Reduction of breast tumor burden in mice by a prenylated flavonoid, Artonin E

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RESEARCH

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ABSTRACT

Background

Breast cancer is still a leading cause of cancer death among women. Thus, therapeutic alternatives from nature should be explored to lessen this burden. This is vital owing to the common occurrences of resistance in conventional therapies alongside their alarming side effects.

Aims

This study was carried out to investigate the inhibitory effect of Artonin E in female mice bearing 4T1 mammary tumour.

Methods

4T1 cells in 100μL PBS were injected into the right mammary fat pad of each female Balb/c mice aged between six to eight weeks. Treatment was commenced when the palpable tumour attained a size of 50–200mm^3. The treatment groups included Artonin E, at dosages of 25mg/kg, 50mg/kg and 100mg/kg per oral bi-weekly, 10mg/kg of paclitaxel weekly and 5 percent tween 20 bi-weekly. Tumour volume and body weight changes were recorded at the staging day and then twice every week throughout the study period. At the end of the study, the vital tissues were collected for histopathologcal assessment and blood samples were taken for serum biochemical analyses.

Results

From the results, the group treated with either 50mg/kg or 100mg/kg of Artonin E showed a significant ($p<0.05$) reduction in tumour volume. Artonin E delayed quadruple tumour growth by more than five days in comparison to the untreated control group. Histopathology and biochemical analysis revealed no toxicity in the dosages of artonin E used in this study. Secondary tumour, which had metastasized to distant organs were seen to reduce upon treatment with Artonin E.

Conclusion

With the capacity to reduce *in vivo* tumour growth, Artonin E has a great prospect to be developed into an anticancer agent.

Key Words

Artonin E, 4T1 cells, Balb/c mice, tumour, breast cancer

What this study adds:

1. What is known about this subject?

Breast cancer is unfortunately the leading cause of cancer death among women.

2. What new information is offered in this study?

To the best of our knowledge, this is the first report on the *in vivo* efficacy of Artonin E in breast cancer.

3. What are the implications for research, policy, or practice?

The result of this study identifies Artonin E as a potential agent to treat breast cancer and reduce occurrence of metastasis.

Background

Breast cancer has been a threat to the female population and a leading cause of death among women.[1,2] This encourages research into natural remedies to abate this disease. We had earlier reported the physicochemical properties of Artonin E, a phytochemical isolated from the root bark of Artocarpus elasticus,[3,4] as well as its *in vitro* anti-breast cancer effect.[4-6] While useful, *in vitro* experiments do not always translate to the complex, biological system, thus, the need for *in vivo* preclinical studies. *In vivo* experiments have been of great importance in cancer drug discovery[7] and served as a guide to the establishment of a first-in-human trial.[8] Today, demonstration of therapeutic efficacy and low toxicity in preclinical models is an essential component of the development of clinical drugs.[9,10]

Animal models currently available for testing breast cancer drugs include: spontaneous, inducible, and syngeneic models of metastasis, xenografts of human breast cancer cell lines growing in immunodeficient mice, chemically induced mouse models, virally induced mouse models[11] as well as humanized xenografts model of breast cancer which involves implantation of human tissue fragments like human bone fragments followed by orthotopic injection of human breast cancer cells to monitor migration of human cancer cells from the primary tumour environment to a human

bone environment.[12] The 4T1-model of breast cancer and metastasis was identified originally as a spontaneous breast cancer model in the BALB/c mouse strain.[13] This murine breast tumour model has several characteristics that make it a suitable experimental animal model for human mammary cancer. Firstly, tumour cells are easily transplanted into the mammary gland so that the primary tumour grows in the anatomic correct site. Secondly, as in human breast cancer, 4T1 metastatic disease develops spontaneously from the primary tumour.[14] Also, the progressive spread of 4T1 metastases to the draining lymph nodes and other organs is very similar to that of human mammary cancer[15] and has been widely employed in breast cancer drug discovery.

In vivo antitumour experiment can either be a tumour growth inhibition study (if treatment is initiated the day after or the day of tumour cell implant) or a tumour growth delay study (if treatment began with an established tumour nodule (50–200 mm^3). According to Corbett *et al*. (1998),[16] a stronger evidence of the clinical potential exist in the growth delay study than in the tumour growth inhibitory assay. Tumour growth delay also serves as a surrogate for disease progression.[17] Indices or endpoints to measure antitumour effectiveness of tested agents include: measurement of tumour volume, tumour growth delay and doubling time, animal body weight throughout the study and the evaluation of biochemical and histopathological examinations.[18,19]

This study is thus focused on the assessment of the antitumour effectiveness of Artonin E in 4T1 breast cancer cell challenged mice.

Method

Animals and environmental control

Female Balb/c mice aged between six to eight weeks old were purchased from the animal research facility, Faculty of Veterinary Medicine, Universiti Putra Malaysia. The mice were housed in cages and allowed to acclimatize to the animal laboratory environment at 24±1°C under a 12 h dark-light cycle for one week before the commencement of the experiment. Pellets and water were provided ad libitum. Ethical approval for the conduct of this animal study was obtained from the Institutional Animal Ethical Committee, University of Malaya with the reference number 2015-180804/PHARM/R/NMH.

