Heliyon 9 (2023) e13869

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Four Togolese plant species exhibiting cytotoxicity and antitumor activities lightning polytherapy approach in cancer treatment

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ARTICLE INFO

Keywords:
Cancer
Cytotoxicity
Antitumor
EAC cells
Anti-inflammatory
Anti-angiogenic

ABSTRACT

Background: Cancer is leading to premature deaths across the globe. Therapeutic approaches are still being developed to enhance the survival of cancer patients. In our previous study, extracts from four Togolese plants, namely, *Cochlospermum planchonii* (CP), *Piliostigma thonningii* (PT), *Paullinia pinnata* (PP), and *Securidaca longipedunculata* (SL), actually used in traditional medicine for cancer treatment, showed beneficial health effects against oxidative stress, inflammation, and angiogenesis.

Purpose: In the present study, we aimed to investigate the cytotoxicity and antitumor activities of these four plant extracts.

Material and methods: Breast, lung, cervical, and liver cancer cell lines were exposed to the extracts, and viability was assessed using the Sulforhodamine B method. *P. pinnata* and *S. longipedunculata* with significant cytotoxicity were selected for *in vivo* tests. The acute oral toxicity of these extracts was assessed using BALB/c mice. The antitumor activity was evaluated using the EAC tumor bearing mice model, wherein mice were orally treated with extracts at different concentrations for 14 days. The standard drug was cisplatin (3.5 mg/kg, i.p), single dose.

Results: Cytotoxicity tests revealed that SL, PP, and CP extracts have more than 50% cytotoxicity at 150 μg/mL. The acute oral toxicity of PP and SL at 2000 mg/kg did not show any toxic signs. At therapeutic doses of 100 mg/kg, 200 mg/kg and 400 mg/kg of PP and 40 mg/kg, 80 mg/kg, and 160 mg/kg of SL, extracts showed beneficial health effects by modulating several biological parameters. SL extract significantly reduced tumor volume ($P < 0.001$), cell viability, and normalized hematological parameters. SL also demonstrated a strong anti-inflammatory activity similar to the standard drug. The SL extract also revealed a significant increase of the life span of treated mice. PP extract reduced the tumor volume and significantly improved the values of endogenous antioxidants. Both PP and SL extracts also exerted significant anti-angiogenic potency.

Conclusion: The study indicated that polytherapy would be a panacea for the efficient use of medicinal plant extracts against cancer. This approach will make it possible to act simultaneously

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https://doi.org/10.1016/j.heliyon.2023.e13869
Received 12 October 2022; Received in revised form 6 February 2023; Accepted 14 February 2023
Available online 23 February 2023

on several biological parameters. Molecular studies of both extracts targeting key cancer genes in several cancer cells are currently underway.

1. Introduction

Nowadays, communicable diseases such as Covid 19 weigh heavily on the world. Noncommunicable diseases (NCDs) such as cancer and cardiovascular diseases have recently become the leading causes of premature deaths worldwide [1,2], with cancer being the leading cause of death in this century, according to NCDs statistics [3]. Based on the International Agency for Research on Cancer (IARC), 19.3 million new cancer cases and 9.9 million cancer-related deaths were recorded in 185 countries worldwide in 2020 [4].

Cancer has a multifactorial etiology, as evidenced by the accumulation of mutations, genetic predispositions, chronic oxidative stress, and inflammation in the affected organs [5]. Many methods for its treatment have been established and are undergoing improvement. These solutions include surgery, radiotherapy, immunotherapy, and chemotherapy. Despite the progress made thus far with these methods, the emergence of numerous unfavorable side effects has remained a challenge for the cancer scientific community. This highlighted the critical need for the development of alternative solutions with selective anticancer activity to counteract these negative effects. Recent efforts to find new approaches have been commendable, and plant products have emerged as a promising alternative for anticancer medicine [6]. In fact, worldwide and especially in developing countries, medicinal plants have been accepted and used for a long time as one of the main sources of anticancer agents. However, it is imperative to make a judicious choice of these plants for the evidence-based medicine approach in drug discovery.

Hence, there is a deep interest in studying several plants to elucidate their presumed safety, cost effectiveness, and sustainable availability for their large-scale usage. Four major anticancer activities are considered when searching for bioactive drugs in promising plants: antioxidant, anti-inflammatory, anti-angiogenic, and cytotoxic [7]. Thus, the first part of our investigations focused on an ethnopharmacological survey on anticancer plants used by traditional healers in the Central and Kara regions of Togo. Traditional healers provided 85 recipes for cancer management in their areas, and approximately 50% of traditional healers receive their patients with a medical diagnosis of cancer that has already been established in a hospital [8]. In the second part, we carried out a presumptive pharmacological tests of four plant species (*Cochlospermum planchonii, Piliostigma thoningii, Paullinia pinnata and securidaca longipedunculata*) resulting from the survey [8]. These medicinal plants are commonly used by traditional healers but have not yet been scientifically tested for anticancer properties *in vitro* and/or *in vivo*. The presumptive study was done to assess their antioxidant, anti-inflammatory, anti-angiogenic, and cytotoxic activity *in vitro* and *ex vivo*. *In vitro*, *Paullinia pinnata* showed good antioxidant and antiinflammatory activity, as well as a reduction in the number of blood vessels in an angiogenic model. *Securidaca longipedunculata* demonstrated also *in vitro* a strong antiinflammatory activity similar to the reference drug and an antiangiogenic activity by reducing the number of blood vessels [9]. The results therefore provided promising evidence that these plants have beneficial effects on health and could motivate their use in traditional medicine to treat many diseases, including cancer. Hence, the aim of the present study was to validate the anticancer activity of the four plants according to the ethnomedical claims and motivate their use in anticancer phytomedicine formulation.

2. Material and methods

2.1. Drug and chemicals

Phosphate Buffered Saline (PBS), Diméthyl Sulfoxide (DMSO), Cisplatin, Trypsin, Penicillin, Streptomycin, DMEM (Dulbecco's Modified Eagle's Medium), Fetal Bovine Serum, Trichloroacetic Acid (TCA), Trypan Blue reagent, Acetic acid, Sulforhodamine B (SRB), and Tris base reagent (Thermo Fisher Scientific, Inc.). All other chemicals used in the study were of analytical grade.

2.2. Plant material and extracts preparation

The hydroethanolic extracts were prepared as previously described by Kola, Metowogo [9]. Briefly, the organs of the plants were collected and authenticated at the Laboratory of Botany and Plant Ecology, University of Lome. Powder of organs were macerated with ethanol - distilled water (60:40 v/v) for 3 days. Macerate was then filtered and evaporated at 45 °C using a Rotary Evaporator.

3. Animals

Female Swiss Albino BALB/c mice (20–25 g b.wt) were used for acute toxicity and *in vivo* antitumor studies.

The experiments involving animal studies have been approved by the Institutional Animal Ethics Committee (IAEC) under No. JSSAHER/CPT/IAEC/072/2021 (Central Animal Facility, Centre for Experimental Pharmacology and Toxicology, JSS College of Pharmacy, Mysuru, India).

