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PHRAGMENTHERA CAPITATA (SPRENG.) *BALLE* METHANOL LEAVES EXTRACT IS USED SAFELY TO REDUCE TUMOR GROWTH IN C57BL/6J MICE BEARING MELANOMA B16-F10

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ABSTRACT

The present study aims to assess the toxicological profile and anti-melanoma effect of methanol extract of Phragmenthera capitata (Spreng.) Balle leaves in mice. MTT assay was used for cell viability assay. Acute and sub-acute toxicity studies in BALB/C mice were carried out. In acute toxicity study, a single dose (2000 mg/kg) was administered to three female mice and any signs of toxicity, morbidity or mortality were determined. In sub-acute toxicity, PCMe-OH (50, 100 and 200 mg/kg) was administered orally to mice for 28 days. Their body weight, macroscopic organs observation and plasma parameters were evaluated. Histopathological analyses were also performed. The effect of PCMe-OH was evaluated on tumor growth in C57BL/6J mice bearing tumor by giving them a single oral dose (100 mg/kg) for 25 days. Body weight and tumor volume of animals were recorded throughout treatment. Final tumor weight was determined followed by Histopathological analyses of tumor tissue. PCMe-OH showed good cytotoxicity (IC50 = 55.35 ±1.17 µg/mL) against B16-F10 cell line. The Lethal Dose 50 (LD50) was greater than 2000 mg/kg. Few signs of toxicity at 200 mg/kg dosage was noted and histological sections reveal the presence of slight hepatic vascular congestion and fibrosis in the lung tissue. Tumor volume was significantly reduced throughout the treatment and tumor weight respectively in all treated mice. Histological sections of tumor tissues showed that PCMe-OH leads to a reduction in melanocyte proliferation and necrosis in treated mice. Therefore, Phragmenthera capitata could be used safely as a potent agent for melanoma management.

KEYWORDS: *Phragmenthera capitata*; toxicity; melanoma; melanocyte.

Abbreviations

ALT: Alanine aminotransferase AST: Aspartate aminotransferase.

DMEM: Dulbecco Modified Eagle Medium; EDTA: Ethylenediaminetetraacetic acid IC50: Half maximal inhibitory concentration LD50: median lethal dose.

LDH: Lactate dehydrogenase.

MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazo liumbromide); RPMI, Roswell Park Memorial Institute medium.

1. INTRODUCTION

In developing countries, herbal medicine is an alternative natural remedy for primary health care.[1] Fighting against many human diseases mainly requires research and discovery of therapeutic substances; the latter may be derived from the herbal medicine system.[2,3] Thereis a perception that herbal remedies, because of their natural derivation, are devoid of adverse or toxic side effects when compared with the synthetic drugs used in conventional medicine[4]; Moreover, medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity.[5]

The use of traditional and herbal medicine is widely practiced in Cameroon and *Phragmenthera capitata* (Spreng.) *Balle* is one of such plants used in Cameroon folkloric medicine. *Phragmenthera capitata* (Spreng.) *Balle* is a mistletoe plant belonging to the Loranthaceae family. It is a mandatory hemiparasite that attaches and enters the stems and branches of its host tree through a haustorium.[6,7] In Cameroon, the leaves, stems, or whole

plant claimed to cure several diseases but mostly used in case of cancers, hypertension and diabetes.[8,9] This plant is widely distributed in Cameroon and in some other African countries. Phytochemical investigations on *Phragmenthera capitata* (Spreng.) *Balle* revealed the presence of the presence of flavonoids, polyphenols, triterpenes, tannins, saponins, and anthraquinones in the leaves justifying therefore their biological activities. Previous studies demonstrated that extracts from this plant have many biological activities, such as anti-inflammatory, antioxidant[10], hematopoietic potentiating[11], antibacterial, antifungal[12], antisecretory, gastroprotective, and antiulcer[13], and that they also enhance steroidogenetic and spermatogenetic activities.[14] The 3-O-β-d glucopyranosyl-28-hydroxy-α-amyrin isolated from methanolic extract of the leaves of *Phragmenthera capitata* (Spreng.) *Balle* have shown good cytotoxic potential on cancerous cells and had better selectivity index values than doxorubicin, which is mostly used in the treatment of many cancers.[14]

Despite the traditional use of *Phragmenthera capitata* (Spreng.) *Balle*, its biological and pharmacological applications, there are insufficient data demonstrating the safety of methanol leaves extract of *Phragmenthera capitata* (Spreng.) *Balle* in treatment of melanoma. Indeed, melanoma classified as a public health hazard is one of the most aggressive cancers with a high frequency of metastasis and a relatively high mortality rate. Unfortunately, the governments of these developing countries are not well prepared to manage the growing burden of cancers[15], which is why the search for a therapeutic alternative remains an effective tool.

Therefore, scientific investigations regarding the safety of the use of this plant as an alternative medicine is very important before it can be further developed into a new medicinal herbal therapy against melanoma. The present study was aimed to evaluate the safety of *Phragmenthera capitata* (Spreng.) *Balle* leaves extract with acute and sub-acute toxicity tests in BALB/c mice and its therapeutic effect on B16F10 murine melanoma model in C57BL/6J mice.

2. MATERIAL AND METHODS

2.1. Collection and Identification of Plant Material.

Phragmenthera capitata (Spreng.) *Balle* from loranthaceae family were collected in June 2021, from the "Mifi" Division in the West Region. The collected plant was identified at the National Herbarium of Cameroon (Yaounde) under the voucher number 29913/SRF/Cam. The plant name has been checked with "World Flora Online".

2.2. Extraction of Plant Material

The dried leaves of *Phragmenthera capitata* (Spreng.) *Balle* (100 g) were ground and macerated in methanol (500 mL) at room temperature for 48 hours. During extraction, the sample was shaken repeatedly. The obtained solution was filtered using Whatman No. 1 paper. Subsequently, the solvent was recovered in a rotary evaporator (BÜCHI R-Rotavapor model R-2000) and the resulting product was dried; the filtrate obtained was evaporated using a rotary evaporator (BÜCHI R - 200) at 70 °C. The crude extract was recovered in a sterile vial and dried in an oven at 40 °C until the solvent completely evaporated. The obtained extract (PCMe-OH) was stored at 4^{o}C for further use.

