = 6 mice per groups). Data was analysed by software GraphPad Prism 5.0. Parameters MST, %ILS, %cell viability were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test and the parameters hematological, biochemical, body weight, %reduction in tumor volume, anti-oxidant, total protein count were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Post hoc test. All p values reported are two-tailed and $p<0.05$ was considered significant.

## Results and Discussion

The %yield of defatted methanolic extract was found to be 26.4% w/w. The extract was found to contain flavonoids, glycosides, alkaloids, saponins, diterpenes and phytosterol (Table I).

The results of trypan blue dye exclusion assay demonstrated that the viable cell count decreased with an increased count of non-viable cells by the extract treatment. These results could indicate either a direct tumorigenic effect of extract on tumor cells or an indirect effect (Dhamija et al., 2013; Zandi et al., 2013).

In the assay for *in vitro* cytotoxicity study, the extract showed direct cytotoxic effect on the EAC cell line in a concentration-dependent manner with 0.956 coefficient of correlation i.e. $R^2$ (Figure 1). The 20 µg/mL

concentration produced 65.2% cytotoxicity whereas 1 µg/mL extract showed 3.1% cytotoxicity. The $IC_{50}$ value of the extract against EAC cell lines was calculated from the slope of the direct relation between concentration and %cytotoxicity (Figure 1) and was found to be 17.0 µg/mL.

The acute toxicity study was carried out as per the guidelines of the organization for economic co-operation and development-423) (OECD, 2001; Schlede et al., 2005). The maximum dose of 2000 mg/kg did not produce any mortality and toxic symptoms. So, for further studies 1/10$^{th}$ and 1/5$^{th}$ of the maximum dose (2,000 mg/kg p.o.) values were taken as treatment dose (Sridharan et al., 2012) i.e. 200 mg/kg i.p. and 400 mg/kg i.p. values were taken as treatment dose.

Intraperitoneal administration of the extract at the dose of 200 mg/kg and 400 mg/kg decreased the %cell viability significantly from 92.5% of EAC control to 34.7 and 25.0% to extract 200 and 400 mg/kg, respectively. Although the mean %cell viability was better in standard (5-FU 20 mg/kg) i.e. 17.8% but was statistically equivalent to extract 400 mg/kg dose (shown by the presence of 'ns'). %Cell viability values for the extract 400 mg/kg was found to be significantly better when compared to the extract 200 mg/kg (Figure 2).

MST was increased significantly from 20.5 to 36.5 days as compared to that of EAC control mice while standard drug 5-FU was showing MST to be 40.5 days as shown in Figure 3A. Similar to that of MTT assay,

**Table I**

**Preliminary phytochemical screening of *Argyreia nervosa***

| | Test | Result |
|---|---|---|
| Phytosterols | Salkowaski reaction | Present |
| | Liberman's burchard reaction | |
| | Liberman's reaction | |
| Glycoside | Keller killani test | Present |
| | Borntrager test | |
| | Legal test | |
| Carbohydrate | Fehling's test | Absent |
| | Benedict's test | |
| Alkaloids | Mayer's test | Present |
| | Wagner's Test | |
| Tannins | 5% $FeCl_3$ | Absent |
| | Dilute $HNO_3$ | |
| Proteins | Millon's test | Absent |
| | Xanthoprotein test | |
| Amino acid | Ninhydrin test | Absent |
| | Million reagent test | |
| Flavonoids | Lead acetate | Present |
| | Sodium hydroxide | |
| Saponins | Froth test | Present |
| | Foam test | Present |
| Diterpenes | Copper-acetate test | Present |
| Fats and fixed oils | Stain test | Absent |
| Resins | Acetone-water test | Absent |

