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Original Research

Cytotoxic and antiproliferative activity of *Securidaca longepedunculata* aqueous extract on Ehrlich ascites carcinoma cells in Swiss albino mice.

Lawal RA[1*], Ozaslan MD[2], Odesanmi OS[1], Karagoz ID[2], Kilic IH[2], Ebuehi OAT[1]

[1]*Department of Biochemistry, College of Medicine, University of Lagos, Lagos, Nigeria.*
[2]*Department of Biology, University of Gaziantep, 27310 Gaziantep, Turkey.*

Summary: *Securidaca longepedunculata* is a savannah shrub found growing in tropical Africa. It is reputed to have more than a hundred medicinal uses and is a major component of anticancer decoctions in Nigeria. An attempt was made in this study to determine the *in vitro* and *in vivo* cytotoxic activity and possible pro-apoptotic effect of *Securidaca longepedunculata* aqueous root bark extract on Ehrlich ascites carcinoma cells. *In vitro* cytotoxic activity was determined using the Trypan blue assay by incubating Ehrlich ascites carcinoma cells with various concentrations of *Securidaca longepedunculata* aqueous extract. *In vivo* study was carried out by intraperitoneal administration of varied doses of *Securidaca longepedunculata* to tumour-bearing mice. Isolated DNA from Ehrlich ascites carcinoma cells in treated and untreated animals was used for DNA fragmentation assay on agarose gel. *Securidaca longepedunculata* Aqueous extract, *Securidaca longepedunculata* was cytotoxic to Ehrlich ascites both *in vivo* and *in vitro*. The IC_{50} of *Securidaca longepedunculata* was 67 µg/ml. *Securidaca longepedunculata* caused a decrease in angiogenesis as observed in the reduction in weight of treated animals and a reduction in volume of ascitic fluid in treated mice. DNA fragmentation assay of Ehrlich ascites carcinoma cells from treated animals depicted a possible pro-apoptotic effect of the *Securidaca longepedunculata* extract due to the ladder forming pattern which was comparable to that of the standard drug (fluorouracil). *Securidaca longepedunculata* aqueous extract had a cytotoxic and pro-apoptotic effect on Ehrlich ascites carcinoma cells.
Industrial relevance: The use of *Securidaca longepedunculata* in traditional medicine in the treatment and management of cancer has been brought to the fore. Development of herbal drugs from the crude extracts could be achieved due to findings suggesting the plant could increase life span in patients with advanced stages of cancer. Herbal supplements could be produce from the plants with low concentration of the extract. Chemotherapeutic applications of the plant also include its potential inclusion in drug development based on the pro-apoptotic potential of the plant.
Keywords : *Securidaca longepedunculata*; cytotoxic; Ehrlich; antiproliferative; fluorouracil; mice.

INTRODUCTION

Chemotherapy is regarded as a major treatment modality for the control of advanced stages of malignancies. Medicinal plants are used in various countries in the treatment and prevention of cancer (Madhuri and Pandey, 2009). Over the years, researchers have focused on the anticancer activity of plants (Saluja *et al.*, 2011; Muthuraman *et al.*, 2008; Sowemimo *et al.*, 2007). Medicinal plants have been known to be good sources of effective anticancer drugs (Cragg and Newman 2005) such as Taxol, vincristine and camptothecin. Despite the development of new drugs, cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year (Abdullaev *et al.*, 2000). Hence, the need to search for new drugs that could prolong the life span of patients. Researchers have recently focused on the use of Ehrlich ascites carcinoma cells in the investigation of plants reported to cure cancer locally (Gupta *et al.*, 2004; Zumrutdal *et al.*, 2008; Bromberg *et al.*, 2012).

Ehrlich ascites carcinoma cells are transplantable tumours used in the testing of anticancer drugs in mice (Thippeswamy and Salimath, 2006; Agrawal *et al.*, 2011; Ozaslan *et al.*, 2011).

Several natural compounds have been reported to induce apoptosis in Ehrlich tumor cells *in*

vivo and *in vitro*. The Swiss albino mice, *Mus musculus* used in this study are timid, social and territorial animals. Swiss albino mice are genetically diverse within a strain, but the genetics of a strain is maintained as a constant. They are rarely aggressive when handled properly and have been used in a lot of experiment (Harkness and

*Corresponding Author.
✉ rilwan_y2k@yahoo.com
☎ +234-8056036852
Available online http.//www.ijarnp.org

Wagner 1995). *Securidaca longepedunculata* is a savannah shrub used locally in Nigeria for a variety of medicinal functions (Dapar *et al.*, 2007). Reported use of the plant in literature include against snakebites, fish poisoning and in different diseases (Olajide *et al.*, 1998; Burkill 1997; Neuwinger, 1996; Odebiyi 1978), anti-rheumatic and anti-inflammatory antihelminthic activity (Neuwinger, 1996) and bacterial and malarial chemotherapy (Msonthi 1986; Akinniyi *et al.*, 1996). The plant has also been shown to cause tissue damage in animals administered doses of the extract intraperitoneally (Dapar *et al.*, 2007).

This research aims to evaluate the *in vivo* and *in vitro* anti-tumour potential of *Securidaca longepedunculata* using Ehrlich Ascites Tumour cells.

MATERIALS AND METHODS

Trypan blue was obtained from Bio-tech Pvt Ltd (India), 5-Fluorouracil from KOCAK FARMA (TURKEY) was used as a standard drug based on previous literature significantly enhanced the life span in EAC tumors (Muthuraman *et al.*, 2008). All other chemicals used in this study were of analytical grade available locally.