2.3. Experimental Animals

After the institute animal ethics committee (IAEC) of NIPER Hyderabad approved the animal procedure under permission number NIP/10/2022/PC/492, female BALB/c and male C57BL/6J mice weighing approximately 6–8 weeks and 25–30 grams were purchased from the accredited suppliers. All the toxicity studies were conducted by strictly following Organization for Economic Cooperation and Development (OECD) guidelines. The animals were permitted free access to food and water ad libitum and the animals were housed in a 22 ± 20C temperature-controlled room with 12/12 hr light/dark cycle having relative humidity of 56 ± 15 % during experimentation period.

2.4. Acute oral toxicity study

The study was conducted by following the step-down procedure of OECD guideline 425 for acute oral toxicity assessment of *Phragmenthera capitata* methanol extract (PCMe-OH). The dose was formulated shortly before dosing. Briefly, the plant extract was weighed accordingly for 2000 mg/kg dose into a tarred plastic eppendorf and appropriate quantity of vehicle was added into it. The mixture was bath sonicated for sufficient time for the plant extract to form a uniform suspension. Six female BALB/c mice of about 6-8 weeks weighing 25-30 grams divided in two groups of 3 animals each were used for single dose acute oral toxicity analysis. In brief, methanol crude extract of *Phragmenthera capitata* at the dose of 2000 mg/kg was administered to 3 female mice through the oral route of administration according to OECD guideline 425. Following administration, tested animals were observed during 14 days for any signs of toxicity, morbidity or mortality.

2.5. Sub-acute oral toxicity study

The sub-acute toxicity was assessed according to the OECD Guideline No. 425 for chemical testing (OECD, 2008). Briefly, after one week of acclimatization, female BALB/c mice were categorized randomly into four groups of 5 animals each for repeated dose toxicity. Mice of test groups were given oral gavage of *Phragmenthera capitata* methanol extract at doses 50, 100 and 200 mg/kg daily for 28 days. Animals of the control group received only the vehicle (water + Tween 5%) daily. Animals were fed with standard diet ad libitum and were allowed free access to water.

2.5.1. Body weight estimation

During the 28 days' administration, animals' body

weights were measured every four days.

2.5.2. Collection of blood samples and organs

On 29th day, all mice were anesthetized and blood were collected at the level of the eye by puncture in the retro-orbital sinus for biochemical analysis. After sacrifice, organs (liver, kidneys, heart, lungs, pancreas, brain, spleen) were removed, and photographs were taken for macroscopic analysis.

2.5.3. Evaluation of biochemical parameters

The collected blood in dry tubes was centrifuged at 4000 rpm for 10 min. They were recovered and stored at -4°C for subsequent biochemical analyses. The following biochemical parameters were explored: aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, amylase, lipases and total proteins. Handling was carried out according to the protocol of the kit manufacturer ACCUREX BIOMEDICAL PVT. LTD. (INDIA).

2.5.4. Histopathological examination

After animal's sacrifice, liver, lungs and kidneys were collected and used for histological analyses as described by Di-Fiore. (1963) with slight modifications. Organs of interest fixed in 10% formaldehyde for 3 weeks were cut into small pieces of 5 to 10 mm, then dehydrated successively in ethanol baths of increasing concentration: 70% ethanol (30 min) → 90% ethanol (30 min) → 100% ethanol (1h). Then the organs were clarified in two baths of 20 min of xylene and embedded in paraffin (two baths of 2 hours each). The latter was eliminated by xylol before being poured into molds containing molten paraffin by heating to 60°C. After cooling the solid block of tissue containing paraffin, it was cut using a microtome to obtain sections of 5 µm thick. Sections were spread dried on steamed slides overnight. They were stained with a hematoxylin-eosin solution. After staining, a few drops of resin were placed on the sections, then the latter were covered with a thin glass slide for observation under microscope. The observation of the stained sections was made with an Olympus light microscope connected to a computer where the images were transferred and analyzed. A comparative histopathological study of the organs was performed.

2.6. Antitumor effect

2.6.1. Cell viability assay

The effect of *Phragmenthera capitata* methanol extract (PCMe-OH) on cell viability was determined using MTT assay. The cell line (B16-F10) was grown in tissue culture flasks at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity in complete growth medium. Flasks with subconfluent stage of growth were selected and cells were harvested by treatment with trypsin-EDTA. The haemocytometer was used to count the number of cells/mL in the suspension. For each cell line, cell density was appropriately adjusted to 5000 cells/100 μL in the cell suspension. 100 μL of cell suspension was added to each well of 96-well plates with the help of multichannel pipette. The plates were incubated at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity for 24 h. Afterward they were immediately treated with different concentrations of crude methanol extract ranging from 7.625 to 125 µg/mL dissolved in 1% dimethyl-sulfoxide (Sigma-Aldrich) and incubated for 48 hours. After incubation period, 100 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazoliumbromide) (5 mg/mL) was added after removing the old media to each well and the plates were incubated for 4 h. The supernatant was then carefully removed from each well plate and the formed formazan crystals were dissolved in 100 µL of DMSO and the absorbance at 570 nm wavelength was recorded using an ELx800 microplate reader (BioTek, USA). Doxorubicin was used as standard drug. The assay was repeated thrice and mean values were considered. Results were expressed as a percentage of viable cells as compared to 100% representing control cells. The IC_{50} value was calculated using nonlinear regression (curve fit) followed by log (cell viability) vs. response equation in GraphPad Prism software.

2.6.2. B16-F10 Xenograft model

The F16-B10 murine melanoma cell line was cultured on DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin (10 mL/L) until 90% confluence was obtained. Viable cells were resuspended in fresh PBS at a concentration of 2×10^6 cells/200 µL, and injected subcutaneously into the right dorsal flank of the mouse paw. Male C57BL/6J Mice were randomly allocated into the following two groups (n = 5) after a week of acclimatization: the control group (1× PBS) and the treated group (100 mg/kg). Every five days, the tumor volume and the body weight of mice were measured. After that, mice were sacrificed, and tumors were collected for weight and histological analyses.

Data Analysis

The results obtained were expressed as the mean ± standard error on the mean (SEM). The analysis was carried out using the ANOVA test (analysis of variance test) followed by Dunnet's post-test (to compare the different groups with the negative control group and the Student's t test (unpaired t-test) (to compare the negative control group to the test group. All of these analysis were carried out using the Graphpad Prism software (version 8.0). The values of probability $p < 0.05$ were considered as significant.