Preparation of cancer cells

The 4T1 mouse breast cancer cell line was purchased from American Type Culture Collection (ATCC, USA). The cells

were maintained in DMEM and the cell cultured appropriately as previously reported.[4] For collection of cells, the cells were tripsinized after washing with PBS and centrifuged at 125×g for 5 minutes. The cell pellet was counted using a hemocytometer and $1×10^6$ cells were resuspended in 100μL PBS for inoculation into the animals.

Breast cancer induction

Each mouse was anesthetized with an intraperitoneal injection of a mixture of Ketamine (90mg/kg) and Xylazine (10mg/kg) and a total of $1×10^6$ cells in 0.1ml PBS were implanted subcutaneously into the mammary fat pad (right flank) of each mouse. The implanted animals were examined every two days for tumour appearance.

Experimental design, drug treatment and compound preparation

Once the tumour became palpable (5 to 12 days post-tumour induction) and the initial solid tumour established in the range of 50–200mm^3, when measured with an electronic vernier caliper, the female BALB/c mice were randomized into seven groups consisting of five animals each (n=5). Group 1 comprised of untreated normal, healthy mice and served as the negative control (animals without the breast cancer burden). Group 2 comprised of mice induced to develop breast cancer and served as the breast cancer control but without any treatment. Group 3 comprised of mice induced to develop breast cancer, but treated with the vehicle and served as the breast cancer vehicle control group, while groups 4, 5 and 6 were breast cancer mice treated bi-weekly with 25mg/kg, 50mg/kg and 100mg/kg body weight of Artonin E dissolved in 5 percent Tween 20 (Sigma Aldrich, USA) respectively. Group 7 was treated intraperitoneally with 10mg/kg body weight of Paclitaxel once every week.[20] Paclitaxel is a naturally derived anticancer agent, thought to inhibit tumours of the breast, lungs and ovary by binding to tubulin.[21] The working solution of this paclitaxel (Taxol, Bristol-Myers Squibb Co., Princeton, NJ) was prepared by diluting the drug in normal saline (NS) to a dose of 10mg/kg in 150μl per injection, once a week for four weeks. The Artonin E treatments were given orally for four consecutive weeks to the animals through gastric intubations. The oral route was chosen for Artonin E since it is the intended route, being non-invasive and had proved a good bioavailability in an earlier *in silico* studies.[4] On the other hand paclitaxel was administered intraperitoneally based on availability, especially as the objective of the work was focussed on therapeutic efficacy of Artonin E and not the former.

The concentrations of Artonin E chosen were based on

preliminary acute toxicity testing (unpublished data) where no clinical sign of toxicity was observed in the mice group treated with 1,000mg/kg of Artonin E. The schedule of Artonin E treatment was similar to that adopted by Ibrahim et al.[22] and was used based on the recommendation of Hollingshead.[17] Paclitaxel dose and treatment schedule were based on a previous report by Liao et al.[20]

Measurement of tumour growth delay and inhibition

To assess the antitumour effect of Artonin E in breast cancer bearing mice, the growth of the solid tumours was monitored using *in situ* caliper measurements to determine the tumour volume. Tumour volumes (mm^3) were calculated from the measurements (mm) of two perpendicular dimensions length (L) and width (W) using the formula for a prolate ellipsoid[23] as follows:-

Volume $(mm^3) = L/2 × W^2$

The measurement was done before the treatment was initiated (staging day) and then twice every week for the study period by a scientist who was blinded to the various treatment as well as the study at large. Some efficacy endpoints were utilized in assessing the antitumour effects as recommended by the Developmental Therapeutics Program of the US National Cancer Institute (http://dtp.nci.nih.gov). This included the relative tumour volume, percent test/control (percent T/C), tumour weights calculated for each day that tumours were measured and tumour growth delay.

The relative tumour volume (V_T/V_0) was calculated for every tumour volume at any given time (V_T) against the tumour volume at staging day (V_0). The relative tumour volume (RTV) – time profile for each group was plotted and tumour growth delay in attaining a specified number of doublings (quadruple) compared to the untreated control was determined (i.e. the time taken for the relative tumour volume to increase fourfold (RTV4).

The RTV was used to calculate the tumour growth inhibition (ratio between the treated (T) and control (C) tumours) as an indication of drug efficacy[10] using the following formulas:

$$T/C \text{ per cent} = RTV_T/RTV_C × 100$$

To evaluate toxicity, body weight was measured twice a week and the relative body weight (BW_T/BW_0) was calculated for every body weight at any given time (V_T) against the body weight at staging day (V_0). The relative body weight – time profile for each group was plotted.

At the end of the study, all the mice were euthanised using

an overdose of Ketamine and Xylazine (180mg/kg and 20mg/kg respectively). The liver, kidney, spleen, lungs tissues were collected for histopathology assessment and blood samples were taken from mice after the course of treatment for biochemistry analysis.

Biochemical analysis

Blood samples from at least three mice representative of each animal group were collected in separate tubes with heparin as anticoagulant and centrifuged (Hettich zent-EBA20, Germany) at 1500×g for 10 minutes, serum was obtained, separated into clean new tubes and stored at -20˚C until analyzed. The liver enzymes, Alanine amino transferase (ALT), Alkaline phosphatase (ALT), creatinine and lactate dehydrogenase were analyzed using standard diagnostic kits (Roche) in an automatic biochemical analyzer (Hitachi 902, Japan).