Plant collection. *Securidaca longepedunculata* root barks were collected from Osogbo, South-West, Nigeria. The plant material was authenticated and identified by Mr Odewo in the Department of Botany, University of Lagos. A voucher specimen was deposited in the University Herbarium, University of Lagos, Lagos, Nigeria with voucher number: LUH 3593.

Preparation of aqueous extract. The plant materials were shade dried for 3 days and pulverized into powder. Aqueous extract of the coarsely powdered material was by macerating 1kg of root bark in 1 L of distilled water for 72 hours. The marc was filtered and the filtrate was concentrated using the Rotary Evaporator and further concentrated to constant weight *in vacuo* using a lyotrap.

In vitro EAC cell culture. Ten days after inoculation of EAC cells in the abdominal cavity of mice, the cells were isolated by needle aspiration, washed in saline, and the erythrocytes removed with a lysing solution. Ascitic tumour cell counts are done in a Cell Counting machine (Cedex, Roche) using the trypan blue dye exclusion method. Cell viability was > 95%. Tumour cell suspensions were prepared in phosphate balanced salt solution (PBS) at pH 7.4 to final concentrations of 1×10^6 viable cells ml^{-1} (Zumrutdal *et al.*, 2008).

In-vitro Cytotoxicity. In-vitro Cytotoxic activity was carried out using the Trypan Blue cytotoxic assay. Briefly, aqueous extract of *Securidaca longepedunculata* was used for the preparation of the stock solution (1000 µg/ ml) in Phosphate buffered saline. Serial dilutions (100, 10, 1 and 0.1 µg/ml of sample solution) were prepared in PBS. 200µl of sample solutions were placed in tubes. The volume in all the tubes was made up to 800 µl with PBS (Phosphate buffered saline). 100µl of EAC with a concentration of 10^6 cells/ml of Phosphate buffered Saline was added to the tubes. A control having solvent alone was also prepared. Incubate at 37^0C for 3 hours and add 100 µl of trypan blue to all test tubes. Ascitic tumour cell counts are done in a Cell Counting machine (Cedex, Roche) using the trypan blue dye exclusion method. Results were expressed as Percentage Cell viability (Saluja *et al.*, 2011).

Animals. Adult Swiss male albino mice (26-33 g) were procured from University of Gaziantep, Turkey and used throughout the study. They were housed in prophylene cages in a controlled environment (temperature 25±2°C and 12 h dark and light cycle) with standard diet and water *ad libitum*. The animal experiments were carried out in accordance with the Institutional Protocols of Animal Care. The animal experiments were carried out in accordance with the Institutional Protocols of Animal Care. The study was conducted after obtaining institutional animal ethic committee clearance of the University of Gaziantep (Ethical Number: 05/2012-13).

EAC Cell Culture. Ehrlich Ascites Carcinoma cells were obtained courtesy of Professor (Dr.) Mehmet Ozaslan, Turkey. They were maintained by weekly *intra peritoneal* inoculation of 10^6 cells/mouse (Ramakrishna et al*.,* 1984). Ascitic tumour cell counts were done in a Cell Counting machine (Cedex, Roche) using the trypan blue dye exclusion method. Cell viability was > 95%. Tumour cell suspensions were prepared in phosphate balanced salt solution (PBS) at pH 7.4 to final concentrations of 1×10^6 viable cells ml^{-1}. Mice were given *intra peritoneal* (i.p.) injection of 1×10^6 viable tumour cells per mouse in a volume of 0.2 ml (Justo *et al.*, 2000).

In-vivo Study. Animals were inoculated with 1 x 10^6 cells/mouse on day '0' and treatment with *intra peritoneal Securidaca longepedunculata* extract started 24 h after inoculation, at a dose of 10, 20 and 40 mg/kg/day. The control group were treated with same volume of 0.9% sodium chloride solution. All the treatments were given for 14 days. Mortality was recorded daily. The mean survival time (MST) of each group, consisting of 5 mice was noted. The antitumor efficacy of SL was compared with that of 5-fluorouracil (5-FU, 20 mg/kg/day, *i.p* for 14 days).

The effect of SL on percentage increase in life span was calculated on the basis of mortality of the experimental mice (Sur and Ganguly 1994).

Mean survival time and Percentage Increased Life Span (% ILS) was calculated using the following equation (Mazumder *et al.,* 1997; Gupta *et al.,* 2000):

$$\text{MST (days)} = \frac{\sum \text{Survival time (days) of each mouse in a group}}{\text{Total number of mice}}$$

$$\text{ILS (\%)} = \frac{\text{MST of treated group}}{\text{MST of control group}} \times 100$$

Ascite Volume. The ascitic fluid from the peritoneal cavity of tumour bearing mice was quantitatively isolated by peritoneal lavage after death into graduated eppendorf tubes and measured (Prakash *et al.*, 2011).