3. RESULTS

3.1. Acute oral toxicity study

Table I summarizes the changes observed during the administration of a single dose of 2000 mg/kg of PCMe-OH in female BALB/C mice. A decrease in locomotion was observed during the 4 hours following administration. Apart from this observation, no deaths were observed in the experimental animals, which continued to lead apparently normal lives. No other clinically detectable signs were observed 48 hours after administration of the

extract or during the 14 days of observation.

Table I: Effect of the extract on the behavior of mice after administration of a single dose of the methanol extract of *Phragmenthera capitata*.

	Extract	
Parameters of study	**Normal control (0 mg/kg)**	**Test (2000 mg/kg)**
Reaction to noise	N	N
Pinch reaction	N	N
Tail state	N	N
Seizures	A	A
Locomotion	N	D
Sleep	A	A
Mortality after 48 hours	0	0
Mortality after 14 days	0	0
Number of spleens used	3	3

N = Normal; D = decreased; A = absent; 0 = Nothing

Therefore, the extract was deemed to be safe at dose of 2000 mg/kg, and the median lethal dose(LD50) was considered to be > 2000 mg/kg.

Macroscopic examination of the various organs (lungs, liver, kidneys, brain, pancreas, and spleen) removed following the autopsy carried out 14 days after administration of the extract revealed that no pathological changes was observed in the treated mice compared to the controlmouse (see figure 1 below).

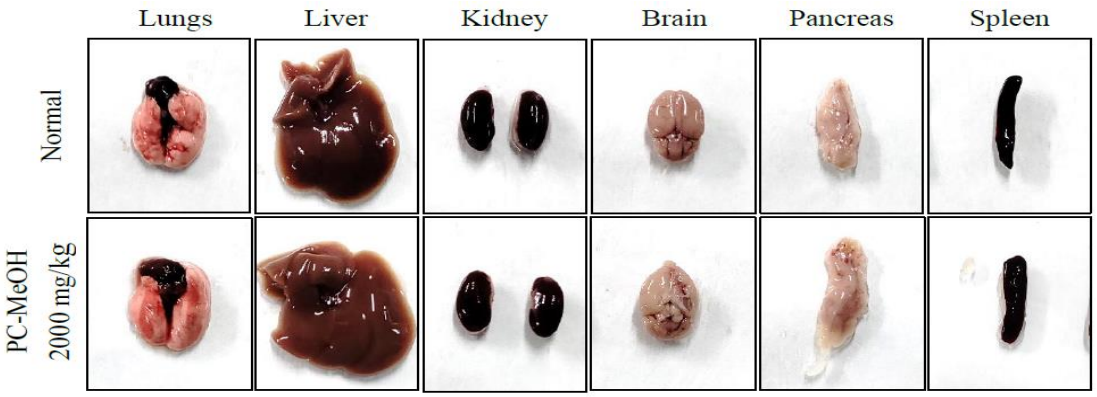


Fig. 1: Effect of extract on the organs of treated mice during acute toxicity.

3.2. Sub-acute toxicity study

*3.2.1.*Effect of extract on mice body weight

We observed that all mice receiving the extract at different doses (50, 100, and 200 mg/kg) havea significantly (**** p <0, 0001) low body weight compared to control mice (Figure 2). However, throughout the administration of the extract, the body weight of the treated mice gradually increased from day 16 to day 28. In addition, the body weight decreases at the start of administration in the treated mice, while in the control mice, it increases gradually until day28.

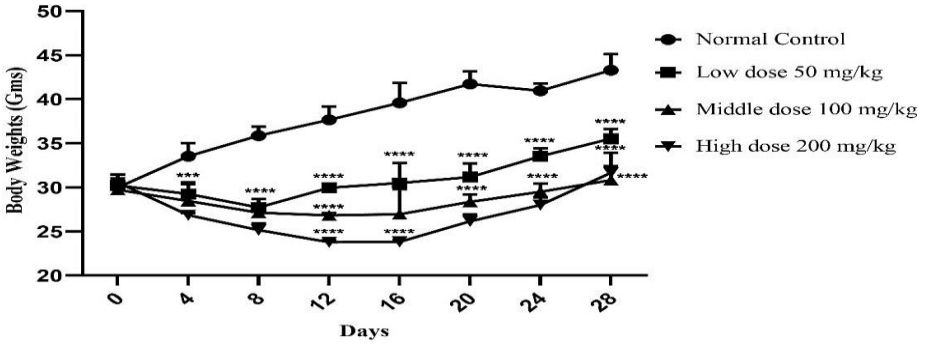


Fig. 2: Effect of the methanol extract of *Phragmenthera capitata* on animal's body weight during 28 days of administration. Each point presents the mean ± sm of 5 animals per group. Values significantly different from control at **** p <0, 0001.

3.2.2. Macroscopic observation of organs

Figure 3 shows the macroscopic examination of various organs (lungs, liver, kidneys, brain, pancreas, and spleen). It revealed that no pathological changes were observed in various organs in the treated mice compared to the control mice.

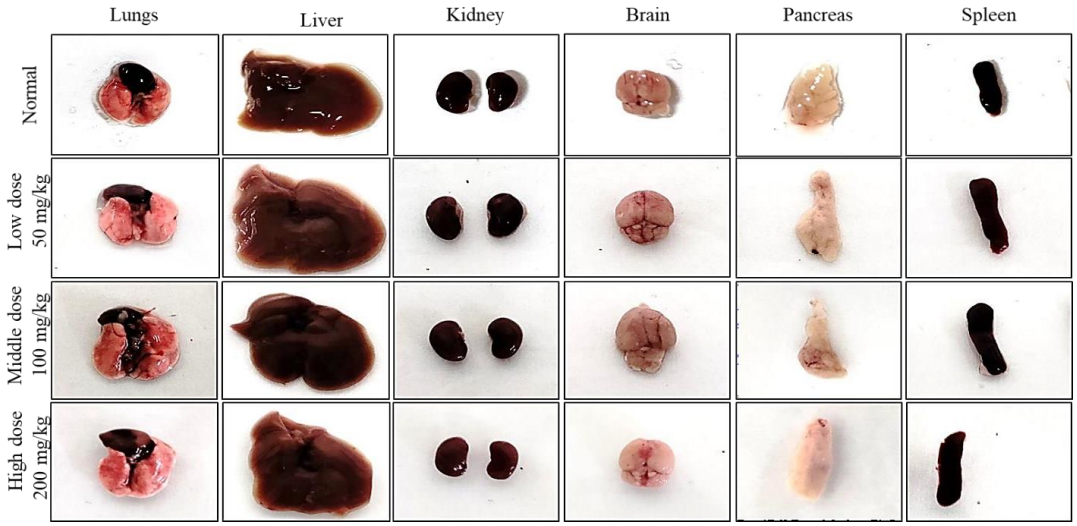


Fig. 3: Effect of extract on organs of treated mice during sub-acute toxicity.