Haematoxylin and Eosin (H&E) histology staining

The excised organs were cut into sections of about $0.5cm^2$ sizes and fixed in 10 per cent formalin for at least 48 h, embedded in paraffin wax (Leica EG1160, Germany) and the blocks trimmed and sectioned to about 5×5×4µm size using a microtome (Leica RM2155). The tissue sections were mounted on glass slides using a hot plate (Leica HI1220, Germany) and subsequently treated with alcohol. The processed tissues were rinsed with tap water and finally stained with the Harris's haematoxylin and eosin (H & E) (Luna, 1968) and examined under a light microscope (Nikon, Japan).

Statistical analysis

Each of the animal group contained five mice to enable statistical analysis. The biochemical and histopathological analysis were done in triplicate and the results expressed as mean ± standard error of mean. The level of significance difference between the mean of each treatment group and the control for each of the investigated efficacy indices was determined using the unpaired two-tailed Student's t-test for independent samples; P-values <0.05 were considered to be statistically significant. Analysis of variance was also employed where necessary to compare the treatment groups per day with the untreated cancer control group followed by Turkey's post hoc test. All the statistical analysis was carried out in the GraphPad prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Artonin E inhibits the growth of Breast cancer *in vivo*

From the results, there was a significant ($p<0.05$) decrease in the relative tumour volumes of mice treated with 50 and 100mg/kg of Artonin E. The standard agent, paclitaxel also showed a significant decrease in the relative tumour volume. On the other hand, the mice group treated with 25mg/kg of Artonin E was not significantly different ($p>0.05$) from the control in terms of the tumour volume (Figure 1). The mice group treated with the vehicle of dissolution, 5 per cent Tween 20, showed no significance ($p>0.05$) difference in mammary tumour volume when compared with the untreated control mice. Hence, the cancer control animals were used to compare treatment effect of Artonin E treatment.The variability in the tumour volumes of the mice at the staging day before any therapeutic intervention is depicted in Figure 2.

Artonin E delayed *in vivo* breast cancer tumour quadruple growth

The effect of Artonin E in delaying mice mammary gland tumours from reaching 400 per cent of the first palpable tumour volume was calculated after extrapolation from Figure 1 From the results, the control group took nine days to increase four times its initial volume from the staging day. Mice treated with 25mg/kg of Artonin E took approximately 10 days, while groups treated with 50 and 100mg/kg of Artonin E took approximately 11 and 14 days to reach the specified relative tumour volume, respectively. The differences in days taken by the treatment group to reach the quadruple volume as extrapolated from Figure 1 was subtracted from the delay experienced by the untreated control groups and presented in Table 1

Artonin E induced tumour growth inhibition *in vivo*

Another index of assessing *in vivo* antitumour activity is the tumour growth inhibition (%T/C). The results were compared with the arbitruary cut-off for *in vivo* growth inhibition (Table 2) reported by Wu.[24] From the results, 100mg/kg of Artonin E had an inhibition of 38 per cent, regarded as having intermediate *in vivo* antitumour activity. The groups treated with 50mg/kg of Artonin E had a T/C value of 57 per cent and the 25mg/kg of Artonin E treated group had 88 per cent value which were both considered as inactive (Tables 2 and 3).

Body weight of Artonin E treated mammary gland tumour-bearing mice

The relative body weight changes of the mice in this study were used as a measure of toxicity to Artonin E. Kruczynski et al.,[25] reported that decrease in body weight exceeding 15 per cent is considered as toxicity due to the treatment. In this study, generally, there was no observed weight loss in the Artonin E treated mice in comparison to the untreated tumour-bearing mice (Figure 3). However, after 24 days of

treatment, mice treated with 50 and 100mg/kg of Artonin E showed lower relative weight gain compared to those treated with 25mg/kg of Artonin E.

Effect of Artonin E on serum biochemical parameters.

In this study ALT, ALP and LDH were chosen as the serum liver enzymes and creatinine as an indicator for renal function. The study revealed a significant ($p<0.05$) elevation of the liver-specific enzyme ALT in the untreated tumour bearing mice in comparison to. treated groups (Table 4). There was a marked increase in LDH in the tumour-bearing mice, which decreased upon treatment with Artonin E.

Histopathology

The livers of mice with 4T1 cell-induced mammary gland showed numerous mutifocal clusters of neoplastic cells, suggesting metastasis of mammary glands tumour (primary site) to the liver. The tumour area also contained numerous leucocytes, predominantly made up of neutrophils and resident Kupffer cells in untreated tumour-bearing mice (Figure 4). Treatment of mammary gland tumour with Artonin E (3C-D) showed reduced metastasis to the liver. This was observed by the fewer neoplastic cell clusters observed in the livers of mice treated with 50 and 100mg/kg Artonin E in comparison to those treated with 25mg/kg of Artonin E.

The lungs of mice with untreated 4T1 induced mammary gland tumour showed multifocal areas of lung consolidation comprising of metastatic tumour cells, numerous lymphocyte and granulocyte infiltration (Figure 5). There were fewer observed metastatic cells following treatment owing to apoptosis shown by the altered and irregular neoplastic cell membrane. A restoration of the lung morphology was observed following Artonin E treatment, as evidenced by the high number of alveoli (Figure 5C-5E).