3.1. Cytotoxicity activity assessment

The protocol used is divided into four main steps: cell fixation in the wells of the plates, incubation of the cells with the extracts,

fixation of the cells with trichloroacetic acid (TCA), staining with the SRB reagent, and absorbance reading at 510 nm [10]. Briefly, two 96-well plates were prepared. Four cell lines of exponentially growing breast, lung, cervical, and liver cancer (1×10^4 cells/well) were placed in these wells. The plates were incubated for 24 h to allow cells to adhere to the wells. Extracts were prepared with 0.1% DMSO, and the cells were subjected to two concentrations of the extracts (15 and 150 μg/ml) for 48 h. The experiment was done in triplicate, and cisplatin was used as the standard drug. After 48 h of incubation, trichloroacetic acid was added to each well and stored at 4 °C for 1 h. After that, the plates were washed four times in a slow-running tap water tank. The excess water was then removed and dried by air. Then, 0.4% SRB was added to each well, left in ambient room air for 1 h and then quickly rinsed with 1% acetic acid to be air-dried again. Finally, 50 μl of 10 mM Tris Base was added to each well and shaken for 5 min on a gyratory shaker. Absorbance was read at 510 nm using a microplate reader (Uvmax Microtiter Plate Reader).

The percentage of tumor growth inhibition was calculated according to the formula:

$$\% \text{ Inhibition} = [(\text{OD Control-OD Sample})/\text{OD Control}] \times 100$$

where OD Control = Optic Density of the control, OD sample = Optic Density of each extract or standard drug. For extracts with more than 50% cytotoxicity activity, increasing concentrations of SL extract (18.75, 37.5, 75, 150, and 300 μg/mL) were prepared for a second test on all cancer cell lines to determine the concentration that produces 50% tumor growth inhibition (IC_{50}).

3.2. Acute oral toxicity and therapeutic doses determination

The acute oral toxicity was assessed following the protocol described by Saleem, Amin [11]. Two plant species was used, *P. pinnata* and *S. longipedunculata*, following the instructions of the Organization for Economic Co-operation and Development (OECD) for toxicity testing (OECD 423). For therapeutic doses calculations, it is generally 1/5th, 1/10th, and 1/20th of lethal dose 50 that is considered [12]. Thus, therapeutic doses were retained based on the limit toxicity test and the toxicity studies reported on the two species of plants, *P. pinnata* [13–16] and *S. longipedunculata* (Auwal et al., 2012; Adeyemi et al., 2010; Gbadamosi et al., 2017; Bankole et al., 2019).

3.3. Transplantation of tumor cells

The specific murine cancer cells, Ehrlich Ascites Carcinoma (EAC), were chosen for the *in vivo* study. Ehrlich ascites carcinoma (EAC) is one of the cell lines commonly used in modeling. These cells first appeared as spontaneous breast cancer in female mice. These cells were searched at the National Centre for Cell Science (NCCS), Pune, Maharashtra 411,007, India. The EAC cells have a high capacity for transplantation, have exponential proliferation, and are able to grow in BALB/c mice because they do not possess a specific transplantation antigen [17].

The cells were maintained in mice's peritoneal cavity by serial transplantation of cells every 15 days. To do this, every 10th day of tumor development, ascites fluid containing EAC cells was aspirated from the peritoneal cavity of a tumor-bearing mouse and then inoculated into a second mouse intraperitoneally. These repetitive passages in mice make it possible to maintain the virulence of cancer cells in a living organism. This gradually increased the rate of proliferation. However, the cells lose differentiation and acquire free growth control mechanisms, gaining the ability to be transplanted into a heterograft and eventually converting to ascites. These cells are the most sensitive to chemotherapy because they are undifferentiated and have a rapid growth rate [17–19].

3.4. Evaluation of in vivo antitumor activity

3.4.1. Treatment schedule

The antitumor activity of extracts was evaluated according to the methods described by Manjula, Kenganora [19], Aruna [20], and Samudrala, Augustine [18].

Healthy female BALB/c mice, 6–8 weeks of age and weighing 20–25 g were used after 5 days of acclimatization. All mice were

Table 1
Groups and treatments received with the EAC model.

Groups	Number of mice	Treatment, Dose, Duration and Route
Normal	06	Normal animals treated with the solvent (1% CMC) 10 ml/kg for 14 days orally
Tumor control	12	Tumor-bearing animals treated with the solvent (1% CMC) for 14 days orally
Standard drug Cisplatin	12	Tumor-bearing animals treated with a single dose of Cisplatin 3.5 mg/kg intraperitoneally
PP 100 mg/kg	12	Tumor-bearing animals treated with corresponding doses of PP and SL extracts for 14 days orally
PP 200 mg/kg	12	
PP 400 mg/kg	12	
SL 40 mg/kg	12	
SL 80 mg/kg	12	
SL 160 mg/kg	12	
PP400 mg/kg only	06	Normal animals treated only with PP extract for 14 days orally
SL160 mg/kg only	06	Normal animals treated only with PP extract for 14 days orally
Total number of animals = 114		

randomized and labeled using picric acid. A fixed number of EAC cancer cells (2.5×10^6 cells/mouse) with 94% viability were inoculated into mice intraperitoneally. That day was considered Day 0, and treatment with extracts and a standard drug began the following day. The treatment was done daily according to the body weight of each mouse and lasted 14 days. The groups and treatments received are listed in Table 1. Animals were maintained under laboratory conditions at 25 ± 2 °C with a relative humidity of 55–65% under a light/dark alternation of 12 h per cycle. They were placed in the polypropylene cages and fed a diet of standard pellets and water ad libitum. The study was approved by the Institutional Animal Ethics Committee; IAEC approval N° JSSAHER/CPT/IAEC/072/2021 (Central Animal Facility, Centre for Experimental Pharmacology and Toxicology, JSS College of Pharmacy, Mysuru, India).

After administration of the last dose on day 14, half of the animals (n = 6) in each group were used for the evaluation of clinical parameters, and rest of the animals were kept for survival time observation, wherein the mortalities in each group were noted every day till 30 days [21,22].

3.4.2. Tumor growth response

Tumor growth response was estimated by changes in mouse weight, tumor volume, number of living and dead cells, median survival time (MST), and percent increase in the lifespan (%ILS).

- Body weight

All mice were weighed every 3rd day until the 15th day of the experiment. Mean of increased body weight of mice in each group was estimated on day 15.

•Tumor volume

Ascitic fluid was collected from the peritoneal cavity of mice. Fluid volume was measured by taking it directly into graduated centrifuge tubes and expressed in milliliters (ml).

❖ Tumor cells (viable and non-viable) count

A suspension of cancer cells was prepared from ascites fluid collected from mice in each treated group. This suspension was diluted 30 times using phosphate buffered solution (PBS), and a cell viability test was done by adding Trypan blue dye (0.4% in normal saline) using a Hemocytometer. Cells that have not aspirated the dye are viable, as their membranes remain intact and prevent the entry of dye. The cells that have been completely stained are not viable, as the dye enters through the dead cells and appears blue [23].

