Cytotoxicity and Oral Acute Toxicity Studies of β-mangostin Isolated from *Cratoxylum arborescens*

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ABSTRACT

Introduction: The objective of this study was to investigate the cytotoxicity and oral acute toxicity of β-mangostin isolated from *Cratoxylum arborescens*. **Material and methods:** Healthy male and female ICR mice (8 weeks) were fed orally with 250 and 500mg/kg of β-mangostin. Body weight of each animal was measured and any gross behavioral change was observed daily. Hematological and clinical biochemical parameters as well as histopathological analysis were carried out on 15th day. The level of oxidative stress was analyzed using MDA and GSH measurement. **Discussion:** The results showed that oral administration of the β-mangostin had no adverse effect on the growth rate, hematological and clinical biochemical parameters. Histological studies showed that the treatments did not induce any pathological changes in the liver and kidney. The compound at both the doses did not alter the oxidative stress biomarkers. The *in vitro* cytotoxicity of β Mangostin was investigated in HepG2, A549, MCF-7, MDA-MB-231 and PC3 cells. There was significant cytotoxicity in both type of breast cancer cells (MCF-7 and MDA-MB-231). In conclusion, our results show that there was no treatment-related acute toxicity in mice following 14-days oral administration of 250 and 500mg/kg of β-mangostin. **Conclusion:** The results showed that the compound can be selected for detailed *in vitro* and *in vivo* breast cancer research.

Keywords: *Cratoxylum arborescens*; β-mangostin; acute toxicity; anti-cancer.

INTRODUCTION

Throughout the history of mankind plants have played a unique role in providing new remedies for various diseases in the world and served to be the foundation of many traditional medicine systems. Plants were served in various formulations including tinctures, teas, poultices, cream, powders etc., and have become the basis of novel drug discovery.[1] They are also the rich source of secondary metabolites or phyto-pharmaceuticals used in pharmaceutical industry. These secondary metabolites behold single or multiple pharmacological properties. Anti-fungal, anti-bacterial, anti-cancer, anti-viral, anti-inflammatory, HIV-inhibitory, anti-diabetioecdc and hepatoprotective properties are some of them. Xanthone, Mangostin, α-Mangostin, γ-Mangostin, Cowaxanthone F, 1,6-dihydroxyxanthone, 1,3,8-trihydroxyxanthone, 1,6- and 3,5-dihydroxyxanthones are few examples of such xanthones.[2–5]

Cratoxylum arborescens (Family: Guttiferae) is a well-known tropical tree from which several xanthones were isolated and these xanthones are present in the leaves, twigs and bark of the tree.[6] From this plant the compounds namely α-Mangostin, β-mangostin (BM) and γ-Mangostin were isolated previously; of which, α-Mangostin and γ-Mangostin where extensively studied pharmacologically.[7,8] β-mangostin (Figure 1), now being focused for its pharmacological benefits, including

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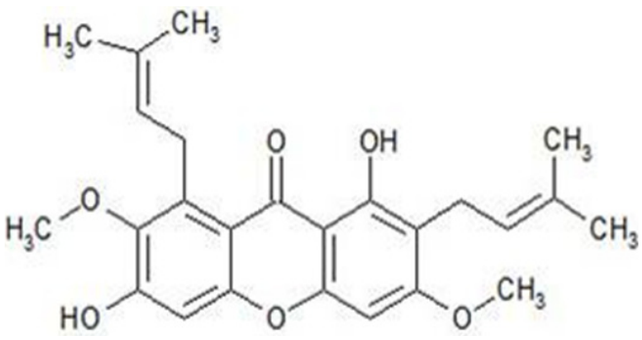

Figure 1. Structure of β-Mangostin [1,6-dihydroxy-3,7-dimethoxy-2,8-bis(3-methylbut-2-enyl)-9H-xanthen-9-one].

anticancer activity,[9,10] however, lacks some important informations with respect to toxicity. As part of a permanent screening program for searching natural products with beneficial biological properties (especially anti-cancer), the current investigation herby reports the toxicity of β-mangostin *in vivo* and its possible effects on various cancer cells *in vitro*. We have designed this study in such a way, the outcome of this study will give better understating of this compound with respect to its suitability in being studied as an anticancer agent, and the safety range that could be used for *in vivo* anticancer mechanisms.

EXPERIMENTAL SECTION

Plant sample

The ground air-dried stem bark of *Cratoxylum arborescens* (1.0kg) was soaked at room temperature in hexane for three days and repeated thrice. The extract was filtered and then concentrated by using rotary evaporator under reduced pressure to give dark gummy semisolid residue. The plant material was then sequentially extracted with chloroform, and methanol. The weights of hexane, chloroform, and methanol crude extracts obtained were 6.12g, 28.18g, and 40.27g, respectively. The hexane extract (6.12g) was separated by vacuum column chromatography and eluted with hexane and followed by mixtures of solvents, hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol to give 26 fractions of 200ml each. Similar fractions based on TLC and observed under UV light were combined. Fraction 14 was further separated by mini column chromatography to give 56 fractions. Fractions 16–52 (eluted with 50% ethyl acetate: 50% methanol) was similarly further purified by preparative thin layer chromatography as well as chromatotron to give yellowish solid and was identified as β-mangostin. Similar separation and fractionation of the chloroform and methanol extracts with series of column chromatography led to the isolation another batch of β-mangostin.