The kidneys of the mice were also examined (Figure 6). Both the treated and untreated kidney tissues showed normal tissue morphology. The glomerular architecture and epithelial lining of the tubules also appeared normal in all groups of mice with no observable signs of toxicity.

Discussion

It is vital to investigate the *in vivo* growth inhibition of a potential anticancer agent. This is because a compound can possess good *in vitro* growth inhibitory properties but fails to reproduce a similar effect *in vivo* within the full complexity of the tumour microenviroment. Consequently, *in vivo* studies, which are usually preclinical trials, have played a crucial role in nearly every medical breakthrough

and has been of great importance in cancer drug discoveries.[7] The 4T1-induced murine mammary gland tumour is an excellent model for human breast cancers because it is aggressive and easily metastasizes.[14,15] Thus, in this study, the female Balb/c mice bearing 4T1 mammary gland tumour were used to investigate the efficacy of Artonin E as a potential antitumour agent.

There are several indices for efficacy evaluation in an *in vivo* model recommended by the Developmental Therapeutics Program of the US National Cancer Institute (http://dtp.nci.nih.gov). One of them is the relative tumour volume. In this study, the relative tumour volume (V_T/V_0) was calculated for every tumour at any given time (V_T) against the tumour volume at staging day (V_0).[26] From the results, Artonin E appeared to produce its antitumoural effect in a dose-dependent manner. Mice treated with Artonin E, at dosages of 50 and 100mg/kg, showed significantly reduced tumour volume. However, there was no significant reduction in the relative mammary tumour volume in mice treated with 25mg/kg of Artonin E. This suggested that 25mg/kg per os of Artonin E was too low for the compound to reach therapeutic concentration at the tumour site. The failure to produce effect at this concentration may as well be attributed to extensive first pass effect[27] which might have caused a decrease in the systemic availability of Artonin E below the *in silico* predicted oral bioavailability of 88 per cent.[4] The multiplexity of the tumour micrenviroment could as well render the dose too low for therapeutic effect. Thus, Artonin E, at appropriate dosages when administered orally, can produce antitumour effects. This oral method of delivery is ideal for therapeutic compound because it is non-invasive, convenient and patient-compliant.

Another modality to assess *in situ* tumour treatment effect is the determination of tumour growth delay in reaching a specified number of doublings.[17] The rate of growth delay was observed to be a function of dose in the treated mice. By direct extrapolation from the graph, at the highest dose of Artonin E, the mice tumour took approximately 14 days to reach a quadruple size from its staging day volume in comparison to the untreated mice that took nine days. Thus, 100mg/kg of Artonin E delayed qudruple tumour growth by approximately five days compared to untreated control.

Tumour growth assessment was also done by assessing the ratio of the relative tumour volume of Artonin E-treated mice to the relative tumour volume of the untreated mice using the arbitrary tumour growth inhibition (T/C) cut-off.

The T/C is a rough measure of growth inhibition. In this study, on the 28th day of the study period, 50mg/kg of Artonin E produced a T/C value of 57 per cent exceeding the antitumour arbitrary cut-off of 45 per cent reported in earlier studies.[24,28,29] Artonin E at a dose of 100mg/kg showed greatly improved antitumour activity with a T/C of 38 per cent. However, Artonin E at this dose still had intermediate level antitumour activity.[24]

Chemotherapy can cause toxicity that can be envisaged from the relative body weight measurement. In the course of this study, the body weight of each mouse in each of the groups was measured and recorded. From the results, there was no significant change in the body weight of Artonin E-treated mice when compared to the untreated mice. It is suggested that decrease in body weight exceeding 15 per cent as a result of treatment is considered an indicator of toxicity.[25] Fortunately, none of the Artonin E treated mice showed significant body weight loss, indicating that it did not cause toxicity at doses used in this study.

To determine the effect of Artonin E treatment on key organs, serum biochemical parameters were evaluated. The ALT, ALP, and LDH were chosen as the serum liver enzymes and creatinine as an indicator for renal function. The serum enzymes are used as indicators of liver damage and toxicity in chemotherapy.[30] The concentrations of these enzymes are only significant when they are elevated. Decreases in these parameters are only a reflection of diurnal and daily fluctuations. ALT is a cytoplasmic enzyme in which the serum activity is increased due to leakage across damaged cytoplasmic membranes. This study showed that the liver-specific enzyme ALT was only marginally ($p>0.05$) elevated in the tumour bearing mice treated with Artonin E. This indicated that there was no rampant hepatic damage related to the treatment.[31] However, there was a significant higher level of the enzyme in the untreated tumour bearing mice and the vehicle control mice compared to healthy mice without tumour burden. This elevation is suggested to be associated with the degeneration of hepatocytes as a result of the metastatic breast cancer.[32]

The level of LDH increased significantly in the untreated mice. This enzyme is often used as a non-specific indicator of cancers and its increase in serum is due to the progression of mammary gland cancer and liver metastasis.[33] LDH concentration increased in mice with untreated mammary gland tumour and mildly decreased upon treatment. The creatinine level was not elevated, indicating that the kidney was neither affected by the tumour nor damaged by Artonin E treatment.