•Percentage increase in the life span (%ILS)

Another important parameter for evaluating the antitumor potential of plant extracts is the extension of the lifespan of tumor-bearing mice [22]. The mean survival time of each treated group was estimated and compared with that of the control group (control). Thus, the percentage of the average survival time was calculated by the following formulas [18]:

$$\text{Mean Survival Time (MST)} = (\text{Day of First Death} + \text{Day of Last Death})/ \quad 2$$

$$\%\ \text{Increase lifespan (\%ILS)} = [(\text{MST of treated group/MST control group}) - 1] \times 100$$

3.5. Hematological and tissue antioxidant parameters

Animals' blood was collected under anesthesia and by retro-orbital puncture for hematological parameters evaluation using the Erba Mannheim H 560 automate or hematological analyzer. Each mouse's liver was perfused with 0.9% NaCl before being removed and weighed to assess antioxidant parameters (malondialdehyde, catalase, glutathione reduced). The methods described by Prabhakar, Reddy [24] and Hadwan [25] were used. The relative liver weight of each mouse in each group was also estimated.

3.6. Determination of extract effect on normal mice

The mice that received the extract only (400 mg/kg of PP and 160 mg/kg of SL) throughout the experiment were used to assess the toxicity of the extracts on hematological parameters.

3.7. Statistical analysis

ANOVA was used to process the data, which was then represented as mean standard error of the mean (SEM) before being subjected to Tukey's multiple comparisons test. A value of $P < 0.05$ was used as the criterion for statistical significance.

4. Results and discussion

4.1. In vitro anti-cancer study: sulforhodamine B method

4.1.1. Cell growth inhibition test

Tumor growth inhibition results at 15 μg/ml (Fig. 1) and 150 μg/ml (Fig. 2) revealed that *C. planchonii*, *P. pinnata* and *S. longipedunculata* produced greater than 50% growth inhibition on all cell lines except for cervical cancer cell line (Fig. 2). Cervical cancer cells are insensitive not only to extracts but also to standard drug Cisplatin. This indicates resistance to chemotherapy, and similar results with these cancer cells have been documented by Wang, Zheng [26]. However, *S. longipedunculata* extract produced strong antiproliferative activity beyond 75% growth inhibition on lung cancer cells with better values (86.47%) compare to standard drug Cisplatin 100 μM (72.62%). This extract was then subjected to further bioassays with low concentrations to determine IC_{50}.

4.1.2. Determination of IC_{50} of S. longipedunculata

The lowest IC_{50} is 67.74 ± 4.78 μg/ml on breast cancer cells after 48 h of incubation (Table 2). Ngulde, Sandabe [27] discovered that the ethanolic extract of *S.* longipedunculata roots on U87 brain cancer cells had a low IC_{50} in a study conducted similarly in Nigeria. This demonstrates how responsive cancer cells are to various therapies. Another determining factor would be the chemical composition of medicinal plants according to their geographic location.

According to the National Cancer Institute (NCI) in the United States, a crude extract is generally considered to have promising high cytotoxic activity or antiproliferative activity *in vitro* if the IC_{50} value is less than 30–40 g/ml [28,29]. Our extracts are not identified as having promising *in vitro* cytotoxic activity based on this criterion. However, in some published studies, extracts with insignificant *in vitro* results produced better *in vivo* outcomes. Additionally, some extracts that don't meet this National Cancer Institute criterion have produced positive outcomes in *in vivo* studies [19,30–32]. Hence, we retained two extracts for the *in vivo* study, both of which produced more than 50% inhibition of tumor growth (Fig. 2). These are *P. pinnata* and *S. longipedunculata*.

4.2. In vivo-anticancer study

4.2.1. Therapeutic doses: an acute toxicity study

Extracts of *P. pinnata* and *S. longipedunculata* were tested for acute toxicity. Both extracts are non-toxic. At an oral dose of 2000 mg/kg body weight, neither abnormal behavioral changes nor mortality were noted. During the 14-day study period, no detectable difference in body weight change compared to the control group was seen (Table 3). Typically, when calculating therapeutic doses, 1/5th, 1/10th, and 1/20th of the lethal dose 50 are taken into account [12]. Accordingly, 100 mg/kg, 200 mg/kg, and 400 mg/kg of body weight of *P. pinnata* extract and 40 mg/kg, 80 mg/kg, and 160 mg/kg of body weight of *S. longipedunculata* extract were kept as therapeutic doses for *in vivo* antitumor studies. This was also based on the findings of prior toxicity studies of *P. pinnata* [13] and *S. longipedunculata* [33–36].

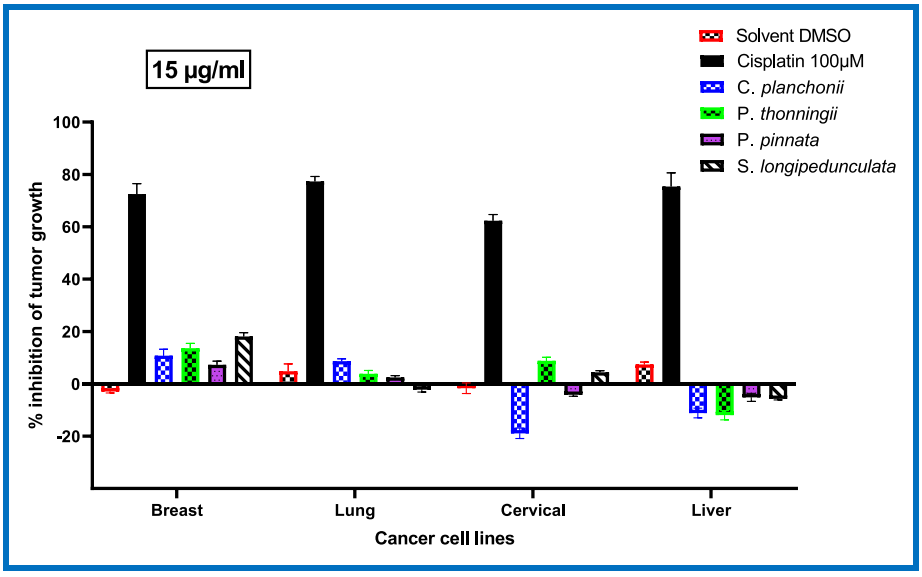

Fig. 1. *In vitro* cytotoxicity on cancer cells at 15 μg/ml.

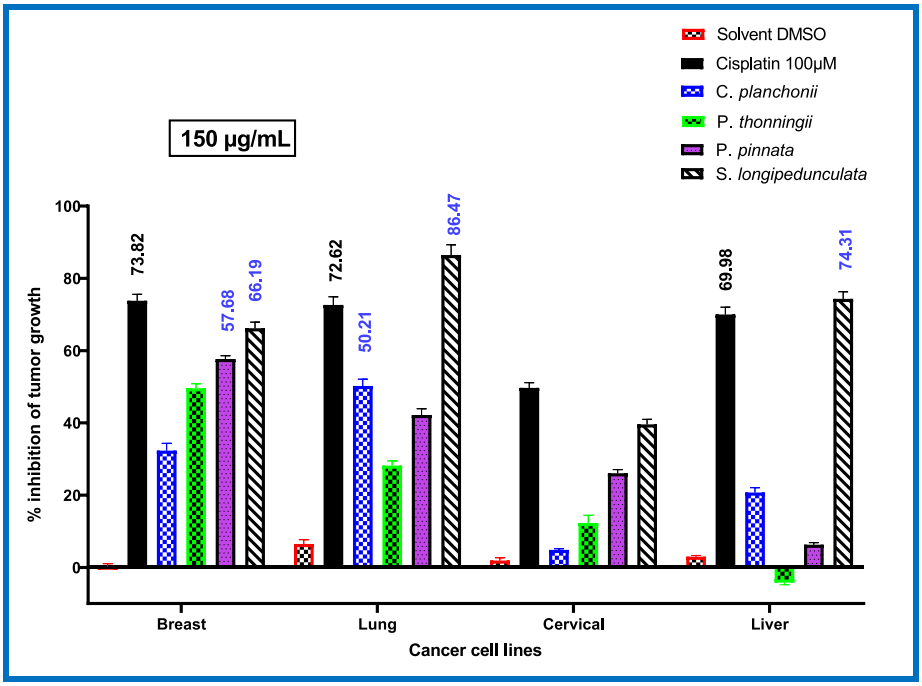

Fig. 2. *In vitro* cytotoxicity on cancer cell lines at 150 μg/ml.