3.2.3. Effect of extract on plasma parameters

The result of the effect of sub-chronic exposure of mice to PCMe-OH on the plasma parameters of female mice is presented in figure 4 below. It appears that the methanol extract caused a significant increase ($p < 0.05$) in the level of ALT in mice at a concentration of 200 mg/kg; on the other hand, the extract had no significant effect on the variation of the AST level. Moreover, the extract caused a significant increase ($p < 0.05$) in the level of total proteins in the animals at the dose of 200 mg/kg compared to the control group. The animals that received the extract at a dose of 200 mg/kg of body weight showed a significant increase ($p < 0.05$) in the levels of albumin, amylase, and lipase; however, the extract at doses of 50 and 100 mg/kg had no significant effect on these parameters when compared to the control group.

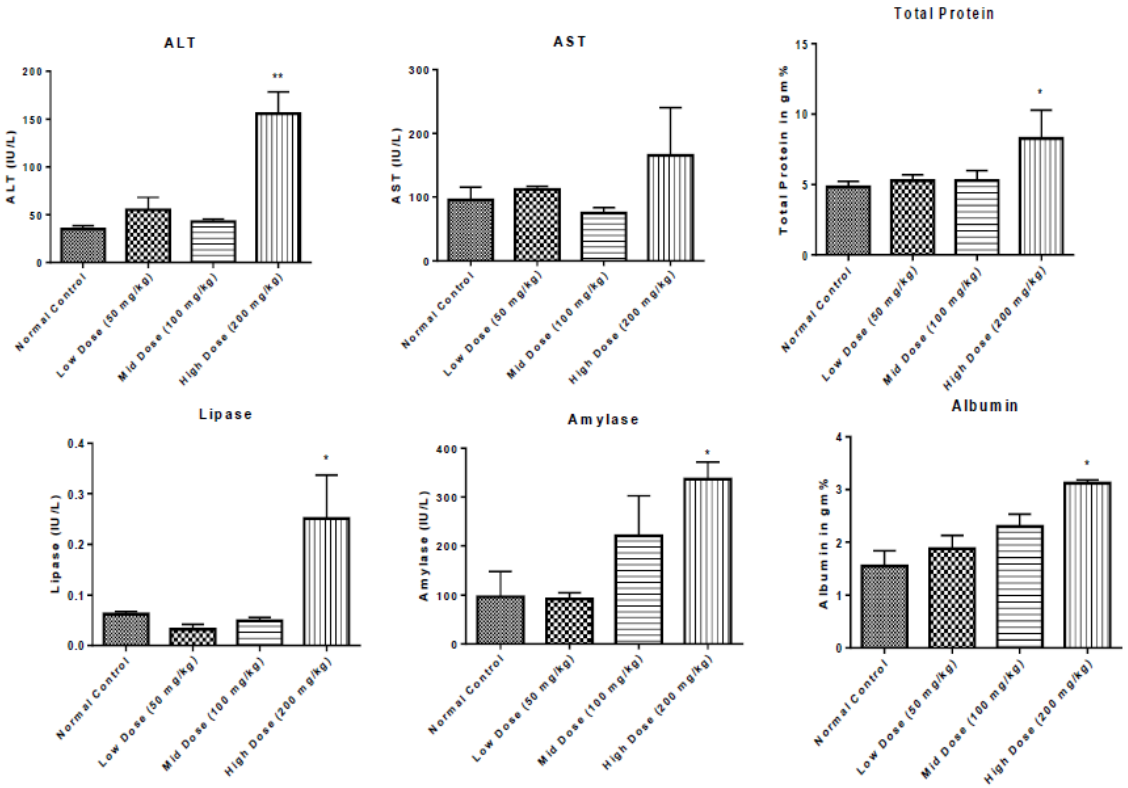
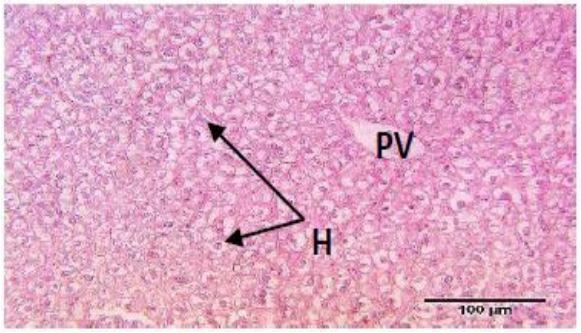


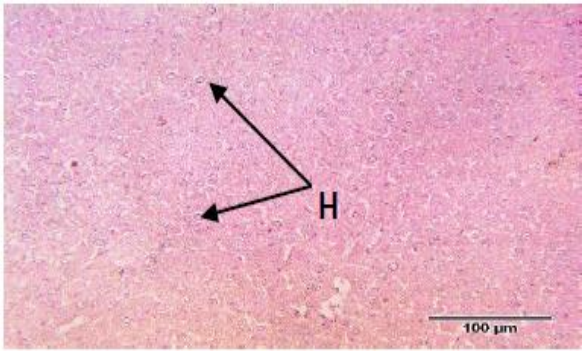
Fig. 4: Dosage of some biochemical parameters in the test groups compared to the control group. Graphical representation showing the mean ± SEM of three independent experiments. *p<0.05, ***p<0.001 compared to control.