To further verify the antitumour activity of Artonin E, the liver, lungs and kidney were subjected to histopathological examination. The murine 4T1-induced mammary gland tumour was shown to have spontaneously metastasized to the liver and lungs within days of tumour inoculation.[13] The liver tissue from the tumour bearing mice showed abundance of infiltrating neoplastic cells as well as leukocytes. The hepatocytes also showed cellular vacuolation. The neoplastic infiltrations and hepatocyte degeneration were reflected in the elevated serum ALT in this group of mice. Upon increasing the concentration of Artonin E in the treatment of the mammary gland tumour, the clusters of neoplastic cells in the liver reduced. It can, thus be deduced that treatment with Artonin E may have prevented massive metastasis or Artonin E had also killed cancer cells in the hepatic tissue.

The histopathology of the lungs of untreated tumour bearing mice showed abundance of metastatic breast cancer cells with numerous lymphocytes and granulocytes. Breast cancer metastasis, has been reported to frequently metastasize to the lungs resulting in the poor survival of patients. Pulmonary metastasis of mammary tumours had been reported to be one of the main causes of death.[34] From the results, therapeutic intervention with increasing doses of Artonin E reduced the metastasis and inflammation in the lungs. The lungs of Artonin E treated mice showed restoration of normal morphology.

The kidneys from the mice with mammary gland tumour treated with Artonin E did not show any renal morphology abnormality. This suggested that Artonin E, at all dosages used in this study was not nephrotoxic. This observation is collaborated by lack of increase of serum creatinine, a parameter used for assessment of renal function.

Conclusion

Artonin E significantly inhibited the *in vivo* growth of the aggressive 4T1-induced mammary gland tumour in mice in a dose-dependent manner. Additionally, Artonin E reduced the rampant metastasis of this aggressive tumour without causing any observed toxicity. Thus, Artonin E shows great promise not only as a potential antimammary gland tumour agent but also as a potential antimetastasis agent.

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PEER REVIEW

Not commissioned. Externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

FUNDING

ETHICS COMMITTEE APPROVAL

Ethical approval for the conduct of this animal study was obtained from the Institutional Animal Ethical Committee, University of Malaya with the reference number 2015-180804/PHARM/R/NMH.

Figure 1: Effect of Artonin E on tumor volume in Balb/c mice induced to develop mammary gland tumour with 4T1 cells. Each point represents means±SEM (n=5/group) of tumour volume relative to staging day. CC=cancer control (untreated positive control), VC=vehicle control (treated with the vehicle, 5%Tween 20) and PTX=paclitaxel. Differences are statistically significant with *(p<0.05) when compared to the cancer control at each given day. The dotted line indicates time taken for tumour to reach quadruple growth
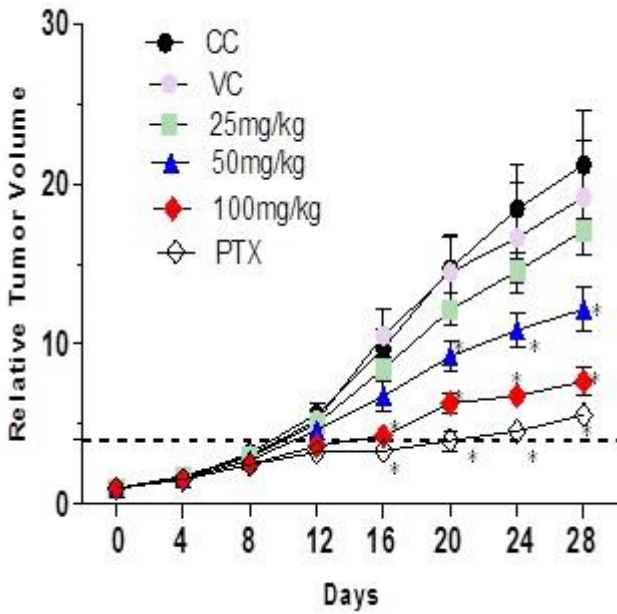
Figure 2: Variability in tumour volume of tumour bearing mice randomised into the various study groups at the staging day
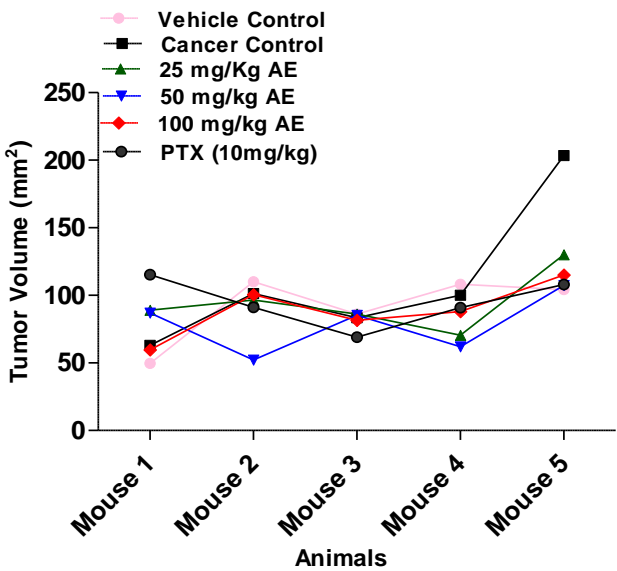
Figure 3: Relative body weight of mice treated with Artonin E. Each point represents means±SEM (n=5/group). Body