Ascite Weight (Biswas *et al.*, 2010). The mice were dissected for collecting ascitic fluid from peritoneal cavity. The ascetic fluid was carefully collected with the help of 5 mL sterile syringe into pre-weighed eppendorf tubes. The ascites weight was calculated using the formula;

Ascite Weight =
Final Weight of Eppendorf – Weight of pre-weighed eppendorf

Ehrlich Packed Cell Volume (Biswas *et al.*, 2010). The mice were dissected for collecting ascitic fluid from peritoneal cavity. 1 of the ascite fluid of the transplantable murine tumor was carefully collected with the help of 5 mL sterile syringe. The fluid was subsequently transferred to a graduated glass centrifuge tube and centrifuged at 1000 rpm for 5 min. The fluid volume was measured. Ehrlich packed cell volume was determined using the following formula;

$$\text{Ehrlich Packed Cell volume (\%)} = \frac{\text{1- volume of fluid}}{1} \times 100$$

Blood Packed Cell Volume (Biswas *et al.*, 2010). 1 ml of blood collected from mice via left ventricular cardiac puncture was centrifuged at 2500 rpm for 10 minutes. The Packed Cell volume was calculated by using the following formula

$$\text{Blood PCV (\%)} = \frac{1 - \text{volume of liquid}}{1} \times 100$$

Antitumor activity. Male Swiss albino mice are divided into 6 groups (n = 5). All the groups were injected with EAC cells (0.2 ml of $1x10^6$ cells/mouse) intraperitoneally (Gupta *et al.,* 2004) except Group I. This was taken as day Zero. Twenty (24) hours after inoculation, animals start receiving daily *intra peritoneal* administration of different concentration of *Securidaca longepedunculata* extract.

Group I - Normal control.

Group II - Disease Control, EAC cell line ($1x10^6$ cell mouse).

Group III - EAC cell line ($1x10^6$ cells) treated with 10 mg/kg i.p SL.

Group IV - EAC cell line ($1x10^6$ cells) treated with standard [5- flurouracil (20 mg/kg i.p.)]

After 14 days of treatment, animals from each group were sacrificed by ether anesthesia. The total number of tumour cells in the peritoneal cavity was counted by the trypan blue exclusion method (Bromberg *et al.*, 2012) using the Cedex counter (Kavimani and Manisenthil kumar 2000).

DNA isolation from ehrlich ascites carcinoma cells. The EAC cells collected from treated and untreated animals were used for DNA fragmentation assay using the modified method of Jun-ya UEDA *et al.*, 2002 as described briefly. Cells were washed twice in 800 µL of PBS and pelleted. Pelleted cells were lysed in 600 µL of Lysis buffer (10 mM Tris-HCl buffer, pH 8.0, 10 mM EDTA and 0.2% Triton X-100) for 10 minutes on ice. The lysate was centrifuged at 6000 rpm for 20 mins. The supernatant was then extracted with 1000 µL of PCIAA (Phenol – chloroform – Isoamyl alcohol solution, 25:24:1). The mixture was then centrifuged at 6000rpm for 20 mins and the upper layer decanted off and precipitated with 50 µL of 3M NaCl and 1000 µL of cold ethanol at -20^0C overnight. After drying, the isolated DNA was dissolved in TE buffer. Contamination by RNA was eliminated by incubation with 40 units of RNase at 37^0C for 30 minutes.

DNA fragmentation assay on 2% agarose gel. Loading buffer was added, and (fragmented) DNA electrophoresed on 2% agarose gel in TBE (40 mM Tris, 20 mM Boric acid, 1mM EDTA) at 100 V for 45 minutes and visualized by EtBr staining.

Light microscopy. The morphological alterations between EAC cells untreated and treated with *Securidaca longepedunculata* were assessed by light microscopy (NIKON TS100, JAPAN) and images were taken using a 100× objective amplified with 5× zoom.

Statistical Analysis. Results are expressed as Mean $\pm$ S.E.M., SPSS package was used for data analysis and t-test was used for determining the significance ($P<0.05$) between mean values within a group and between groups.

RESULTS

It is evident from the results in Table 1 that the death rate of Ehrlich ascites tumour cells *in-vitro* increases with the concentration of *Securidaca longepedunculata* root bark extract. *Securidaca longepedunculata* at concentrations of 0.1, 1, 10, 100, 1000 µg/ml caused mortalities of 7.3, 10.6, 27.9, 45.1 and 82.5 % respectively in Ehrlich ascites carcinoma cells

The aqueous root bark extract of *Securidaca longepedunculata* (SL) has been shown to be cytotoxic to Ehrlich ascites tumour cells. The IC_{50} was calculated to be 67µg/ml.

The effect of SL on the survival of tumour-bearing mice is shown in Table 2. The Mean Survival time (MST) for the control group was 14.4 days whereas MST was 17.4, 5.6, 3.6 and 17.4 days respectively for the groups treated with 10, 20 and 40 mg/kg body weight SL and 20 mg/kg body weight 5-fluorouracil respectively. The percentage increase in life span of tumour-bearing mice treated with 10, 20, 40 mg/kg SL and 20 mg/kg 5-fluorouracil was found to be 20, -61.1, -75.0 and 20 % respectively as compared to the diseased control (Table 2). Treatment with 10 mg/kg body weight of extract also caused a significant ($P < 0.05$) decrease in viable tumour cell count as compared to the EAC tumour control while there was an increase in the dead cell count when compared to the control (Table 3).

Table 1: *In vitro* cytotoxic effect of Aqueous extracts of *Securidaca longepedunculata* on Ehrlich Ascites Carcinoma cells.

Concentration in µg/ml	% Mortality
1000	82.5
100	45.1
10	27.9
1	10.6
0.1	7.3

Table 2: Mean Survival Time and Increased life span of Ehrlich ascites-bearing mice treated with *Securidaca longepedunculata* extract and 5-fluorouracil*.