*3.2.4.*Histopathological examinations

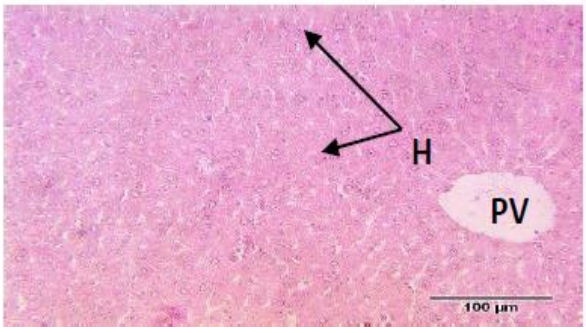
Histopathological examinations were performed on the liver, kidney and lung, to assess whether or not organs or tissues had been damaged (see figures 5, 6 and 7 below). The PCMe-OH at a dose of 200 mg/kg revealed the presence of slight vascular congestion in the liver tissue. Moreover, weak lesions were noted on the glomerular architecture in the kidney tissue.*Phragmenthera capitata* leaves extract at a dose of 200 mg/kg showed early pulmonary fibrosis. In summary, at the doses of 50 or 100mg/kg we found that none of the organs of the female mice showed any morphological alterations or abnormalities under the light microscope. Nevertheless, at the dose of 200 mg/kg some minor alterations were observed.



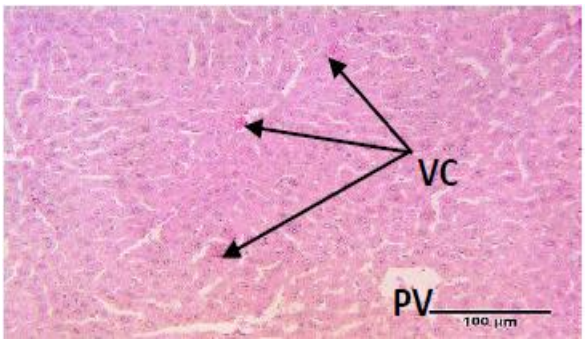
Normal control (0 mg/kg): Normal architecture of liver tissue; normal hepatic portal vein (PV), normal hepatocytes (H).



Low Dose (50 mg/kg): Normal architecture of liver tissue; normal hepatocytes (H**).**

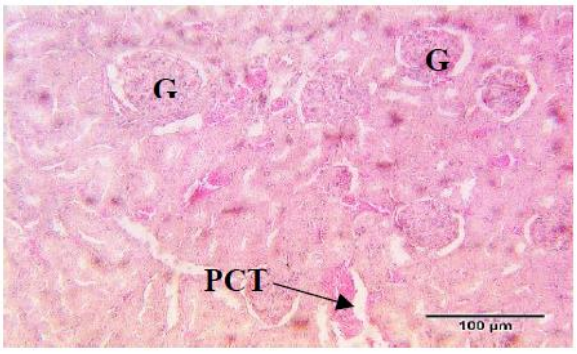


Middle Dose (100 mg/kg): Normal architecture of liver tissue; normal hepatic portal vein (PV), normal hepatocytes (H).

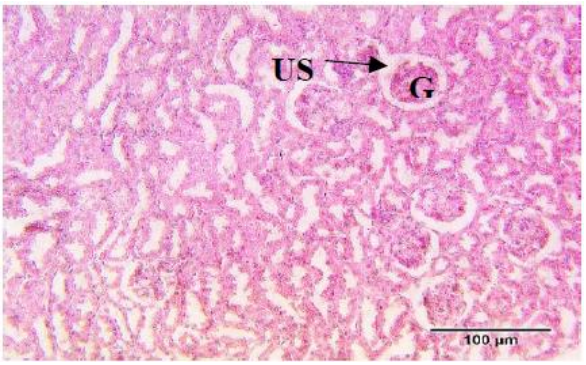


Low Dose (50 mg/kg): Normal architecture of liver tissue; normal hepatocytes (H**).**

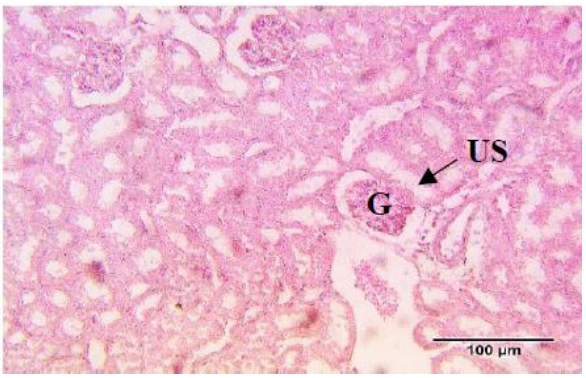
Fig. 5: Photomicrographs (hematoxylin-eosin staining, 20X) of liver of female BALB/C mice after 28-day exposure to *Phragmenthera capitata* methanol extract.



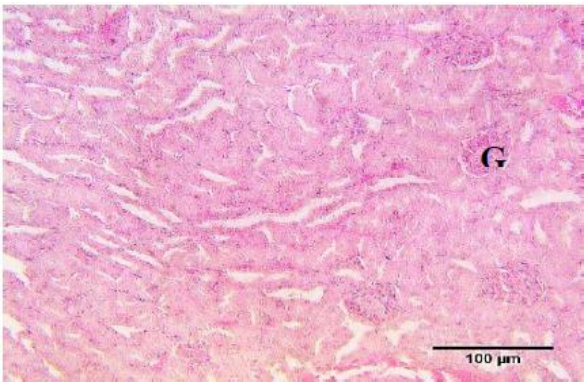
Normal control (0 mg/kg): Normal architecture of kidney tissue; proximal convoluted tubule (PCT)glomerulus (G)



Low Dose (50 mg/kg): Normal architecture of kidney tissue; urinary space (EU) glomerulus (G)

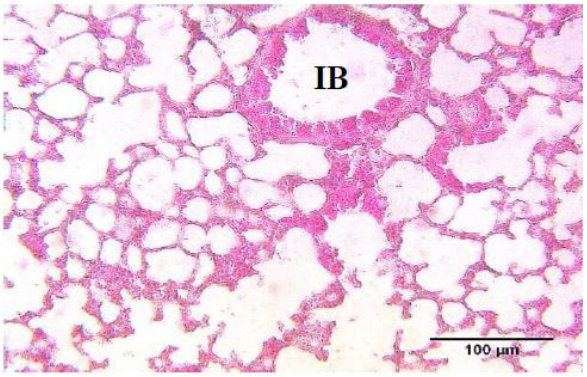


Middle Dose (100 mg/kg): Normal architecture of kidney tissue; urinary space (EU) glomerulus (G).