Table 2
Cytotoxicity (IC_{50}) of SL extract.

	Timing	Breast	Lung	Liver	Cervical
IC_{50} (μg/ml)	24 h	175.58 ± 5.81	110.69 ± 2.90	160.99 ± 2.76	282.84 ± 7.58
	48 h	67.74 ± 2.48	105.76 ± 3.01	110.18 ± 3.08	130.16 ± 4.56

Values are expressed as mean ± S.E.M. (n = 3).

Table 3
Body weight change during acute toxicity.

Groups	Weight (g) Day1	Weight (g) Day7	Weight (g) Day14
Control 0.25% CMC	25.80 ± 1.06	26.90 ± 0.64	27.93 ± 1.01
PP 2000 mg/kg	25.10 ± 0.64	27.67 ± 0.67	28.10 ± 0.72
SL 2000 mg/kg	26.07 ± 1.24	27.93 ± 1.15	28.10 ± 1.21

Values are expressed as mean ± S.E.M. (n = 5); One-way ANOVA followed by Tukey's multiple comparison test.

4.3. EAC tumor-bearing mouse model

4.3.1. Effect of extracts on body weight change

Tumor growth was visible as early as the sixth day after tumor cells were inoculated.

Figs. 3 and 4 (A-D), and 5 (A-D) show mice from the untreated and treated groups on the 15th day of the experiment. These figures depict tumor development as well as the effect of extracts on tumor regression. From the sixth day of cancer cell inoculation until the end of the study, a consistent increase in body weight was observed (day 15th). The EAC control group experienced the greatest weight gain. When compared to the control group, it was highly significant ($P < 0.001$). Body weight was significantly reduced in treated groups at 3.5 mg/kg of Cisplatin ($P < 0.001$), 400 mg/kg of *P. pinnata* ($P < 0.05$) (Fig. 6A) and all therapeutic doses of *S. longipedunculata* (Fig. 6B). Normal mice treated with the maximum therapeutic dose only showed no significant difference when compared to the control group. Ehrlich ascites carcinoma (EAC) cells grow in all mouse strains and have been used for decades to study the anticancer activities of numerous natural and synthetic products [18]. Our findings indicate that the increase in body weight observed is due to an increase in the volume of ascitic fluid caused by tumor cell exponential growth. Treatment with extracts resulted in a significant reduction in body weight, most likely due to a decrease in ascitic fluid volume and/or cell cycle arrest (see Fig. 5).

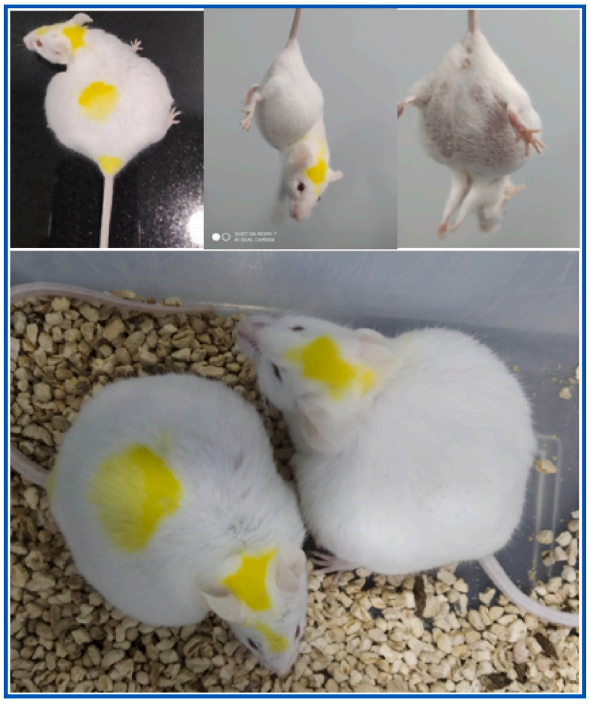

Fig. 3. Images of untreated mice (EAC control) on the 15th day of the experiment.

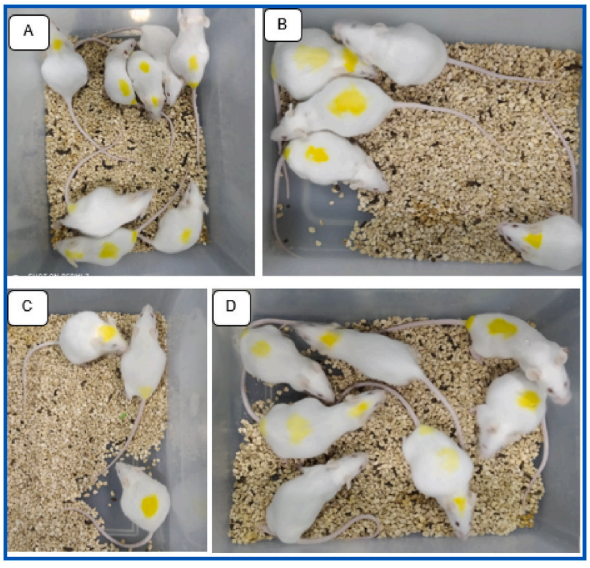

Fig. 4. Images of treated mice: (A) Cisplatin 3.5 mg/kg, (B) PP100 mg/kg, (C) PP 200 mg/kg, (D) PP400 mg/kg.

4.3.2. Effects of extracts on tumor growth and survival parameters

When compared to the EAC Control group, the tumor volume and number of living cells in the Cisplatin-treated group were significantly ($P < 0.001$) lower. Tables 4 and 5 show the results. When compared to the control, administration of *P. pinnata* extract at 200 mg/kg and 400 mg/kg resulted in a significant decrease ($P < 0.05$) in tumor volume and living cell count and a significant increase ($P < 0.05$) in dead cell count. In addition to a significant increase in survival time of tumor-bearing mice compared to control, cell viability was reduced in treated groups (Table 4). When compared to the control, administration of *S. longipedunculata* extract resulted in a very significant ($P < 0.001$) improvement in survival and life expectancy at doses of 80 mg/kg and 160 mg/kg (Table 5).