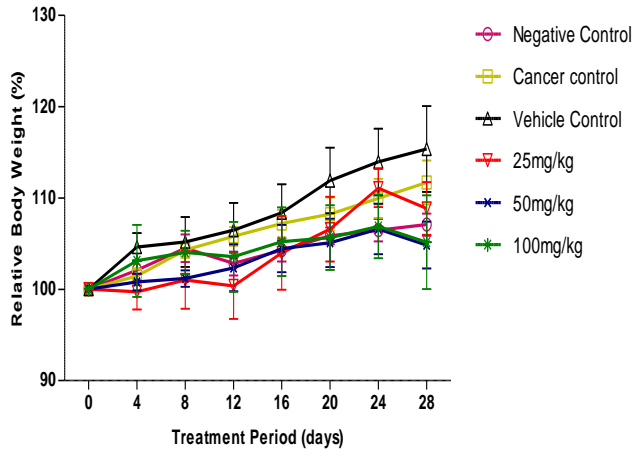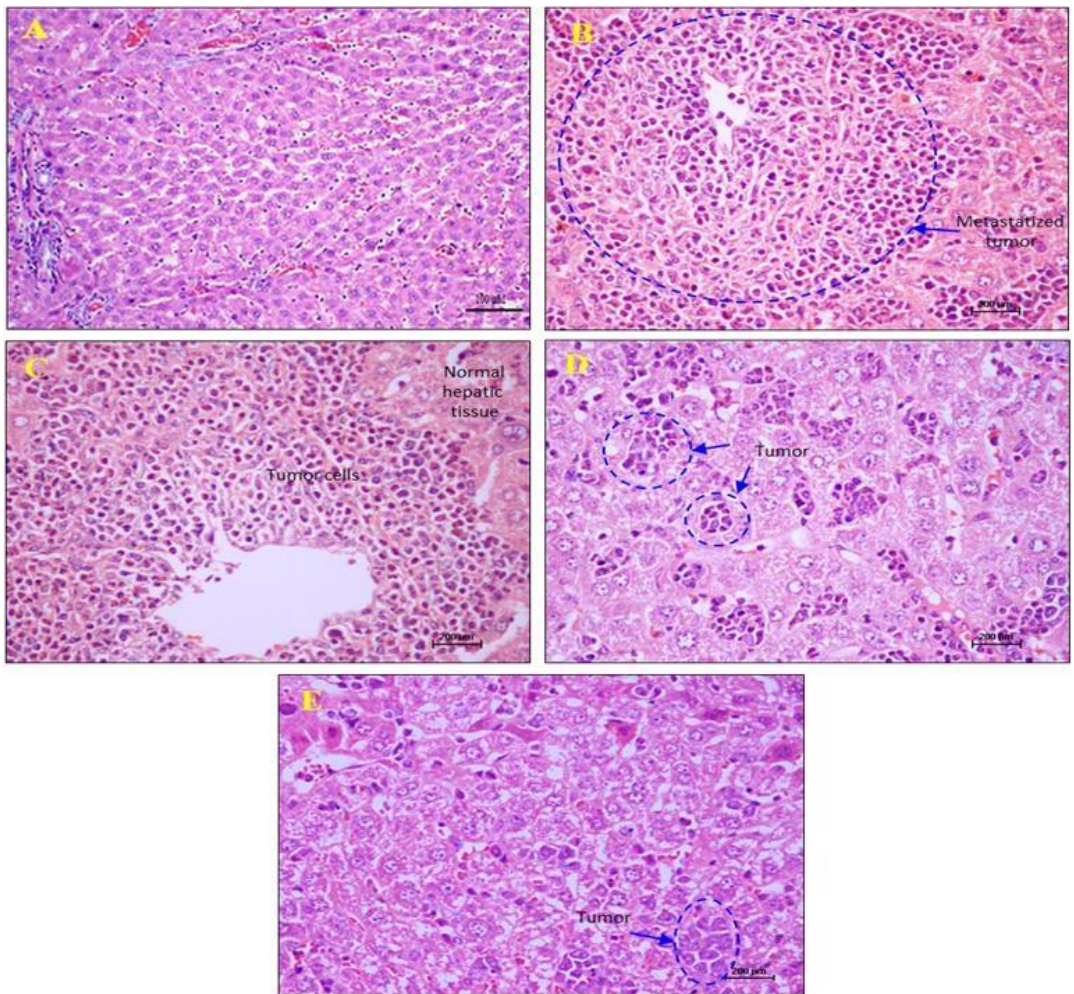