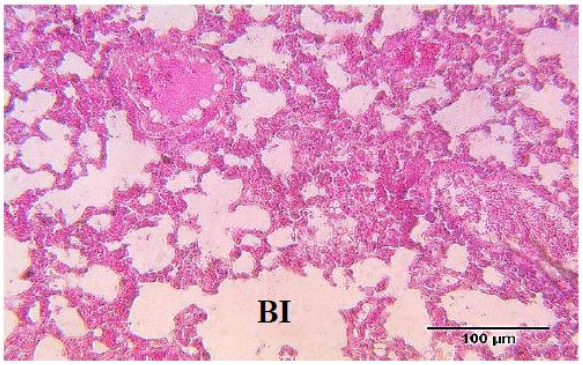


High Dose (200 mg/kg): Normal architecture of kidney tissue; glomerulus (G).

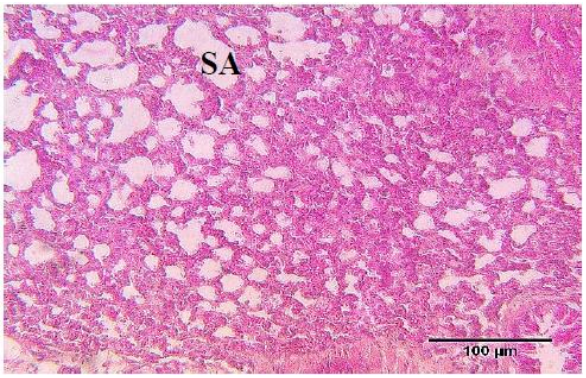
Fig. 6: Photomicrographs (hematoxylin-eosin staining, 20X) of kidney of female BALB/C mice after 28-day exposure to *Phragmenthera capitata* methanol extract.



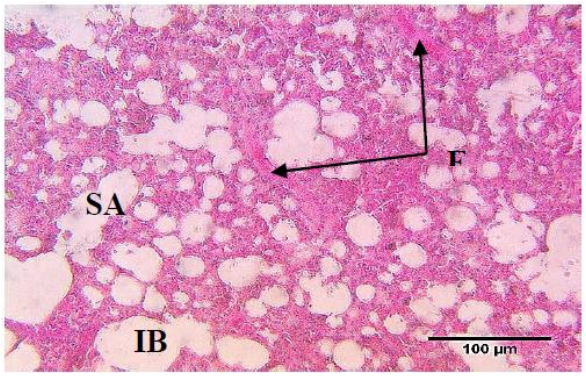
Normal control (0 mg/kg): Normal architecture of lung tissue; interlobular bronchus (IB).



Low Dose (50 mg/kg): Normal architecture of lung tissue; interlobular bronchus (IB).



Middle Dose (100 mg/kg): Normal architecture of lung tissue; satellite artery (SA).



High Dose (200 mg/kg): Normal lung tissue architecture with slight fibrosis (F).

Fig. 7: Photomicrographs (hematoxylin-eosin staining, 20X) of lungs of female BALB/C mice after 28-day exposure to *Phragmenthera capitata* methanol extract.

3.3. Antitumor effect of *Phragmenthera capitata* methanol extract on B16F10 murinemelanoma

*3.3.1.*Effect on B16F10 Cell viability

The outcomes from fig.8 revealed a significant decrease in cell viability in B16-F10 melanoma cells after 48 hours of exposure to methanol extract of *Phragmenthera capitata* (fig.8) at different concentrations ranging from 7.8125 to 125 ug/mL and in a dose-dependent manner. The IC_{50} value of treated B16-F10 cells was calculated to be 55.35 µg/ml.

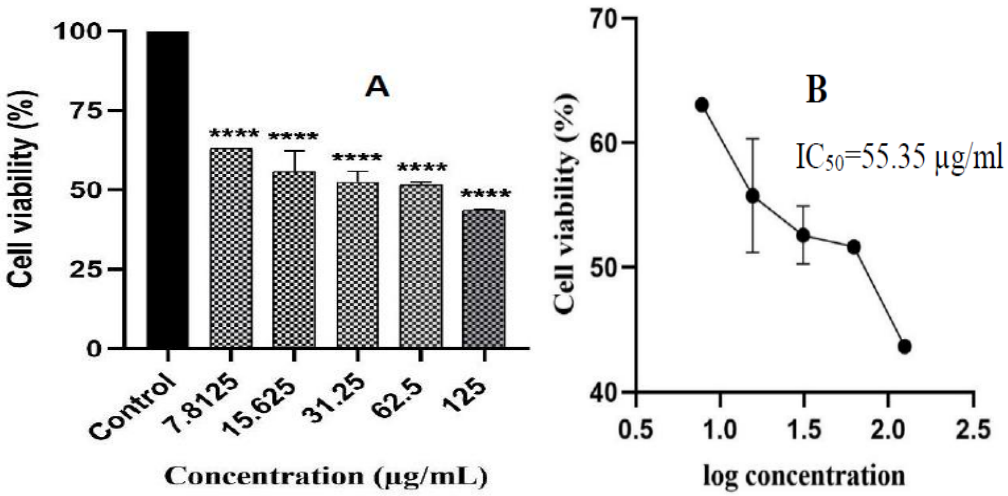


Fig. 8: Cell viability percentage of B16-F10 cells after 48h treatment with *Phragmenthera capitata* methanol extract (A). IC50 of melanoma cells treated with increasing concentration of the extract was calculated as 55.35 µg/ml using dose response curve (B). The percentage viability of extracts treated cells was calculated as absorbance of the drug-treated sample/absorbance of the non-treated sample × 100.

3.3.2. Effect of extract on body weight of C57BL/6J mice bearing melanoma

The body weight of animals bearing the tumor increased until the last day of treatment in negative control. On the other hand, a slight increase in the body weight of treated mice (100 mg/kg) was observed and remained lower in a highly significant manner (*** p <0.001) from day 20 of treatment in comparison to tumor control (Fig.9.).