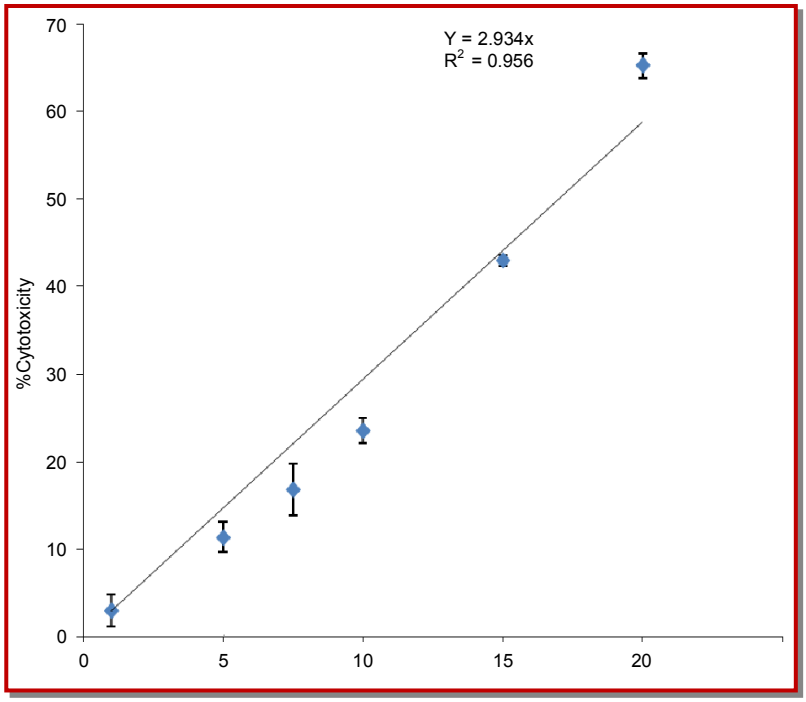


Figure 1: %Cytotoxicity of the extract of *A. nervosa* at various concentrations in MTT assay
Results are presented in form of mean ± SD with n=3

**Table II**

**Effect of extract of *Argyreia nervosa* on hematological count and biochemical, antioxidant and total protein levels**

| Parameters | Group | | | | |
|---|---|---|---|---|---|
| | Vehicle control | EAC control | 5-Flourouracil (20 mg/kg) | Extract (200 mg/kg) | Extract (400 mg/kg) |
| Hb (g/dL) | 12.2+ 0.7 | 4.1+ 0.5[a] | 11.7 + 0.9[b] | 9.9 + 0.7[b] | 10.8 + 0.8[a] |
| RBC count ($10^6$ cells/µL) | 6.1 + 0.4 | 3.4 + 0.2[a] | 5.3 + 0.3[b] | 4.3 + 0.3[a,b] | 4.8 + 0.4[a] |
| WBC count ($10^3$ cells/µL) | 5.6 + 0.4 | 11.0 + 0.6[a] | 5.2 + 0.5[b] | 8.1 + 0.4[a,b,c] | 6.2 + 0.4[a] |
| SGOT (IU/L) | 43.2 + 1.8 | 111.1 + 2.2[a] | 45.0 + 1.7[b] | 75.1 + 2.4[a,b,c] | 55.1 + 1.8[a,b,c,d] |
| SGPT (IU/L) | 34.6 + 1.2 | 76.2 + 2.0[a] | 41.0 + 1.28[a,b] | 62.3 + 1.6[a,b,c] | 43.5 + 1.4[a,b,d] |
| reduced GSH (µM/mg wet liver tissue) | 28.1 + 1.2 | 8.7 + 0.6[a] | 28.0 + 1.09[b] | 21.3 + 0.8[a,b,c] | 25.0 + 0.9[b] |
| NO (µM ) | 24.2 + 0.9 | 33.8 + 0.9[a] | 25.4 + 0.8[b] | 30.4 + 0.9[a,b,c] | 28.1 + 0.9[a,b] |
| Protein (g/dL) | 1.9 + 0.5 | 8.1 + 0.9[a] | 3.5 + 0.8[b] | 5.9 + 0.8[a,b,c] | 4.0 + 0.8[a,b,d] |

All values are Mean + S.E.M., (n= 6); 'a' represent p<0.05 as compared to vehicle control, 'b' represents the p<0.05 as compared to EAC control, 'c' represented the p<0.05 as compared to 5-FU i.e standard, 'd' represented the p<0.05 as compared to extract (200 mg $kg^{-1}$), were analysed by one-way ANOVA followed by Tukey's Post hoc test. EAC= Ehrlich ascites carcinoma, extract = defatted methanolic extract of *A. nervosa*, 5-FU= 5-Flourouracil, NO= nitric oxide