Indeed, tumor transplantation provokes a local inflammatory reaction. This causes increased vascular permeability, which causes edema, cell migration, and an increase in ascitic fluid production. This is most likely justified by an increase in vascular endothelial

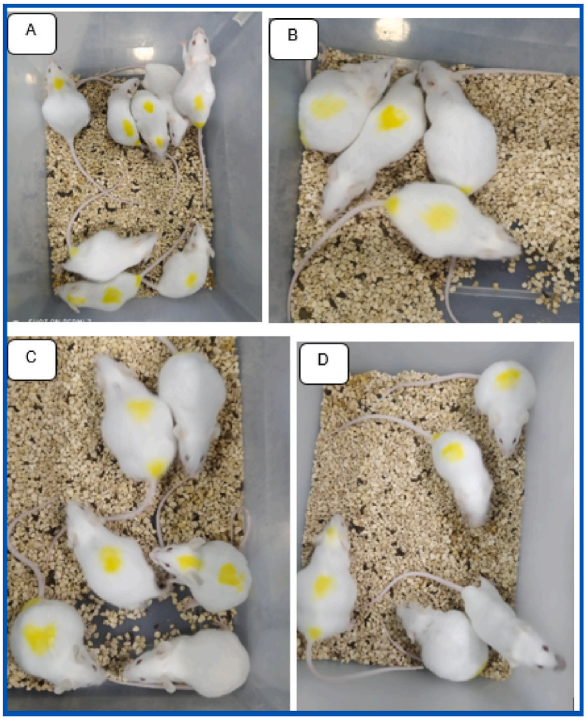

Fig. 5. Images of treated mice: (A) Cisplatin 3.5 mg/kg, (B) SL40 mg/kg, (C) SL80 mg/kg and (D) SL160 mg/kg.

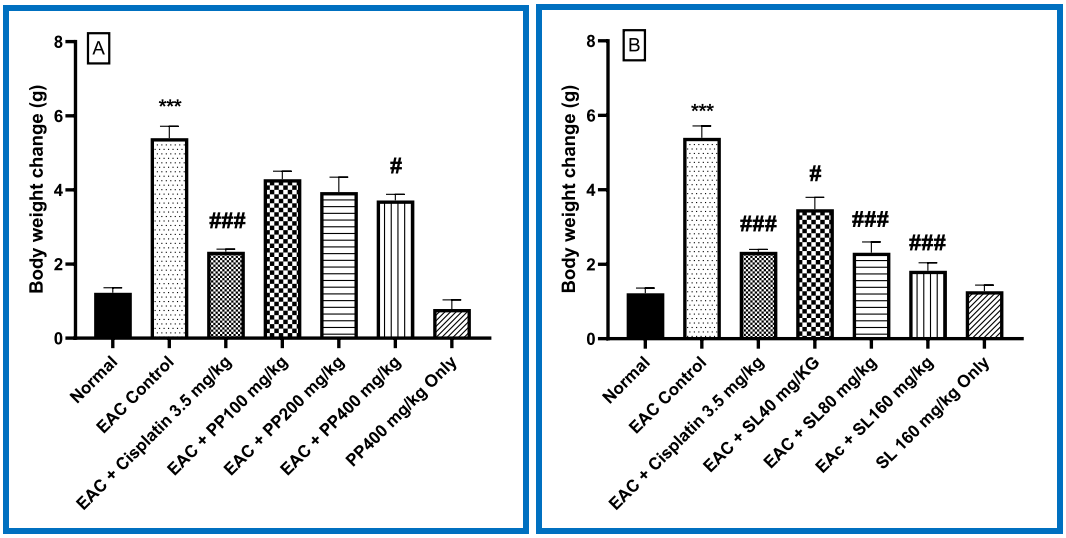

Fig. 6. Effect of extracts on body weight change. (A) P. *pinnata* (PP), (B) S. *longipedunculata* (SL). Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. ***p < 0.001: EAC Control vs Normal #p < 0.05; ###p < 0.001: Treated groups vs EAC Control.

growth factor (VEGF), which is intrinsically involved in tumor vascularization and angiogenesis. Ascites fluid is necessary for cancer cells and thus a direct source of nutrients. As a result, a rapid increase in this fluid corresponds to exponential tumor growth [37].

A substance or plant extract that can reduce the volume of ascites fluid and living cells while increasing the number of dead cells could be a good candidate for anticancer drug development. This substance would increase the lifespan of tumor-bearing mice, aiding in the fight against cancer [21,22]. Indeed, it is widely acknowledged that one of the obvious criteria for assessing the efficacy of anti-cancer drugs is increased lifespan. A percentage of ILS greater than 25%, according to National Cancer Institute criteria, indicates that the drug has significant antitumor activity [38,39]. Our current findings indicate that administering extracts orally had a positive

Table 4
Effect of *Paullinia pinnata* extract.

Parameters	EAC Control	EAC + Cisplatin 3.5 mg/kg	EAC + PP100 mg/kg	EAC + PP200 mg/kg	EAC + PP400 mg/kg
Tumor volume (ml)	6.12 ± 0.55	2.18 ± 0.42***	5.52 ± 0.41	4.15 ± 0.58*	3.56 ± 0.21**
Viable cells (x10^7 cells)	7.79 ± 0.88	2.47 ± 0.65***	6.29 ± 0.49	5.19 ± 0.54*	4.96 ± 0.48*
Nonviable cells (x10^7 cells)	0.48 ± 0.15	2.83 ± 0.38***	0.85 ± 0.12	1.50 ± 0.30	1.89 ± 0.15*
Viability (%)	94.19	46.60	88.09	77.58	72.41
MST (days)	19.5	37	21.5	22	24
%ILS (%)	–	89.74	10.23	12.82	23.08

Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. Treated groups vs EAC Control: *P < 0.05, **P < 0.01, ***P < 0.001. MST = Mean Survival Time, %ILS = Percentage Increase Life Span.

Table 5
Effect of *Securidaca longipedunculata* extract.

Parameters	EAC Control	EAC + Cisplatin 3,5 mg/kg	EAC + SL40 mg/kg	EAC + SL80 mg/kg	EAC + SL160 mg/kg
Tumor volume (ml)	6.12 ± 0.55	2.18 ± 0.42***	4.50 ± 0.34	2.84 ± 0.26***	2.77 ± 0.43***
Viable cells (x10^7 cells)	7.79 ± 0.88	2.47 ± 0.65***	3.84 ± 0.29***	2.51 ± 0.18***	2.36 ± 0.25***
Nonviable cells (x10^7 cells)	0.48 ± 0.15	2.83 ± 0.38***	1.36 ± 0.22	2.05 ± 0.31**	2.86 ± 0.35***
Viability (%)	94.19	46.60	73.85	55.04	44.36
MST (days)	19.5	37	28	29.5	32.5
%ILS (%)	–	89.74	43.59	51.28	66.67

Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. Treated groups vs EAC Control: **P < 0.01, ***P < 0.001. MST = Mean Survival Time, %ILS = Percentage Increase Life Span.

effect on survival parameters. The percentage ILS of *S. longipedunculata* extract treated animals was greater than 25%, meeting the anticancer substance criterion [40].

However, aside from its beneficial effect on tumor volume reduction, the extract of *P. pinnata* did not increase lifespan (%ILS <25%). Furthermore, even at high therapeutic doses, the viability of cancer cells treated with *P. pinnata* remained constant, highlighting a presumed minor antiproliferative activity previously observed *in vitro* on cancer cell lines.

Nonetheless, the anti-angiogenic effect as measured by tumor volume reduction is consistent with our findings from presumptive anti-angiogenic tests [9]. *P. pinnata's* antiproliferative effect in this study could be attributed to its polyphenol-rich composition. These compounds are well known for their antioxidant and antitumor properties [19].

The cytotoxic activity of plant-derived products may be due to either direct cytolytic effects on the tumor inducing apoptosis or inhibition of tumor neovascularization.