Groups	Treatment	MST (days)	ILS (%)
2	EAC + Normal Saline	14.40 ± 0.93	-
3	EAC +10 mg/kg b.wt SL	17.40 ± 1.36	20.8
4	EAC +20 mg/kg b.wt SL	5.60 ± 0.51	-61.1
5	EAC +40 mg/kg b.wt SL	3.60 ± 0.24	-75.0
6	EAC +20 mg/kg b.wt 5-FU	17.40 ± 1.91	20.8

*Results are expressed as Mean ± Standard error of mean (S.E.M)
Values with different superscripts are significantly different from control
Keys: MST (Mean Survival time), ILS (Increased life span), 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma).

Table 3: Effect of *Securidaca longepedunculata* and 5-fluorouracil on viability of Ehrlich Ascites carcinoma cells in tumour-bearing mice*.

Groups	EAC only	EAC + 20 mg/kg FU	EAC + 10 mg/kg SL
Viable Cell Count	1091 ± 11.61^{a}	153.33 ± 18.49^{b}	350.33 ± 67.78^{bc}
Dead Cell Count	413.67 ± 14.49^{a}	537.33 ± 39.18^{b}	2660.00 ± 820.75^{a}
Total Cell Count	1505 ± 2.91^{a}	690.67 ± 25.95^{b}	3050.33 ± 913.40^{a}
Total Cell Concentration ($\times 10^5$) (cells/ml)	423.81 ± 3.43^{a}	175.16 ± 5.14^{b}	414.93 ± 126.25^{a}
Viability (%)	72.46 ± 0.91^{a}	22.30 ± 3.30^{b}	12.83 ± 2.05^{b}

*Results are expressed as Mean ± Standard error of mean (S.E.M)
Values with different superscripts are significantly different from control
Keys: 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma) SL (*Securidaca longepedunculata*)

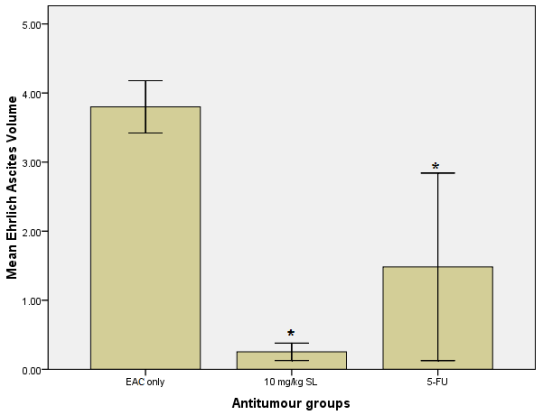

Figure 1: Effect of *Securidaca longepedunculata* and 5-fluorouracil on tumour volume of Ehrlich Ascites carcinoma cells in tumour-bearing mice.

*Values are significantly different from control (P < 0.05). Keys: 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma). SL- *Securidaca longepedunculata*

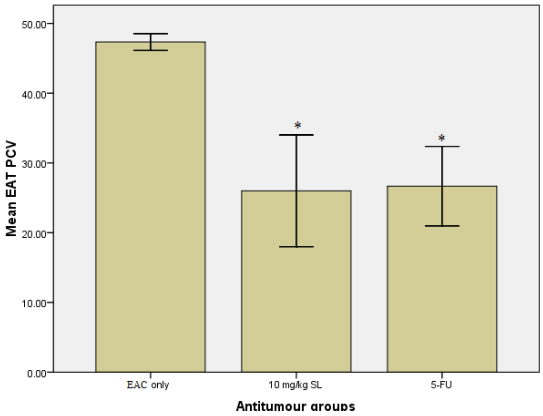

Figure 3: Effect of *Securidaca longepedunculata* and 5-fluorouracil on ascites packed cell volume of Ehrlich Ascites carcinoma cells in tumour-bearing mice.

*Values are significantly different from control (P < 0.05). Keys: 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma). SL- *Securidaca longepedunculata*

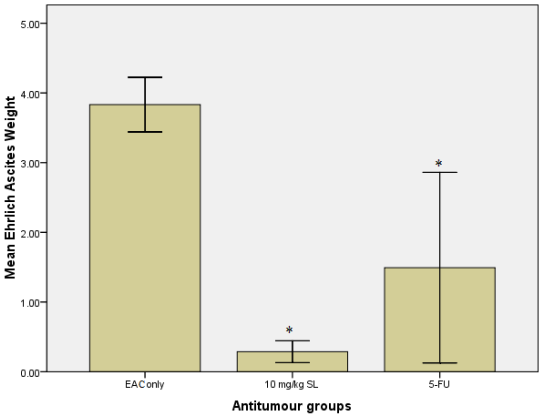

Figure 2: Effect of *Securidaca longepedunculata* and 5-fluorouracil on weight of Ehrlich Ascites carcinoma cells in tumour-bearing mice.