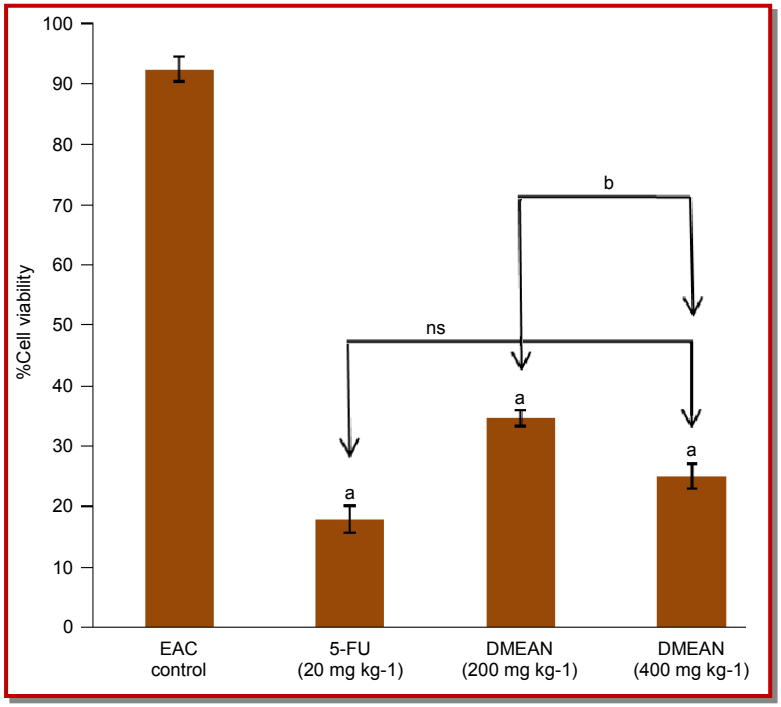


Figure 2: Effect of extract of *A. nervosa* on percentage cell viability

EAC= Ehrlich ascites carcinoma; DMEAN= Defatted methanolic extract of *Argyreia nervosa;* 5-FU= 5-Flourouracil. All values are mean + S.E.M., (n= 6); analyzed by one way ANOVA followed by Tukey's test; [a]represent p<0.05 as compared to control, 'ns' means non significant and [b]represent p<0.05 as compared to extract 200

again the extract (400 mg/kg) was showing statistically equivalent effect to standard drug (5-FU, 20 mg/kg) (represented by 'ns'). Values for MST for extract 400 mg/kg was found to be significantly better than extract 200 mg/kg values (Figure 3A).

Similarly, percentage increase in life span (%ILS) was observed (97.6, 46.3 and 75.6% for 5-FU 20 mg/kg, extract 200 mg/kg and extract 400 mg/kg treatment, respectively) in comparison to EAC control as zero % ILS (Figure 3B).

A pre-clinical study conducted on EAC induced liquid tumor mice using an ethanolic extract from *A. nilagirica*