This inhibition causes the tumor microenvironment to be destroyed, resulting in a decrease in ascitic fluid volume [19,37]. *P. pinnata* extract significantly reduced tumor volume without improving mouse survival, according to our findings. *S. longipedunculata* extract, on the other hand, reduced tumor volume, improved mouse survival by more than 25% at a low therapeutic dose, and then reduced cell viability to levels comparable to the standard drug Cisplatin. These *in vivo* findings are consistent with those obtained *in vitro* [9] and may be explained in part by the strong anti-inflammatory properties of *S. longipedunculata* extract, as well as its anti-angiogenic and cytotoxic properties.

4.4. Extracts' effects on hematological parameters

Myelosuppression and anemia are major challenges in cancer chemotherapy. These issues are primarily caused by iron deficiency, hemolysis, or myelopathy, which results in a decrease in red blood cell or hemoglobin content. Tables 6 and 7 show the results of

Table 6
Effect of P. *pinnata* extract on hematological parameters.

Parameters	Normal	EAC Control	EAC + Cisplatin 3,5 mg/kg	EAC + PP100 mg/kg	EAC + PP200 mg/kg	EAC + PP400 mg/kg
HGB	13.23 ± 0.82	9.53 ± 0.76***	12.39 ± 0.91$^{\#\#}$	10.00 ± 0.50	12.47 ± 0.63$^{\#\#}$	12.51 ± 0.39$^{\#\#}$
HCT	45.63 ± 1.44	43.4 ± 4.95	52.03 ± 3.03	54.43 ± 2.14	45.1 ± 2.68	53.7 ± 2.00
RBC	7.30 ± 0.58	4.71 ± 0.30***	6.50 ± 0.78$^{\#}$	5.17 ± 0.28	6.09 ± 0.51	6.82 ± 0.62$^{\#\#}$
WBC	5.53 ± 0.62	8.92 ± 0.38**	5.97 ± 0.38$^{\#\#}$	7.01 ± 0.58	6.53 ± 0.24$^{\#}$	5.83 ± 0.54$^{\#\#}$
%Mon	1.91 ± 0.27	0.91 ± 0.22	2.18 ± 0.55	1.87 ± 0.71	1.38 ± 0.54	1.73 ± 0.59
%Neu	9.63 ± 0.54	68.3 ± 2.56***	20.77 ± 1.89$^{\#\#\#}$	48.1 ± 1.30$^{\#\#}$	40.96 ± 1.51$^{\#\#}$	31.47 ± 2.18$^{\#\#\#}$
%Lym	87.96 ± 3.02	30.6 ± 1.55***	76.87 ± 2.38$^{\#\#\#}$	49.83 ± 1.12$^{\#\#}$	52.8 ± 1.62$^{\#\#}$	66.60 ± 1.6$^{\#\#\#}$

Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. **P < 0.01, ***p < 0.001: EAC Control vs Normal; #p < 0.05; ##p < 0.01; ###p < 0.001: Treated groups vs EAC Control. HGB (g/dL), HCT (%), RBC (106 cells/μL), WBC (103 cells/μL), Mon (%), Neu (%) and Lym (%).

hematological parameters. These findings show that the EAC control group has a significant decrease ($P < 0.05$) in hemoglobin level and red blood cells ($P < 0.01$) and a significant increase ($P < 0.001$) in white blood cells when compared to the Normal group. Treatment with both extracts brought back the values to normal (Tables 6 and 7). These findings indicate that the extracts have a protective effect on the hematopoietic system. Several studies have confirmed that several plant extracts contribute to myelotoxicity reduction by stimulating the immune system and scavenging free radicals [21]. In the differential immune cell count, lymphocytes decreased and neutrophils increased significantly ($P < 0.001$) in the EAC control group compared to the Normal group. This increase in neutrophils could be due to an acute inflammatory response or stress caused by cancer cell proliferation [41]. Treatment with extracts resulted in a significant change ($p < 0.001$) towards normal values (Tables 6 and 7). This decrease in neutrophil levels in treated groups could be attributed to an immunostimulatory effect of the extracts as well as the host organism's defense against tumor cells. These findings point to the extracts having significant anti-inflammatory activity. The current findings corroborated our previous *in vitro* studies in which these extracts demonstrated antioxidant and anti-inflammatory potential [9]. Ojewole [42] and Gbadamosi, Adeyi [35] found that the extract of *S. longipedunculata* had significant anti-inflammatory activity *in vivo*, confirming our findings. Aiyelero, Salawu [43] also found that *P. pinnata* had anti-inflammatory activity in a carrageenan-induced paw edema model.

4.5. Effect of extracts on mice relative liver weight

To assess the toxicity of any new compound, vital organs such as the liver and kidney must be examined. Several parameters, such as relative liver weight, can be used to assess this [44]. The results are shown in Fig. 7. When compared to control groups, repeated administration of extracts over 15 days had no significant negative impact on the relative weight of the liver in all treated groups. Indeed, an increase in the relative weight of the liver after repeated administration of a substance indicates toxicity. As a result, our findings support the safety of the extracts on the liver during the period of study.

4.5.1. Effects of repeated extract administration in healthy mice

Acute and subacute toxicity testing is required prior to any pharmacological validation and development of a phytomedicine from any medicinal plant, according to standard guidelines [45]. As a result, toxicity provides accurate information on potentially relevant adverse effects for the substance under consideration. Table 8 shows the results of repeated administration of the extracts. The administration of the highest therapeutic dose of each extract on a daily basis for 14 days resulted in no significant changes in the hematological parameters of mice. These findings indicate that at therapeutic doses, there is no toxicity on the hematopoietic system of healthy mice.

4.6. In vivo effects of extracts on oxidative stress markers

Fig. 8 shows the effects of extracts on endogenous antioxidants. *In vivo*, oxidative stress can cause lipid peroxidation and damage vital macromolecules such as lipids. Thus, in carcinogenesis, the decrease in the level of endogenous antioxidant enzymes following the generation of free radicals and malondialdehyde (MDA) is well documented [22]. Reduced glutathione (GSH), a potent antioxidant and powerful inhibitor of neoplastic processes, is also found in low concentrations during tumorogenesis. The primary function of free radical scavenging enzymes like SOD and CAT is to protect cells from superoxide anions and hydrogen peroxide.

In carcinogenesis, inhibition of this function has also been reported [18]. Tumor development significantly increased ($P < 0.001$) the level of malondialdehyde in the EAC control group compared to the Normal group in the current study (Fig. 8A and D). GSH and CAT levels in the EAC control group were significantly ($P < 0.001$ and $P < 0.01$ respectively) lower than in the Normal group (Fig. 8B, E, C and F). Treatment with extracts and a standard drug (Cisplatin) reversed the trends and returned the values near to normal. When compared to the EAC control group, cisplatin significantly reduced MDA ($P < 0.01$) and increased catalase activity ($P < 0.05$).