*Values are significantly different from control (P < 0.05). Keys: 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma). SL- *Securidaca longepedunculata*

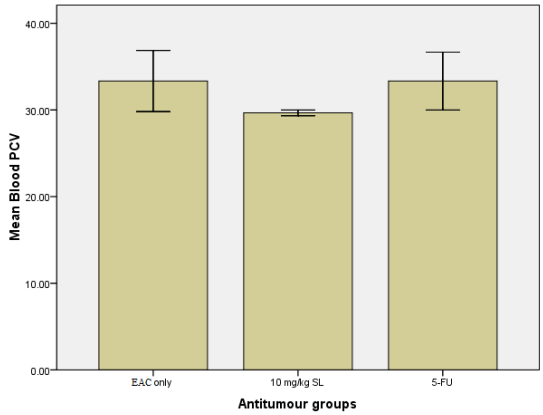

Figure 4: Effect of *Securidaca longepedunculata* and 5-fluorouracil on blood packed cell volume of Ehrlich Ascites carcinoma cells in tumour-bearing mice.. *Values are significantly different from control (P < 0.05). Keys: 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma). SL- *Securidaca longepedunculata*

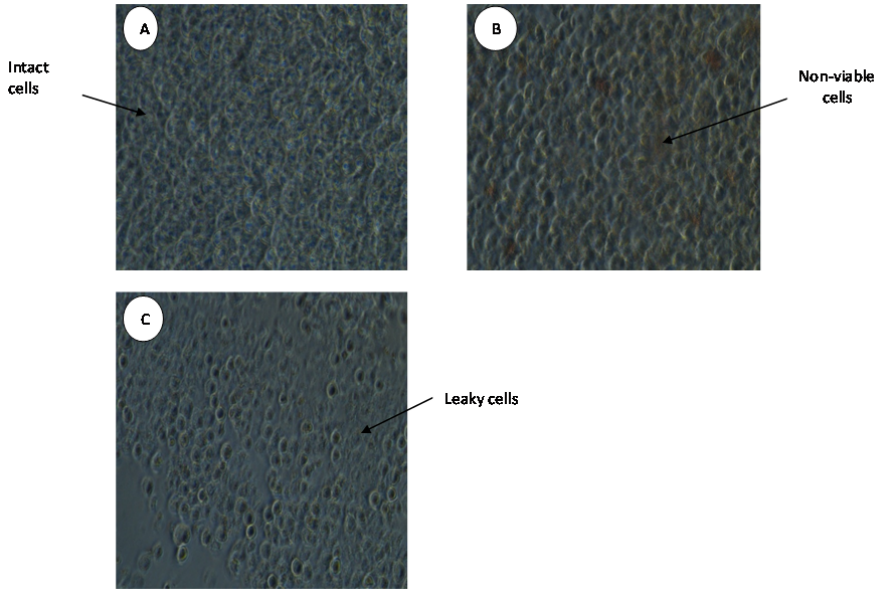

Figure 5. Morphological features of EAC cells from the peritoneal cavity of Mice treated with Aqueous extract of *Securidaca longepedunculata*.
*A - EAC only, B – EAC + 5-FU, C – EAC + 10 mg/Kg SL.
Keys: 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma). SL- *Securidaca longepedunculata*

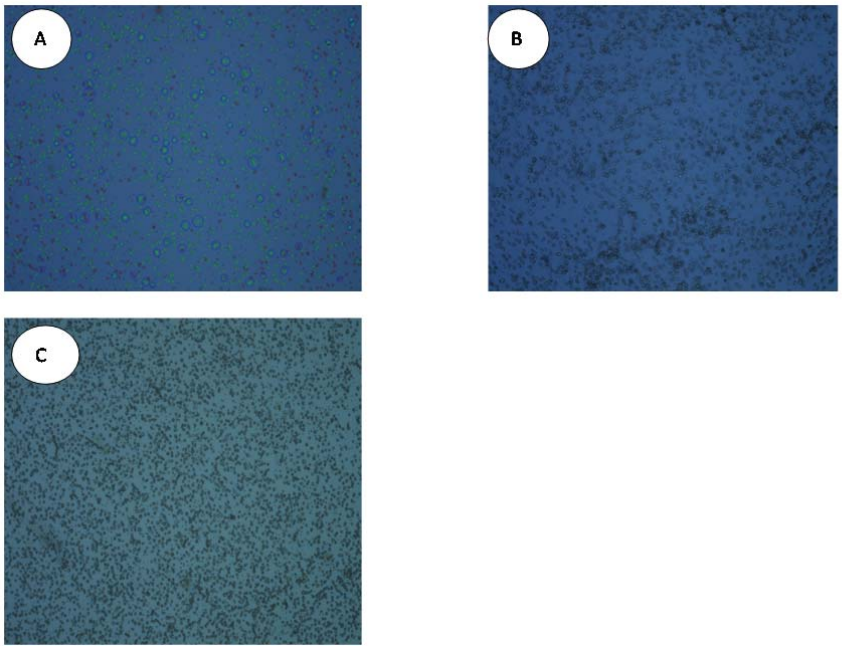

Figure 6. Morphological features of EAC cells stained with Trypan blue from the peritoneal cavity of Mice treated with Aqueous extract of *Securidaca longepedunculata* and 5-fluorouracil.
*A - EAC only, B - EAC+5-FU, C – EAC + 10 mg/Kg SL. Blue stained cells are leaky and damaged, while unstained cells are viable.

Morphological features as described in Figure 5B and C indicate a high percentage of tumour cells with damaged and leaky cell membranes in the groups treated with 5-fluorouracil and *Securidaca longepedunculata* aqueous root bark while the tumour cells from the group administered normal saline alone had more intact cells.