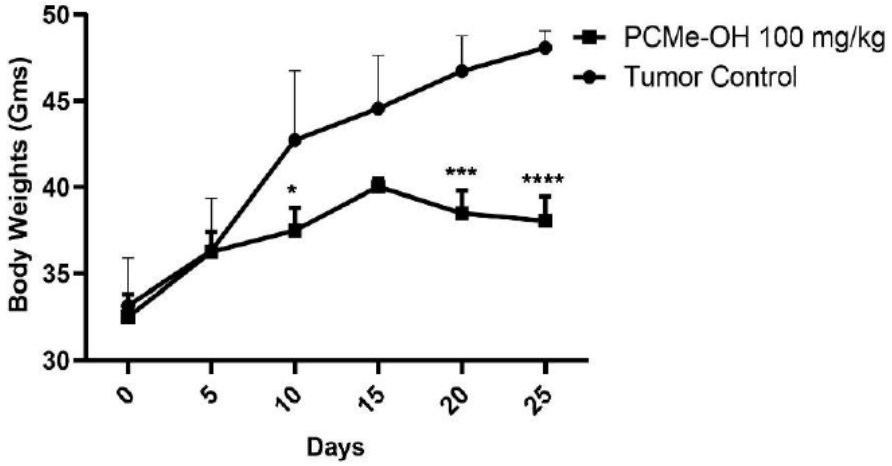


Fig. 9: Effect of the extract on the body weight of animals bearing melanoma. Each point presents the mean ± sm of 5 animals per group. Values significantly different from control at, *p<0.05, ***p<0.001, ****p<0.0001.

3.3.3. Effect of extract on tumor volume

Fig. 10 below depicts the effect of the extract on tumor volume (A) and percentage inhibition of tumor volume in animals (B) throughout treatment. It revealed that the tumor volume increased during the 25 days of treatment in tumor control mice, whereas a constant low volumeof the tumor was noted in treated mice, which remained significantly (**** p <0.0001) low from day 10 until the end of treatment in comparison to the untreated group (Tumor control). At the end of the treatment, the tumor volume decreased by 79.48 ± 1.37% in comparison to tumor control.

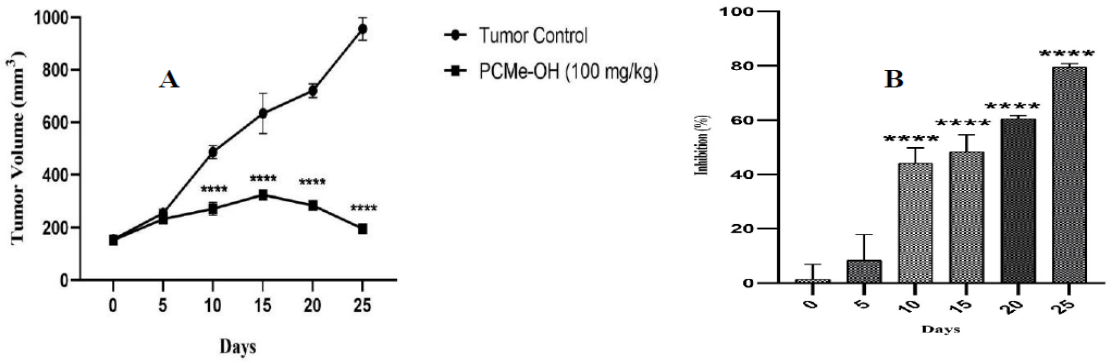


Fig. 10: Effect of extract on tumor volume of animals (A) and percentage inhibition of tumor volume in animals (B). Each point presents the mean ± sm of 5 animals per group. Values significantly different from negative control at ****p<0.0001.

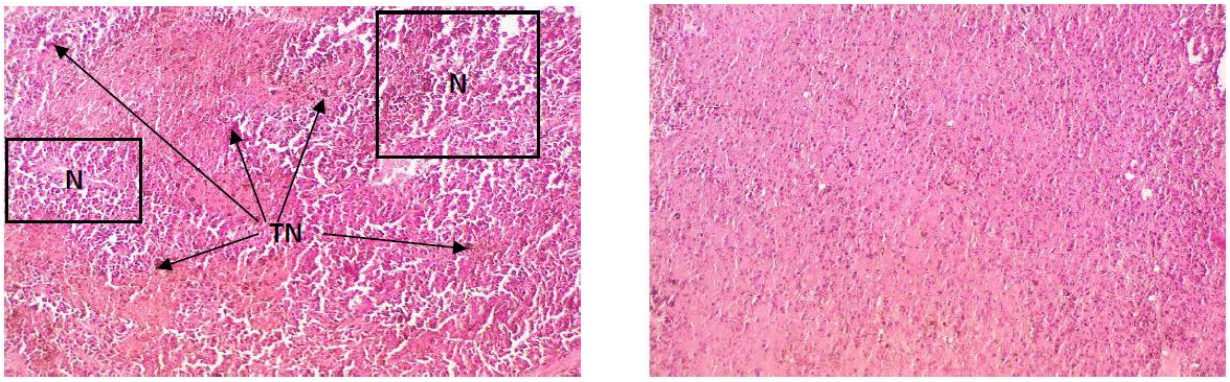
3.3.4. Effect of extract on tumor weight

Fig. 11 below represents the effect of extract on tumor weight in animals at the end of 25-days treatment. It revealed is that the extract at a dose of 100 mg/kg caused a significant reduction (* p <0.05) in tumor weight in the treated animals compared to tumor control at the end of thetreatment.

Fig. 11: Effect of extract on tumor weight (A) and images of excised tumors (B). of Each pointpresents the mean ± sm of 5 animals per group. Values significantly different from negative control at *p<0.05. PCMe-OH: *Phragmenthera capitata* methanol extract.

3.3.5. Histopathology of tumor tissue

Histopathological examinations were performed on tumor tissue to assess whether or not tissues had been repaired (fig 12). Haematoxylin & Eosin (H&E) staining showed cancerous pattern with increased melanin content and perfect Tumor nuclei in the tumor control tissues, while there is decreased melanin content and necrotic tissue observed in the treated tissue.



Tumor control (0 mg/kg): Tumor tissue architecture, tumor nuclei (TN), necrotic tissue(N).

Dose (100mg/kg): Tumor tissue architecture.

Fig. 12: Photomicrographs (hematoxylin-eosin staining, 20X) of tumor tissue in C57BL/6Jmice after 25-day exposure to *Phragmenthera capitata* methanol extract.

4. DISCUSSION

Because the medical conditions they have been utilized to treat are chronic in nature, herbal medicines are typically taken throughout life. The negative effects of these medicines receive little or no attention.[16] Based on this, an acute toxicity test and a 28-day sub-acute toxicity research were carried out in experimental animals to assess the safety profile of *Phragmenthera capitata* methanol leaf extract which therefore can be explore in the treatment of melanoma. Since the use of herbal medicines has recently been investigated, it's crucial that medicinal plants have a safety profile. Thus, toxicological profile and Anti-tumour effect of methanol extract of *Phragmenthera capitata* (Spreng.) *Balle* leaves in mice have been carried out in thisstudy.