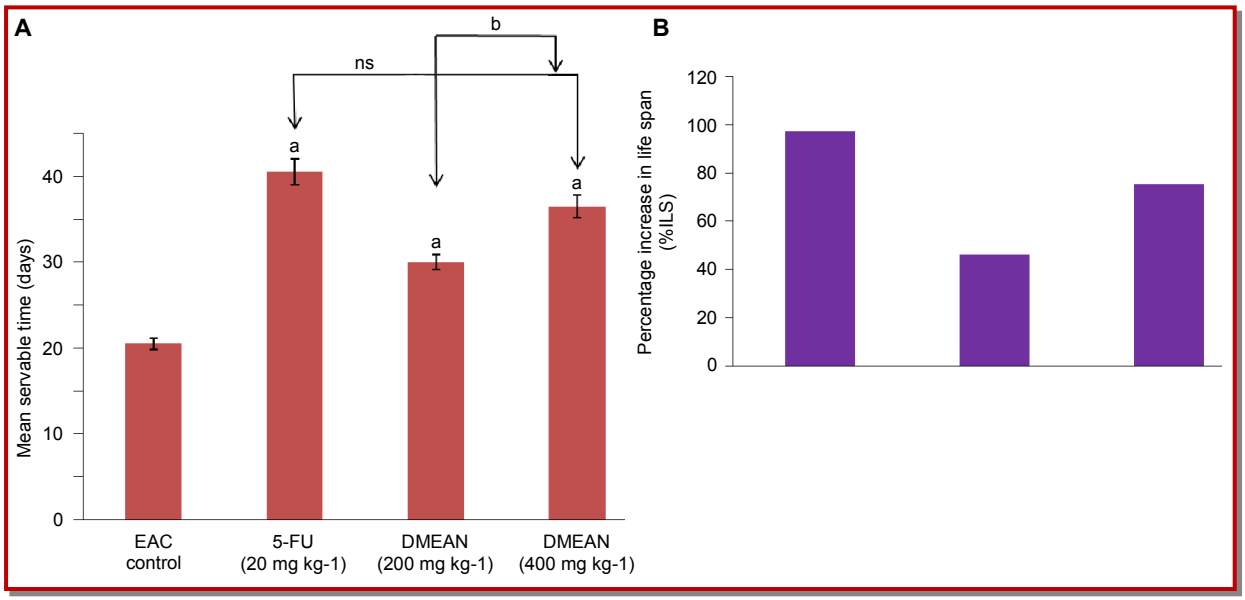


Figure 3: Effect of extract of *A. nervosa* on (A) mean survival time and (B) percentage increase in life span

EAC control is not shown as it was taken as zero %ILS in its calculation; DMEAN= Defatted methanolic extract of *Argyreia nervosa;* 5-FU= 5- Flourouracil; All values are mean ± S.E.M.; (n= 6); analyzed by one-way ANOVA followed by Tukey's test; [a]represent $p<0.05$ as compared to control, 'ns' means non-significant and [b]represent $p<0.05$ as compared to extract 200

revealed that viable cell count was found to be significantly reduced in mice treated with 200 mg/kg and 400 mg/kg (7680 and 6760 cell/mL respectively) dose when compared to viable cell count in EAC control mice (11,180 cells/mL) (Perumal et al., 2010).

A study related to clinical research also used an extract from plant *Viscum album* in patients recognized as advanced or metastatic local pancreatic cancer. The therapy of extract showed a clinically and statistically significant prolongation of survival time of patients (2.7 to 4.8 months) along with least toxicity and thus it is strong and effective second-line therapy in the concerned cancer type (Kienle and Kiene, 2010).

The level of Hb, RBCs count, Level of reduced glutathione was decreased while WBCs count, level of SGOT, SGPT and nitric oxide was increased very significantly ($p<0.05$) (Table II). 200 and 400 mg/kg extract treated animals brought all these parameters near to baseline i.e. normal level of vehicle control. Standard 5-FU (20 mg/kg) showed similar pattern for normalization of above mentioned parameters at 20 mg/kg dose and was more effective. The treatment brought the level to normal level. 5-FU standard drug was showing normal level in each parameter except (level of SGPT) while the extract 400 mg/kg was showing effect equivalent to normal level in some parameters. Every treatment (standard and tests) was showing significant effect as compared to control in each parameters (Table II). Absence of '$' sign showed the equivalence between test and standard drug. Extract 400 mg/kg showed equivalent effects as compared to standard (5-FU, 20 mg/kg) to normalize the parameters (except level of SGPT) while extract 200 mg/kg was effect significantly lower than the standard except in level of Hb and RBCs count where the effects were equivalent to standard. The SGOT, SGPT and total protein values for extract 400 mg/kg were found to be statistically better than compared to extract 200 mg/kg in rest of parameters extract 200 mg/kg was equivalent to extract 400 mg/kg.

Chemotherapy in cancer causes anemia due to reduction in RBCs count (Bala et al., 2010) so treatment should enhance the RBCs level to normal. In this study the treatment with standard and extract treatment group, both showed significant increase in RBCs count. One of reliable criteria for judging the value of any anticancer drug is decrease the WBC level to normal (Bala et al., 2010). The WBC level decreased when compared with the EAC control, indicating that extract possess less toxic effect on hematological system.