Morphological features as described in Figure 6B and 6C indicate a high percentage of tumour cells stained blue (leaky and non viable cells) in the groups treated with 5-fluorouracil and *Securidaca longepedunculata* aqueous root bark while the tumour cells from the group administered normal saline alone had more unstained cells (viable cells)

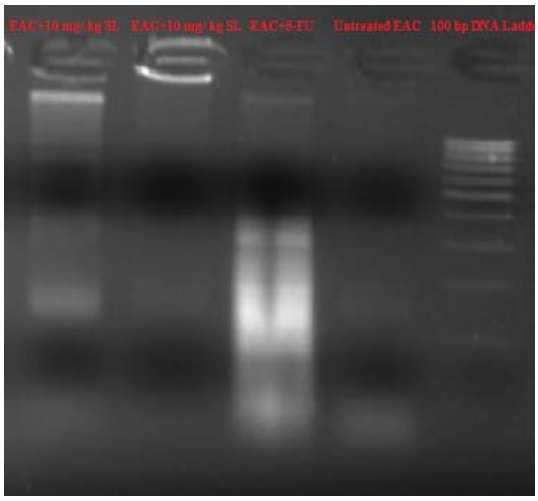

Figure 7. The ethanol extract of *Securidaca longepedunculata* from Osogbo, Nigeria induced DNA fragmentation in Ehrlich ascites carcinoma comparable to the effect of 5-fluorouracil. Keys: 5-FU (5-fluorouracil), EAC (Ehrlich ascites carcinoma). SL- *Securidaca longepedunculata*

Treatment with *Securidaca longepedunculata* root bark extract also caused a significant reduction in Tumour volume, tumour weight, tumour packed cell volume and blood packed cell volume as compared to the EAC tumour control. 5-fluorouracil also caused a reduction in the tumour volume and tumour weight.

DNA fragmentation was evident as seen in agarose gel electrophoresis (Fig 7) of DNA extracted from *Securidaca longepedunculata* - treated cells. The DNA from the control was found to be intact while the fragmentation pattern of 5-fluorouracil-treated cells though more intense, was comparable to the pattern of SL-treated cells.

DISCUSSION

The results of the in-vitro cytotoxicity assay revealed that *Securidaca longepedunculata* is toxic to the EAC cells as there was an increase in the number of cells stained with trypan blue dye, with an increase in the concentration of SLE (Muthuraman *et al.*, 2008). This cytotoxic activity might be due to mechanisms other than direct cytolytic effects such as directly on the tumour cells and cause their lysis and/or indirectly by destroying the microenvironment i.e the ascites fluid produced by the tumour cells. The use of Ehrlich Ascites Carcinoma (EAC) as a model in anti-cancer research was proven by many authors to give accurate and reliable results (Clarkson and Burchenal, 1965; Kuttan *et al.*, 1990; Sheeja *et al.*, 1997; Ramnath *et al.*, 2002; Gupta *et al.*, 2004). The reliability of such test lies in its ability to determine the value of any anticancer drug through prolongation of experimental animal life span in addition to the changes in number and viability of the cell line itself in addition to the volume of the liquid generated by the tumor inside the peritoneal cavity (Maity *et al.* 1999). The present study was aimed at evaluating the anti-tumour potential of *Securidaca longepedunculata* aqueous extract in EAC-bearing mice. It was observed that SLE increased the life span of EAC-bearing mice by reducing the viability of the EAC cells, decreasing the tumour volume and tumour burden. Adreani *et al.*, 1983 had suggested that an increase in the lifespan of ascites-bearing animals by 25% is considered indicative of significant drug activity. In this experiment, although 5-FU had an approximately 21% increasing rate in the lifespan, 10 mg/kg dose of *Securidaca longepedunculata* has a 21% increased lifespan.

5-flourouracil has been shown to significantly enhance the life span in EAC tumors (Fodstat *et al.*, 1977) and was used as reference drug in this study. One of the most reliable criterions for assessing the value of any anticancer drug is the prolongation of the life span of animals treated. It was observed that SLE increased the life span of EAC-bearing mice by reducing the viability of the EAC cells. The rationale for injecting crude extract on the next day after inoculation with exponentially growing EAC is to simulate a natural state in human where a partially or wholly grown tumour has to be treated.

In order to ensure that these malignant cells receive the nourishment they need to thrive, angiogenesis occurs. This is the formation of new blood vessels (Yarbro *et al.*, 2005). The Ehrlich ascites tumour implantation induces a local inflammatory reaction, with increasing vascular permeability (due to rise in the VEGF), which results in an intense edema formation, cellular migration and a progressive ascetic fluid formation (Fecchio *et al.*, 1990). Ascitic fluid has severally been shown to be the direct source of nutrition for tumour cells. The results of this study show that intraperitoneal administration of SLE reduced the ascites burden as depicted by the reduction in ascites volume.

Apoptotic cells are characterised by a number of morphological features such as cell shrinkage, membrane blebbing, chromatin condensation and the formation of apoptotic bodies (Zimmerman *et al.*, 2001; Orienius, 2004). Some of the morphological changes associated with apoptosis occur as a result of the activation of endogenous endonucleolytic

and proteolytic (caspases) enzymes that, in turn, mediate the cleavage of DNA into fragments as well as protein substrates, which usually determine the integrity and shape of the cytoplasm and organelles (Seraste and Pulkki 2000; Denault and Salvesen 2002; Kasibhatla and Tseng 2003).

A cell undergoing apoptosis would exhibit the following specific morphological changes: cell shrinkage, a dense cytoplasm and tightly packed

cell organelles, condensation of chromatin, a breakdown of the nuclear envelope, irregular buds known as blebs in the cell membrane, and finally the breaking apart of the cell into several vesicles (Darzynkiewicz *et al*., 1994).