Acute toxicity is a qualitative and quantitative study of the toxic phenomena that may result from a single administration of a substance to be tested. It makes it possible to determine the major harmful effects that appear in the animal in the short term. Body weight and general behavioral changes in animals have been shown to be important indicators for the assessment of primary signs of toxicity caused by a drug or chemical.[17] After single-dose administration of the crude methanol extract of *Phragmenthera capitata*, no death was recorded at the dose of2000 mg/kg of body weight, which shows that the LD_{50} may be greater than 2000 mg/kg. Apart from a weak locomotion observed in mice receiving the extract, no changes in the general behaviour of mice treated with *Phragmenthera capitata* extract was observed. It can therefore be concluded that the extract is almost non-toxic or relatively harmless according to the scale of Delongeas *et al.*[18] These results corroborate those obtained by Etame[19], who demonstrated that the aqueous extract of *Phragmenthera capitata* had a LD_{50} greater than 2000 mg/kg. The decrease in locomotion observed in mice could be reflected in the sedative or tranquilizing effect of the extracts[20]; or in their muscle relaxant or tranquilizing effect on nerve centres or motor fibers.[21] In the case of exposure to plant extracts, it is possible that certain vital organs, such as the liver, kidneys, and lungs, are affected. No necrotic lesions or congestion were observed in these organs. Daily administration of the methanol extract of *Phragmenthera capitata* for four weeks at different doses resulted in a significant reduction in the body weight of the mice. However, their weight increased gradually from the 20th day untilthe end of the administration.

According to Liu *et al*.[22], any injury to liver cells often causes an increase transaminases level in blood and can be viewed as a precursor to cell damage that ends in the release of enzymes into the serum. High ALT levels in serum indicate liver tissue enlargement and damage.[23] The AST level, in addition to being an indicator of liver function, is also used for the diagnosis of muscle and heart diseases.[24] In the present study, regarding the assessmentof liver function, *Phragmenthera capitata* extract did not produce significant changes ($P > 0.05$) in serum ALT and AST levels in treated groups at doses of 50 and 100 mg/kg compared to the control groups. These results may suggest the absence of apparent hepatotoxic effects in mice. However, a significant ($P > 0.05$) increase in ALT levels was observed in treated mice at the dose of 200 mg/kg compared to control mice, which suggests that the extract may have a harmful effect on the liver at this dosage for long-term treatment due to a possible saturation of the receptors by phytoconstituents present in the extract. This significant increase ($P > 0.05$) would be due to the action of certain metabolites present in the extract, which alter the phase Iand II enzymes involved in the metabolic processes of the liver.[25,26] Serum total protein levels may reflect major functional variations in the liver and kidney. Abnormal serum proteinlevels may be associated with liver infections or chronic inflammation.[27] In our study, there were no significant changes in total protein and albumin levels in treated mice at doses 50 and 100 mg/kg in comparison to control groups. However; there was a significant increase ($P > 0.05$) in the serum level at the dose of 200 mg/kg, thus reflecting infection or hepatic and/or renal inflammation. These results correlate with the results of liver enzyme parameters, which showed that the extract has toxic effects on the liver at a dose of 200 mg/kg. Their increase in serum levels could be caused by the blood's breakdown of proteins from other organs or couldbe a sign of high-dose toxicity.[28] In mice given a dose of 200 mg/kg, histopathological examination of the liver and lungs revealed modifications characterized by the development of lung fibrosis and a slight vascular congestion in the liver. They may result from the liver's function in the biotransformation of xenobiotics[29], the immune system's reaction to the plant extract's components[30], the saturation of the receptors affected by high doses of extract, or even the extract's vasoconstrictor action on blood vessel walls or stress experienced by the animals at the time of sacrifice.

With regard to toxicological studies, the administration of the extract at doses below 200 mg/kg appears to have no remarkable toxic effect in mice. Therefore, we verified the therapeutic effect of the extract at a single dose of 100 mg/kg on B16F10 murine melanoma model. We noted that in addition to the in vitro anticancer effect of the methanol extract of *Phragmenthera capitata*, this extract also demonstrated beneficial effects in a B16F10 murine melanoma model. The methanol extract of *Phragmenthera capitata* showed significant ($P > 0.05$) reduction in tumor growth and tumor weight in vivo without any side effects. These results corroborate those obtained by Njateng *et al.*[31] who showed that the methanol extract of *Piper capense* at a dose of 100 mg/kg reduced tumor size in a melanoma model in C57BL/6J mice. These observations would reflect the presence of secondary metabolites endowed with anti-cancer potential in our extract. Moreover, numerous studies have demonstrated the antiproliferative effect of secondary metabolites such as alkaloids, phenolic

compounds and triterpenes.[32,33] For example, quercetin has been shown to have anti-tumor activity in a variety of cancers, including lung cancer, colorectal cancer and others.[34] Also, kaempferol has anti-tumor properties due to its modulation of Akt/mTOR and FAK signaling pathways.[35,36] These activities could be explained by the fact that the extract inhibits the formation and development of VM tubes which are newly formed vessels playing a role in supplying cancer cells with nutrients at the level of the primary tumor.[37]

CONCLUSION

As a result, while *Phragmenthera capitata* (Spreng.) *Balle* may be well tolerated in short-term therapies, caution should be used when utilizing the extract for long-term therapy because continued administration for up to 28 days may result in certain toxic effects on the liver, kidneys and lungs at high doses. Tissue pathologic analysis revealed that treated mice given *Phragmenthera capitata* methanol extract (100 mg/kg) had tumor suppression potential compared to the tumor control group. Thus, *Phragmenthera capitata* (Spreng.) *Balle* can be used safely as a potent agent for the management of melanoma.

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Author Contributions

Telefo Phelix Bruno, Chandraiaih Godugu and Tagne Simo Richard, designed the study. Azabadji Ashu performed the experimental work. Azabadji Ashu and Tagne Simo Richard wrote the manuscript. Biswajit Panda, Geetanjali Devabattula and Shrilekha Chilvery maintained the cells and help handling animals. Shrilekha Chilvery prepared cell culture media and the required materials. All authors have reviewed the manuscript. All authors have reviewed the manuscript and approved its submission.

Competing interests

Authors state no conflict of interest.

Informed consent

Informed consent was obtained from all individuals included in this study.

Ethical approval

The local Institutional Review Board deemed the study exempt from review.

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