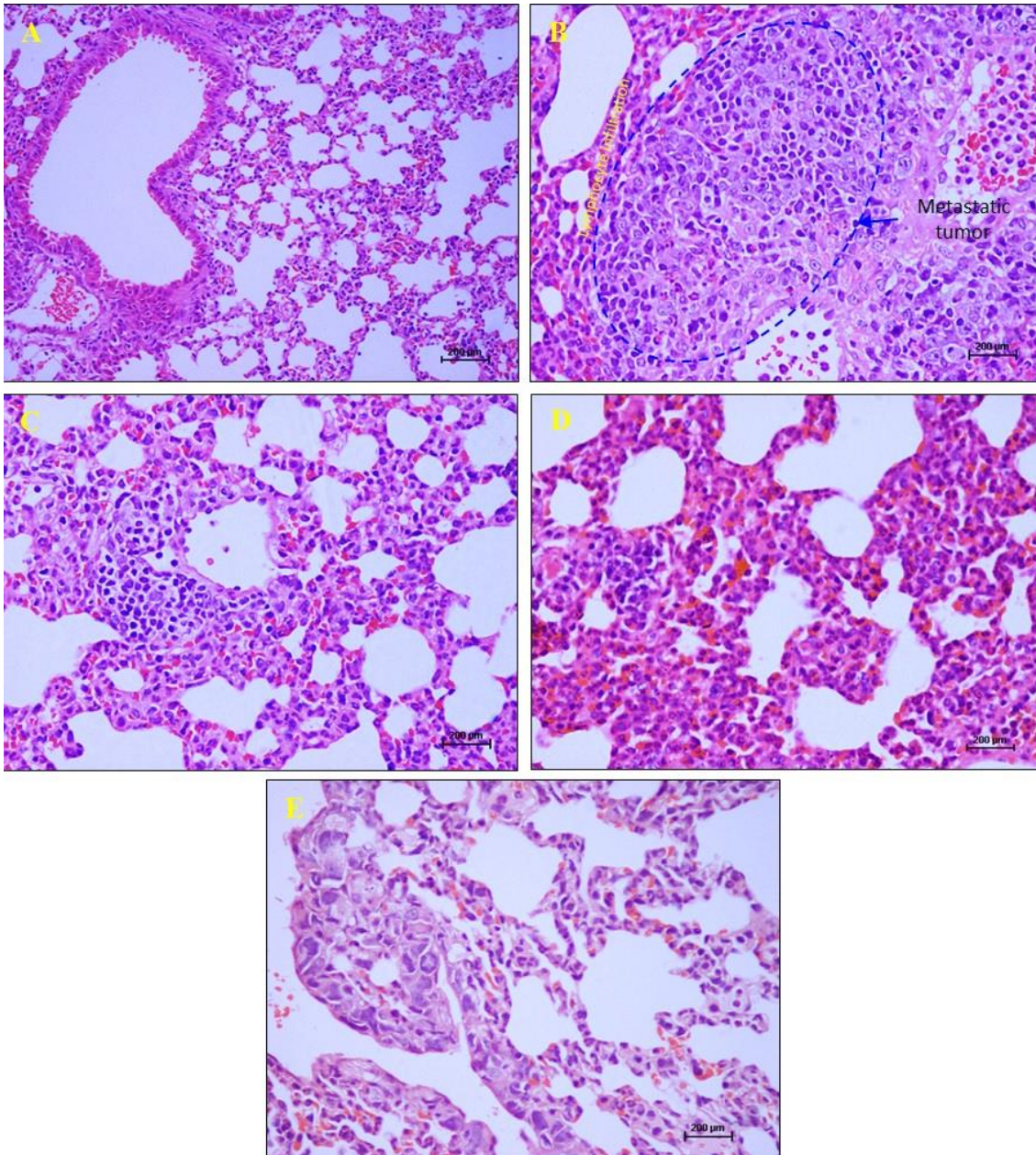**weight changes were not significant from the negative control**