The extract inhibited the growth of tumour cells and induced morphological changes typical of apoptosis. DNA fragmentation assay shows that the aqueous extract of *Securidaca longepedunculata* induced ladder-like DNA fragmentation which is characteristic of DNA damage. Most anticancer drugs of plant origin or synthetic have been known to cause DNA damage or suppress its replication, not necessarily killing the cells directly but inducing apoptosis. During apoptosis, a specific nuclease (now known as caspase-activated DNase or CAD and pre-existed in living cells as an inactive complex) cuts the genomic DNA between nucleosomes and generates DNA fragments. This ladder has been used extensively as a marker in studies on apoptotic cell death (Wyllie, 1980; Nagata, 2000). However, further studies are needed for better understanding of how *Securidaca longepedunculata* activated apoptotic cascade.

S. longepedunculata has proved to be a potential anti-proliferative and apoptosis-inducing agent which could be further developed and translated into a therapeutic regime for treatment of human cancer.

REFERENCES

Abdullaev FI, Luna RR, Roitenburd BV, Espinosa AJ. 2000. Pattern of childhood cancer mortility in Mexico. *Arch Med Res.* 31: 526-31.

Adreani A, Scapini G and Galatulas I. 1983. Potential antitumor agents. IX synthesis and antitumor activity of two analogues of ketocaine. *J. Pharm. Sci.* 72: 814-819.

Agrawal S.S., Sarita Saraswati, Rajani Mathur, Maneesha Pandey. 2011. Antitumor properties of Boswellic acid against Ehrlich ascites cells bearing mouse. *Food and Chemical Toxicology* 49:1924–1934

Akinniyi JA, Manawadu D, Sultanbawa M. 1996. Ethnobotany and Ethnopharmacology of Nigerian medicinal Plants Research and Traditional Medicine in Malaria: In: State of Medicinal Plants Research in Nigeria: pp. 35-349.

Biswas M., Samit Bera, Biswakanth Kar, Tarun K. Karan, Sanjib Bhattacharya, Ashoke K. Ghosh, Pallab K. Haldar. 2010. Antitumour effect of Dregea volubilis fruit in Ehrlich Ascites Carcinoma bearing mice. *Global J. Pharmacol.* 4(3): 102-106

Bromberg N., Juliana L. Dreyfuss, Caio V. Regatieri, Marcelly V. Palladino, Nelson Duran, Helena B. Nader, Marcela Haun, Giselle Z. Justo. 2010. Growth inhibition and pro-apoptotic activity of violacein in Ehrlich ascites tumor. *Chemico-Biological Interactions.* (186) 43–52.

Burkill H. M. 1997. The Useful Plants of West Africa, Royal Botanic Gardens Kew 4: 447-458.

Clarkson B.D., Burchenal J.H. 1965. Preliminary screening of antineoplastic drugs. *Prog. Clin. Cancer* 1:625- 629.

Cragg G. M., Newman D. J. 2005. Plants as a source of anti-cancer agents. *J. Ethnopharmacol.* (100):72–79.

Dapar, L. P. Maxwell, Aguiyi, C. John, Wannang, N. Noel, Gyang, S. Steven and Tanko, M. Naanlep. 2007. The histopathologic effects of *Securidaca longepedunculata* on heart, liver, kidney and lungs of rats. *Afr. J. Biotechnol.* 6 (5): 591-595.

Darzynkiewicz, Z., Li, X., and Gong, J. 1994. Assays of cell viability: Discrimination of cells

Denault JB, Salvesen G.S. 2002. Caspases: keys in the ignition of cell death. *Chem. Rev*. 102(12): 4489-4500.

Fecchio, D., Sirois, P., Russo, M. and Janear, S. 1990. Studies on inflammatory response induced by Ehrlich tumor in mice peritoneal cavity. *Inflammation* 14: 125–131.

Fodstadt, O., Olsnes, S., Alexander Pihl, A. 1977. Inhibitory effect of abrin and ricin on the growth of transplantable murine tumors and of abrin on human cancers in nude mice. *Cancer Res*. 37, 4559–4567.

Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. 2000. Antitumor activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma. *J. Ethnopharmacol.* 72: 151-156.

Gupta M, Mazumder UK, Sambath Kumar R, Sivakumar T, Mohan vamsi ML. 2004. Antitumor Activity and Antioxidant Status of *Caesalpinia bonducella* Against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *J. Pharmacol. Sci.* 94: 177-184.

Harkness, John E., Wagner, Joseph E. 1995. The Biology and Medicine of Rabbits and Rodents. Williams & Wilkins. pp. 382.

Jun-ya UEDA, Yasuhiro TEZUKA, Arjun Hari BASKOTA, Quan Le TRAN, Qui Kim TRAN, Yuko HARIMAYA, Ikuo SAIKI, and Shigetoshi KADOTA. 2002. Antiproliferative Activity of Vietnamese Medicinal Plants. *Biol. Pharm. Bull.* 25(6) 753—760

Justo G.Z., N. Duran, M.L.S. Queiroz. 2000. Myelopoietic response in tumor-bearing mice by an aggregated polymer isolated from Aspergillus oryzae. *Eur. J. Pharmacol.* 388: 219–226.