P. *pinnata* extracts at 200 mg/kg and 400 mg/kg significantly reduced MDA while increasing GSH and CAT levels when compared to the EAC control group. Similarly, at a therapeutic dose of 400 mg/kg in healthy mice, *P. pinnata* extract significantly improved ($P < 0.001$) endogenous antioxidants. This *in vivo* antioxidant activity is consistent with our *in vitro* results, particularly the extract's high polyphenol and flavonoid content and strong antioxidant power [9]. These findings support those of Jimoh, Sofidiya [46] and Aiyelero, Salawu [43] studies on antioxidant and anti-inflammatory activities *in vitro* and *in vivo*. *S. longipedunculata* extract, on the other hand, had no effect on endogenous antioxidants at any concentration. These findings are consistent with those obtained *in vitro* during antioxidant tests using DPPH, FRAP, and Total Antioxidant Capacity (TAC) [9]. Similar values of antioxidant potency were also obtained by Obasi [47]. It appears important to emphasize that a significant improvement in endogenous antioxidants was observed at the maximum therapeutic dose of SL administered to healthy mice over 14 days of experimentation (Fig. 8). These findings suggest that SL lacks the antioxidant power to produce significant results during tumor development, when oxidative stress is at its peak. This could be explained by *S. longipedunculata's* low content of polyphenols and flavonoids [9,48]. The extraction solvent used is a determining factor that influences the content of extracts in bioactive compounds and thus determines antioxidant, anti-inflammatory, and even toxicity activity. Obasi (2020) found that polyphenol content varies as follows: ethylacetate (147.52 mg/g) versus ethanol (76.75 mg/g) and water (45.09 mg/g). The hydroethanolic extract used in the study had 69.44 ± 8.88 mg gallic acid/g [9]. This low polyphenol content could explain the antioxidant power observed *in vitro* and *in vivo*.

5. Conclusion

Current *in vitro* and *in vivo* studies using four human cancer cell lines and an EAC tumor-bearing mouse model, respectively, have

Table 7
Effect of S. *longipedunculata* extract on hematological parameters.

Parameters	Normal	EAC control	EAC + Cisplatin 3,5 mg/kg	EAC + SL40 mg/kg	EAC + SL80 mg/kg	EAC + SL160 mg/kg
HGB	13.23 ± 0.82	9.53 ± 0.76***	12.39 ± 0.91$^{\#\#}$	11.93 ± 0.91$^{\#}$	12.07 ± 0.86$^{\#}$	13.07 ± 0.64$^{\#\#\#}$
HTC	45.63 ± 1.44	43.4 ± 4.95	52.03 ± 3.03	46.47 ± 3.28	46.07 ± 1.11	55.8 ± 0.70$^{\#}$
RBC	7.630 ± 0.58	4.71 ± 0.30***	6.50 ± 0.78$^{\#}$	6.23 ± 0.46	6.88 ± 0.89$^{\#\#}$	7.73 ± 0.82$^{\#\#\#}$
WBC	5.53 ± 0.62	8.92 ± 0.38**	5.97 ± 0.38$^{\#\#}$	6.42 ± 0.93$^{\#}$	6.54 ± 0.46$^{\#}$	5.88 ± 0.58$^{\#\#}$
%Mon	1.91 ± 0.27	0.91 ± 0.22	2.18 ± 0.55	1.5 ± 0.58	1.4 ± 0.26	0.9 ± 0.40
%Neu	9.63 ± 0.54	68.3 ± 1.58***	20.77 ± 1.89$^{\#\#}$	40.6 ± 1.75$^{\#\#}$	21.7 ± 1.70$^{\#\#\#}$	20.73 ± 2.89$^{\#\#\#}$
%Lym	87.96 ± 3.02	30.6 ± 1.55***	76.87 ± 2.38$^{\#\#\#}$	57.7 ± 2.30$^{\#\#\#}$	76.7 ± 3.30$^{\#\#\#}$	80.83 ± 2.32$^{\#\#\#}$

Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. Values are expressed as mean ± S.E. M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. **P < 0.01, ***p < 0.001: EAC Control vs Normal; #p < 0.05; ##p < 0.01; ###p < 0.001: Treated groups vs EAC Control. HGB (g/dL), HCT (%), RBC (106 cells/μL), WBC (103 cells/μL), Mon (%), Neu (%) and Lym (%).

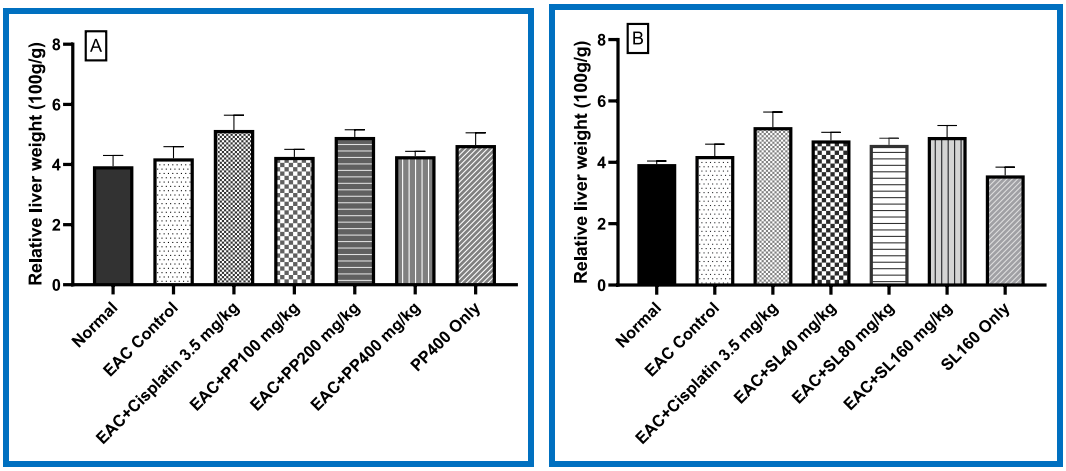

Fig.7. Effect of PP and SL extracts on relative liver weight of EAC mice. (A): P. *pinnata*; (B): S. *longipedunculata*. Values are expressed as mean ± S.E. M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test.

Table 8
Effects of extracts on hematological parameters of healthy mice.

Parameters	Normal	PP400 mg/kg	SL160 mg/kg
HGB	13.23 ± 0.82	12.62 ± 1.10	13.30 ± 1.00
HCT	45.63 ± 1.44	50.53 ± 2.04	43.87 ± 2.27
RBC	7.30 ± 0.58	7.09 ± 0.63	7.70 ± 0.82
WBC	5.53 ± 0.62	5.49 ± 0.35	5.88 ± 0.67
%Mon	1.91 ± 0.27	1.95 ± 0.71	0.73 ± 0.29
%Neu	9.63 ± 0.54	19.40 ± 2,54	15.1 ± 2.50
%Lym	87.96 ± 3.02	73.13 ± 2.84	83.97 ± 2.61

Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. HGB (g/dL), HCT (%), RBC (106 cells/μL), WBC (103 cells/μL), Mon (%), Neu (%) and Lym (%).

shown that the PP and SL extracts possess beneficial health effects in cancer. *S. longipedunculata* extract was found to significantly reduce tumor growth *in vitro* and *in vivo* in this study. This extract also reduced cell viability and normalized hematological parameters while exhibiting strong anti-inflammatory activity comparable to reference drugs. It also increased the lifespan of mice with tumors. This is a deciding factor that confirms its ability to fight cancer. However, it has not demonstrated sufficient antioxidant activity to reduce oxidative stress during tumor development. *P. pinnata*, in addition to its antioxidant and anti-inflammatory actions *in vitro* and *in vivo*, demonstrated antiangiogenic activity by decreasing tumor volume. Toxicity studies have revealed that the extracts at the tested dose have no adverse effects on health during the experiment. Furthermore, studies are being conducted to assess the molecular effects of these extracts on cancer target genes as well as the active principle (s) involved in demonstrating antitumor, antioxidant, and anti-inflammatory properties. Our group is working on molecular studies that could lead to the development of antitumor herbal formulations.