Figure 4: Liver from Balb/c with 4T1 cell induced mammary gland tumour treated with (C) 25mg/kg, (D) 50mg/kg and (E) 100mg/kg of Artonin E. (A) is healthy mice, (B) is untreated tumourous mice (H & E)

Figure 5: Lungs from Balb/c mice with 4T1 cell induced mammary gland tumour treated with (C) 25mg/kg, (D) 50mg/kg and (E) 100mg/kg of Artonin E. (A) is healthy mice, (B) is untreated tumourous mice (H & E)

Figure 6: Kidney from Balb/c mice with 4T1 cell induced mammary gland tumour treated with (C) 25mg/kg, (D) 50mg/kg and (E) 100mg/kg of Artonin E. (A) is healthy mice, (B) is untreated tumourous mice (H & E)
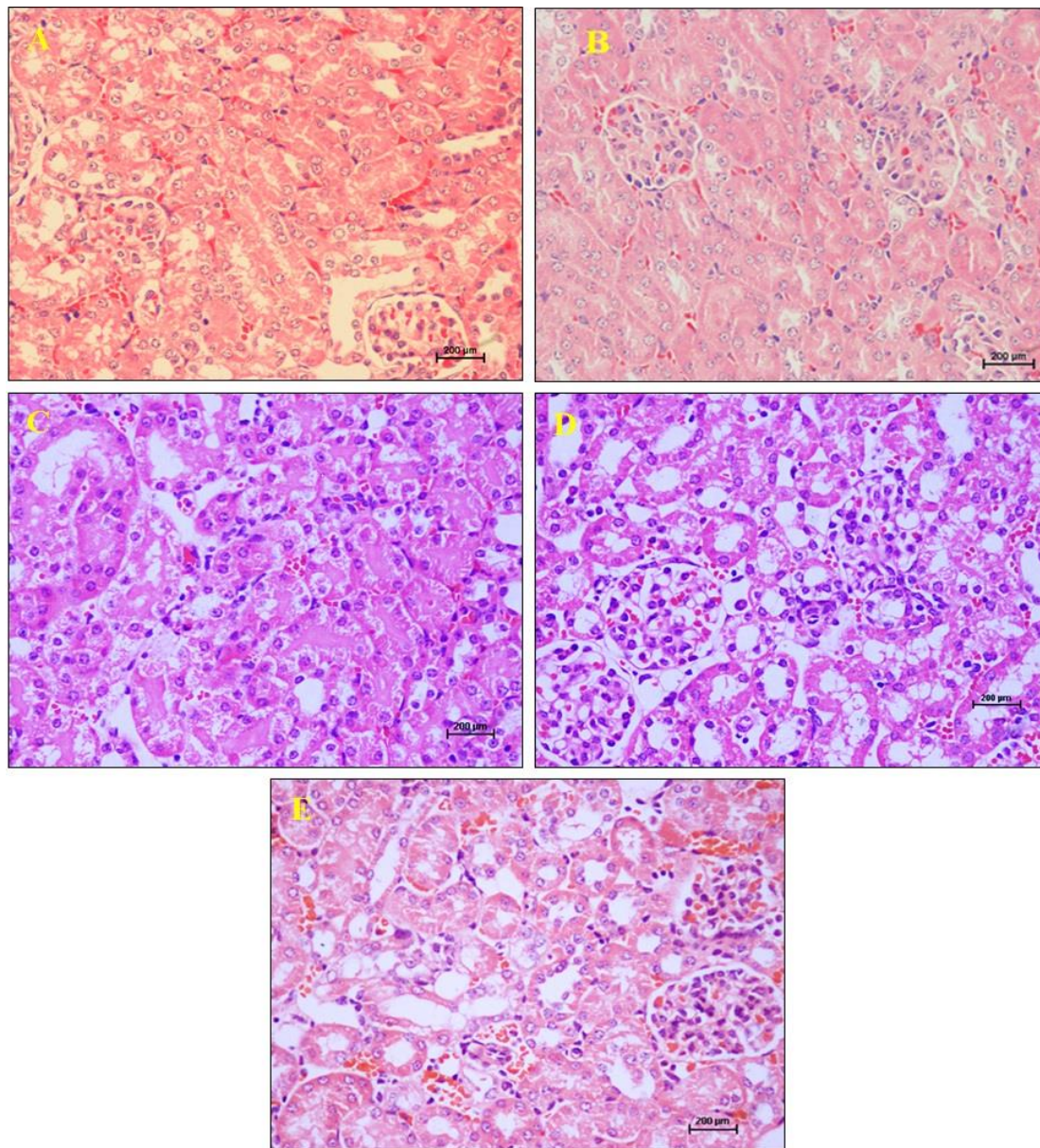
Table 1: Tumour growth delay in artonin E treated breast cancer bearing mice

Compound	Dose (mg/kg)	Quadruple growth delay (T-C) (Days)
Artonin E	25	0.7
	50	1.3
	100	5.3
Paclitaxel	10	10.8

T and C represent time taken for treated tumours to reach 400% of the first palpable tumour volume, while C represents the number of days for the untreated group to reach similar tumour volume. T-C is the difference in days between the treated and untreated group in achieving 400% of the firat palpable tumour volume. The first palpable tumour volume was the initial volume of tumour before treatment was commenced

Table 2: In Vivo Antitumor Growth Inhibition Rating

Measurement	Cutoff point	Activity rating
T/C	≤15%	Highly active
	>15% and ≤45%	Intermediate active
	>45%	Inactive

Table 3. T/C* values for Artonin E treated murine mammary tumour

Dose	Experimental Period (day)							
	0	4	8	12	16	20	24	28
	T/C values							
25mg/kg Artonin E	100	103	93	90	87	83	79	81
50mg/kg Artonin E	100	98	86	82	69	63	59	56
100mg/kg Artonin E	100	96	78	63	44	44	40	38
10mg/kg Paclitaxel	100	96	86	56	34	27	25	26

Value was calculated from the mean relative tumour volume of treated mice and control, untreated mice

Table 4: Serum biochemical parameters of mammary gland tumor-bearing mice treated with Artonin E

Treatment	ALT (U/L)	ALP (U/L)	LDH (U/L)	Creatinine(µmol/L)
Healthy Control	43.0±1.2	116.5±7.8	273.3±25.1	39.5±0.9
Cancer Control	125.7[a]±47.2	54.0±1.2	2095.3[a]±30.9	36.7±2.9
Vehicle (Control) (100µL 5% Tween 20)	112.9[a]±3.0	41.5±3.2	2107.5[a,b]±7.2	42.0±0.6
25mg/kg Artonin E	75.7±0.2	53.5±3.8	1779.5[a,]±77.1	42.1±1.0
50mg/kg Artonin E	39.3[a]±3.0	52.0±5.0	1835.7[a,]±105.1	29.7±1.9
100mg/kg Artonin E	53.0[a]±2.1	57.0±5.7	1945.0[a]±36.7	43.0±0.6
10mg/kg Paclitaxel	59.2[a]±4.8	68.5[ac]±3.3	1965.5[a]±8.9	30.5±5.5

Values are mean±SEM. a=significant elevation between treatment and healthy control, b=significance between treatment and cancer control and c=significance between treatments and vehicle control.

ALT=Alanine aminotransferase; ALP=Alkaline phosphatase; LDH= Lactate dehydrogenase