Serum enzymes have been studied as both early possible indicators of neoplasia and as an aid in following the progression and regression of the disease. In certain circumstances they can be carcinogenic and may endanger hepatotoxicity. In the present study, results of the experiment concluded that EAC control group exhibited raised levels of liver enzymes such as SGOT and SGPT. Treatment with extract restored the elevated biochemical parameters almost within the normal range indicating the protection against tumor cell induced hepatotoxicity (Kathiriya et al., 2010; Saha et al., 2011). Neoplastic growth has been found to co-exist with impairment in the endogenous anti-oxidant status (Dhamija et al., 2014). Depletion in GSH content

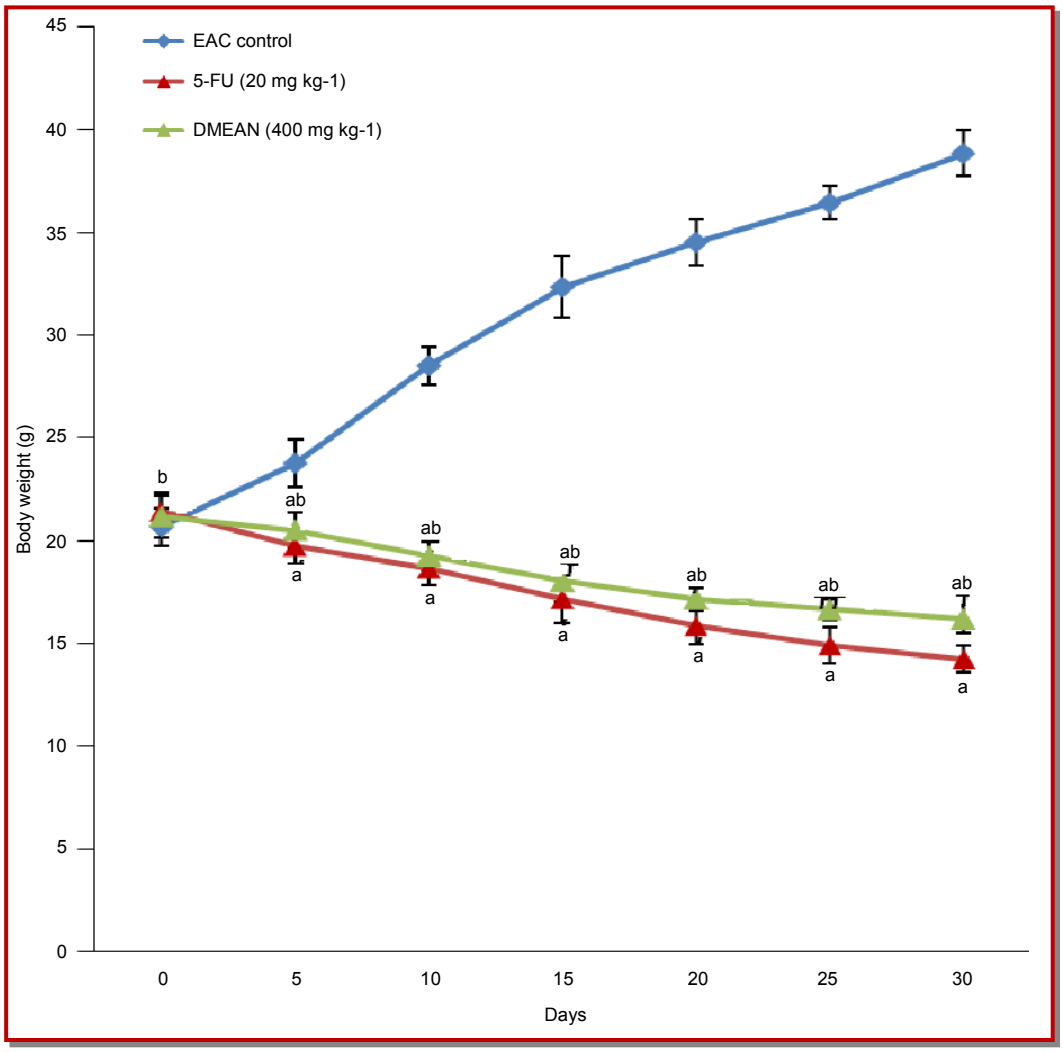


Figure 4: Effect of extract on body weight (g)