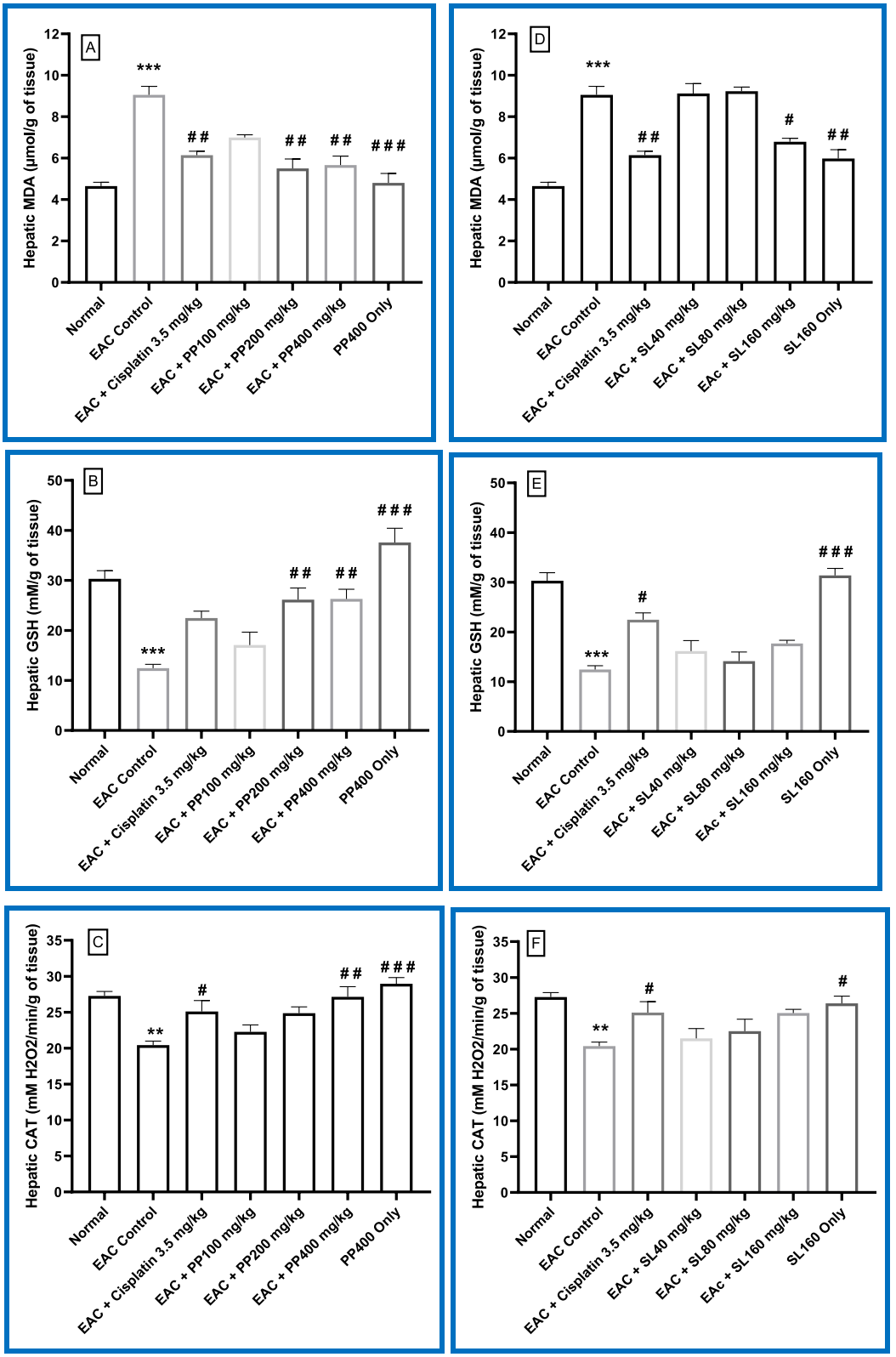

Fig. 8. Effect of extracts on MDA, GSH and CAT. A to C: P. *pinnata*; D to F: S. *longipedunculata*. Values are expressed as mean ± S.E.M. (n = 6); One-way ANOVA followed by Tukey's multiple comparison test. **p < 0.01; ***p < 0.001: EAC Control vs Normal #p < 0.05; ##p < 0.01; ###p < 0.001: Treated groups vs EAC Control.

Data availability

The data used to support the findings of this study are included in the article.

Authorship contribution statement

P. Kola: PhD Scholar, designed protocols, performed the experiments, Formal analysis, Writing – original draft, analyzed the data, and drafted the manuscript. **K. Metowogo:** Thesis Supervisor, designed the study, supervised and validated the study, gave final approval to the manuscript. **S.N. Manjula**: Supervision of the whole study, provided chemicals, animals and facilities, validated results, and reviewed the manuscript. **S. V. Madhunapantula:** Biochemistry Department**,** Leader of Special Interest Group in Cancer Biology and Cancer Stem Cells (SIG-CBCSC), provided facilities, supervised *in vitro* studies, and validated results. **K. Eklu-Gadegbeku**: Head of the Research Unit, provided administrative support.

Funding statement

This work was partly supported by TWAS Fellowship for Research and Advanced Training (Award 2020 FR Number: 3240313434).

Declaration of competing interests

We wish to confirm that there have no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgment

Poyodi Kola testify his gratitude to University of Lomé and to JSS Academy of Higher Education & Research institutions mainly to Pharmacology Department, JSS College of Pharmacy, Mysuru, to Central Animal Facility - Centre for Experimental Pharmacology and Toxicology, JSS College of Pharmacy, Mysuru and to Centre of Excellence in Molecular Biology and Regenerative Medicine (CEMR) - Department of Biochemistry, JSS Medical College, Mysuru, for providing the necessary support and facilities to carry out this research. Special acknowledgment to **Professor Saravana Babu Chidambaram**, Coordinator Central Animal Facility, JSS Academy of Higher Education & Research, Karnataka, Mysuru for the facilities during our *in vivo* studies.

Abbreviations

CAT	Catalase
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Diméthyl sulfoxide
DPPH	2,2-dyphenyl-1-picrylhydrazyl
EAC	Ehrlich Ascites Carcinoma
FRAP	Ferric Reducing Antioxidant Power
GSH	Glutathione
IAEC	Institutional Animal Ethics Committee
IARC	International Agency for Research on Cancer
IC50	Inhibition Concentration 50
ILS	Increase Life-Span
MDA	Malondialdehyde
MST	Median Survival Time
NCCS	National Centre for Cell Science
NCI	National Cancer Institute
OD	Optic Density
OECD	Organization for Economic Co-operation and Development
PBS	Phosphate Buffered Saline
SOD	Superoxide Dismutase
SRB	Sulforhodamine B
TAC	Total Antioxidant Capacity
TCA	Trichloroacetic Acid
VEGF	Vascular Endothelial Growth Factor

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