Kasibhatla S, Tseng B. 2003. Minireview: Why target apoptosis in cancer treatment. *Mol. Cancer Ther*. 2: 573-580.

Kavimani S, Manisenthil kumar KT. 2000. Effect of methanol extract of *Enicostemna littorale* on Dalton's lymphoma. *J. Ethnopharmacol.* 71: 349-352.

Kuttan G, Vasudevan D & Kuttan R. 1990. Effect of a preparation from *Viscum album* on tumour development *in vitro* and in mice. *J. Ethnopharm*. 29: 35-41.

Madhuri S., Pandey G. 2009. Some anticancer medicinal plants of foreign origin. *Current Science*, 96 (6) 779-783.

Maity P, Chakrabory S & Bhattacharya P. 1999. Angiogenesis, a putative new approach in glutamine related therapy. *Path. Onco. Res. J.* 5(4): 309-314.

Mazumder U.K., Gupta M, Maiti S, Mukherjee M. 1997. Antitumor activity of *Hygrophilaspinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian J. Exp. Biol.* 35: 473-477.

Mehmet Ozaslan, Isik Didem Karagoz, Ibrahim Halil Kilic and Muhammed Emin Guldur. 2011. Ehrlich ascites carcinoma. *Afr. J. Biotechnol.* 10 (13), 2375-2378.

Mehmet Ozaslan, Isik Didem Karagoz, Ibrahim Halil Kilic and Muhammed Emin Guldur. 2011. Ehrlich

ascites carcinoma. *Afr. J. Biotechnol.* 10(13) 2375-2378

Msonthi J. D. 1986. The Status of Medicinal Plant Research And Traditional Medicine In Malaria. In: The state of Medicinal plants Research in Nigeria. pp. 335-349

Muthuraman M. S., Sundarsanam Dorairaj, Parthasarathy Rangarajan, Brindha Pemaiah. 2008. Antitumour and Antioxidant Potential of Tragia Plukenetii R.Smith on Ehrlich ascites carcinoma in mice. *Afr. J. Biotechnol.* 7 (20): 3527-3530.

Nagata S. 2000. Apoptotic DNA fragmentation. *Exp. Cell. Res.* 256: 12-18.

Neuwinger H. D. 1996. African Ethnobotany, Poisons and Drugs Chemistry, Pharmocology, Toxicology Chapman and Hall, London, UK, pp. 743-753.

Olajide O. A., Awe S. O., Makinde J. M. 1998. Pharmacological Screening of the Root Extract of *Securidaca longepedunculat*a. *Fitoterapia,* 69(3): 245-248

Orrenius S. 2004. Mitochondrial regulation of apoptotic cell death. *Toxicol. Lett*. 149: 19-23.

Prakash N.S., R. Sundaram, S.K. Mitra. 2011. *In Vitro* and *In Vivo* Anticancer Activity of Bacoside A from Whole Plant of *Bacopa Monnieiri* (Linn) *Am. J. Pharm. Toxicol.* 6 (1): 11-19.

Ramakrishna Y., Manohar AL, Mamata P, Shreekant KG. 1984. Plants and novel antitumor agents: A review. *Indian Drugs* 21: 173-185.

Ramnath V., Kuttan G, Kuttan R. 2002. Anti-tumour effect of abrin on transplanted tumours in mice. *Indian J. Physiol. Pharmacol.* 46: 69-77.

Saluja M. S, B. Sangameswaran, I. S. Hura, Ajay Sharma, S.K.Gupta and M. Chaturvedi. 2011. In-vitro cytotoxic activity of leaves of Madhuca longifolia against Ehrlich Ascites carcinoma (EAC) cell lines. *Int. J. of Drug Discovery & Herbal Research* 1(2): 55-57.

Seraste A, Pulkki K. 2000. Morphologic and biochemical hallmarks of apoptosis. *Cardiov ascular Res*. 45(3): 528-537.

Sheeja K.R., Kuttan G., Kuttan R. 1997. Cytotoxic and antitumour activity of Berberin. *Amala Res Bull.* 17: 73-6.

Sowemimo, A.A., F.A. Fakoya, I. Awopetu, O.R. Omobuwajo, S.A. Adesanya. 2007. Toxicity and mutagenic activity of some selected Nigerian plants. *J. Ethnopharmacol.* 113:427–432

Sur, P., Ganguly, D.K. 1994. Tea plant roots extract (TRE) as an antineoplastic agent. *Planta Med*. 60, 106–109.

Thippeswamy G., Bharathi P. Salimath. 2006. Curcuma aromatic extract induces apoptosis and inhibits angiogenesis in Ehrlich ascites tumour cells in vivo. *mySCIENCE* 1(1): 79-92.

Wyllie A. H. 1980. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284: 555-556.

Yarbro, C., Frogge, M., Goodman, M. 2005. *Cancer Nursing: Principles and Practice*, 6th edn, Jones and Bartlett Publishers, Boston, MA.

Zimmerman KC, Bonzon C, Green D. R. 2001. The machinery of cell death. *Pharmacol. Ther*. 92: 57-70.

Zumrutdal M. E., Mehmet Ozaslan, Mehmet Tuzcu, Mehmet Emin Kalender, Kenan Daglıoglu, Atilla Akova, Isık Didem Karagöz, Ibrahim Halil Kilic, Omer Colak and Fatih Köksal. 2008. Effect of *Lawsonia inermis* treatment on mice with sarcoma. *Afr. J. Biotechnol.* 7 (16) 2781-2786.