All values are mean ± S.E.M., (n= 6); #represent p<0.05 as compared to control and *represent p>0.05 (i.e. non significant) as compared to standard and analyzed by one-way ANOVA followed by Tukey's Post hoc test; EAC= Ehrlich ascites carcinoma; DMEAN= Defatted methanlic extract of *Argyreia nervosa;* 5-FU= 5-Flourouracil

was found to be associated with impaired immune response and increased risk of malignancy. Also GSH is a potent inhibitor of neoplastic proliferation process, so lowered glutathione content was reported in human cancer cell lines (Saha et al., 2011). The depleted reduced GSH may be due to reduction in its synthesis or to its degradation by oxidative stress in EAC bearing animals. Extract treatment significantly increased the reduced hepatic glutathione content in tumor bearing mice in a dose-dependent manner.

Nitric oxide (NO) is an important regulator of tumor growth and involved in various pathophysiological process includes inflammation and carcinogenesis (Hong et al., 2002). Treatment with extract reduces the level of Nitric oxide production in liver tissue, when compared with EAC control animals significantly. More significant decrease in NO level was observed in 400 mg/kg extract treated animals. The present study demonstrates that the protein levels were high in EAC control mice as the number of cells in EAC control mice was high. The administration of extract reduces the protein count in dose-dependent manner.

A pre-clinical study was conducted using ethanolic extract of *Curcuma zedoaria* against B16F10 melanoma cell line revealed that the ethanolic extract returned decreased and increased levels of RBCs and WBCs respectively to the normal levels (Carvalho et al., 2010).

A study conducted on DAL induced liquid tumor mice using methanolic extract of *Acacia nilotica* showed that the levels of SGOT & SGPT were significantly ($p<0.01$) reduced as compared to the tumor control groups. The cellular nitric oxide level in the DAL cells was found to be reduced significantly from 13.1 ± 0.5 µM in EAC

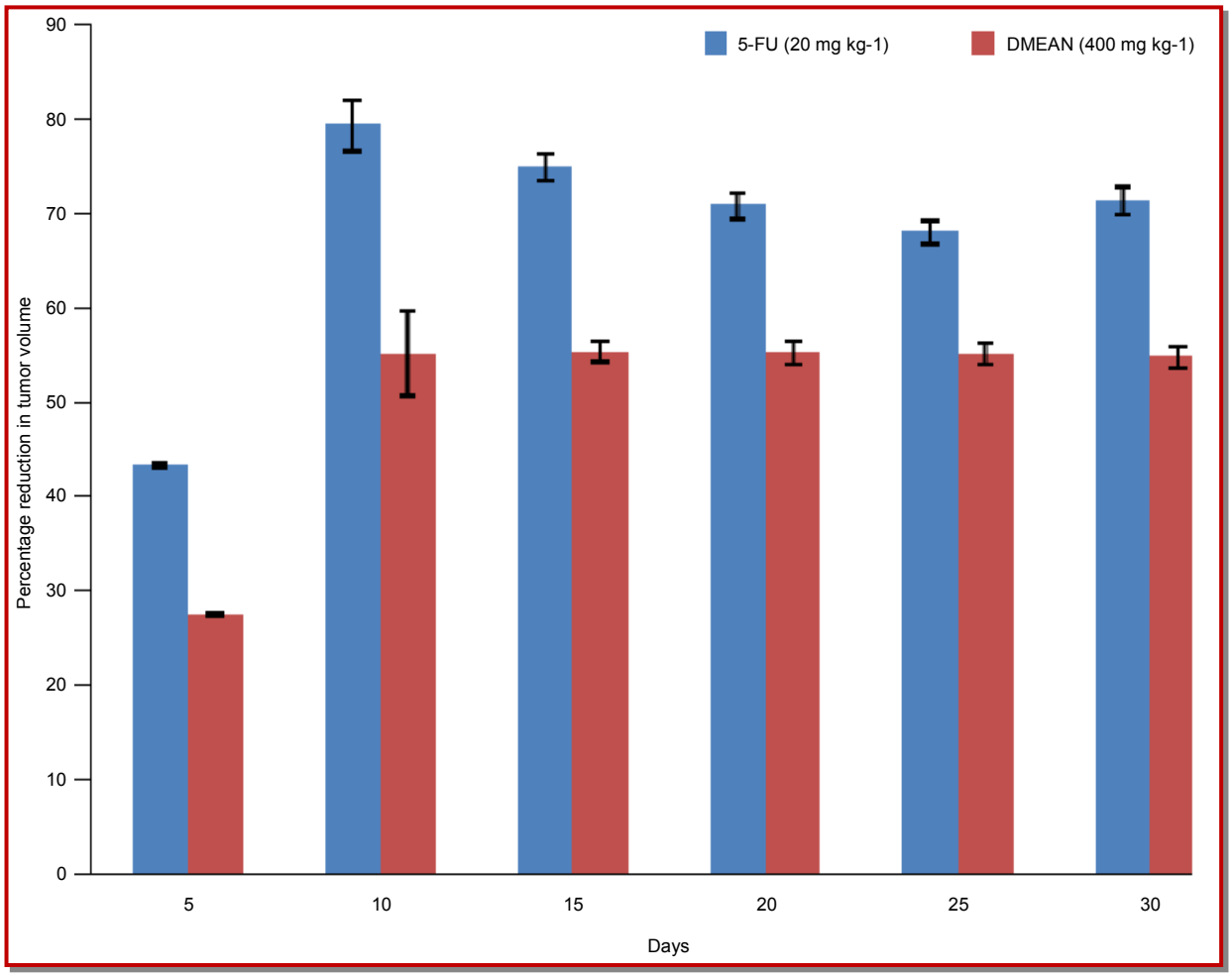


Figure 5: Effect of extract on percentage reduction in tumor volume

The values are mean ± S.E.M.; (n=6); DMEAN= Defatted methanolic extract of *Argyreia nervosa;* 5-FU = 5-Flourouracil

DAL induced mice to 8.9 ± 0.8 µM in extract treated mice (Sakthivel and Guruvayoorappan, 2013).

The study on liquid tumor showed that extract 400 mg/kg has more significant result which were comparable to standard drug i.e. 5-FU so extract 400 mg/kg dose was used for further study on solid tumor bearing mice. EAC control animals showed a significant increase in body weight from day 0 to day 30 as compared to extract and 5-FU treated animals which showed a significant decrease in body weight from day 0 to 30. Extract and 5-FU treated animals showed significant decrease in the body weight during all the days when compared to EAC control animals, which showed a significant increase in the body weight (represented by #). The body weight values for extract 400 mg/kg was found to be significantly better than 5-FU 20 mg/kg (Figure 4). Body weight is an indirect parameter of health index of animals and recovery from the disease (Morton and Griffiths, 1985). Significant loss of body weight was observed in extract treated groups when compared with EAC control groups.

In EAC control mice, there was a regular and rapid increase in tumor volume. Significant %reduction in tumor volume was observed in extract treated group when compared to EAC control group. This inhibitory effect on body weight and %reduction in tumor volume suggests that the effect of extract is systemic and not only related to its local cytotoxic effect. 5-FU treated animals also showed an increase in %reduction in tumor volume (Figure 5).

A preclinical study performed against DAL cell lines induced solid tumor using extract of *Acacia ferruginea* showed significant decreased in body weight and tumor volume when compared with DAL control mice (Sakthivel and Guruvayoorappan, 2013).

## Acknowledgement

The significant results at the extract 400 mg/kg dose suggest that the plant *A. nervosa* has the potential as anticancer. Thus it may provide a novel compound for the concerned disease after studies on isolation and

characterization of pure compounds involved.

## Acknowledgement

One of author (Bhawna Sharma) acknowledge the College of Pharmacy, PDM Group of Institution, Bahadurgarh and DCRS, INMAS, DRDO for providing necessary academic and research facilities, respectively.

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