Acute oral toxicity study of β-mangostin

The experimental protocol with animals in the study was in compliance with the regulations set by the Institutional Animal Care and Use Committee, Faculty of Medicine, UM. Healthy male and female ICR mice (8 weeks) used for the acute oral toxicity study were bred and reared at the Animal House, University of Malaya, Malaysia. The animals were housed in polypropylene cages with stainless steel grill tops and provided with bedding of clean paddy husk. The animals were acclimatized to laboratory conditions for 1-week prior to treatment. The temperature in the animal room was maintained between 25±2 °C with a relative humidity of 30–70% and illumination cycle set to 12h light and 12h dark. The mice were fed with commercial pellet diet. All animals had free access to tap water and food except for an overnight fasting before treatment. The mice were then allowed to take food after an hour of treatment.

Treatment

Being a dietary constituent from fruits, we expected β-mangostin to be relatively safe.[11] Thus, two doses (low and high), as recommended by Organisation for Economic Co-operation and Development[12] guidelines of 250 and 500mg/kg of compound was suspended in 10% Tween20 and administered by gavage to mice weighing between 25–30g (n=5). The control group received tween 20 alone. The general behaviour of mice was observed every one hour for six hours and thereafter every 24 hour until 14 days. Animals were observed for any sign of toxicity and for mortality up until 14 days and daily weight were noted as described earlier.[13] Other features observed included changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavior pattern. Upon completing 14 days, the animals were sacrificed and vital organs (lungs, heart, spleen, liver, kidneys) were carefully examined macroscopically and weighed. Hematological parameters and histological (liver and kidney) parameters were determined by standard methods.[14] Lipid peroxidation was also performed on fresh liver and kidney necropsies.

Assessment of kidney and liver functions

All biochemical assays were performed spectrophotometrically using Hitachi-912 Autoanalyser (Mannheim,

Germany) with kits were supplied by Roche Diagnostics (Mannheim, Germany). The common indicators of kidney function such as serum creatinine, blood urea nitrogen, sodium, potassium, chloride, carbon dioxide and anion gap levels were measured. Serum Alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total protein, albumin, globulin, total bilirubin and conjugated bilirubin levels were measured so as to evaluate the liver function. To establish a good sensitivity and validity of the data, serum samples were analyzed in triplicates and blindly.

Assessment of lipid profile

Analysis of total cholesterol and high density lipoprotein (HDL) cholesterol concentrations were performed with the aid of commercial kits of Span Diagnostics as described previously.[15] GPO-PAP end point assay was used to assess the triglycerides concentration.

Histopathological examinations

Fixed (10% formalin) renal and hepatic tissues were embedded in paraffin, sectioned at 5μm and stained with haematoxylin-eosin. All sections were examined using a photomicroscope (Olympus BH-2, Japan). Samples were blindly analysed for histopathology by an independent histopathologist.

Lipid peroxidation

Lipid peroxidation was assessed using malondialdehyde (MDA) as indicator (Kei, 1978). Briefly, 10% (weight/volume) homogenates of kidney and liver were made by 0.1mol/l phosphate buffer. The homogenates were centrifuged at 3500 rpm (4ºC) for 10 min. 0.2ml supernatant was mixed with 0.67% 2-thiobarbituric acid (TBA) and 20% trichloroacetic acid solution, and heated in a boiling water bath for 30 min. The pink-colored chromogen formed by the reaction of TBA with MDA was measured at 532nm. The results were expressed as MDAnmol/mg protein. Protein concentration of the supernatant was measured by Lowry's method.[16]

Measurement of tissue glutathione

The tissue samples were homogenized in 10 volumes of ice-cold 10% trichloroacetic acid and centrifuged at 1000g (4˚C) for 15 min. The supernatant was discarded and centrifuged at 35,000g (4˚C) at for 8 min. GSH was determined spectrophotometrically by Ellman procedure with slight modification.[17]

Cytotoxicity study

Cell culture

MCF-7 (oestrogen receptor-positive human breast adenocarcinoma cell line), MDA-MB 231 (oestrogen receptor-negative human breast adenocarcinoma cell line), HepG2 (human liver hepatocellular cells), A-549 (human lung cancer cell line) and PC3 (Human prostate cancer cell line) were obtained from American Type Culture Collection (ATCC, Va, USA). All cells were maintained in RPMI-1640 medium that is supplemented with 10% Fetal Bovine Serum (FBS) and incubated at 37°C. Celll viability assay was determined by MTT assay as previously described by Mossman et al.[18,19] Briefly, 1×10^6 cells/ml were treated with the compound at different concentration in 96-well plate and incubated for 24h. The colorimetric assay is measured and recorded at absorbance of 570nm. Results of cell viability were expressed as percentage of control. The potency of cell growth inhibition for test agent was expressed as IC_{50} value.

STATISTICAL ANALYSES

Data was presented as mean ± standard deviation. Significant differences were determined by using the Student's t-test and ANOVA, where $*p < 0.05$ denotes a statistically significant difference. All the samples were measured in triplicates.

RESULTS

Isolation of β-Mangostin

β-mangostin was initially obtained as yellowish solid and yellowish needle-shaped crystals were obtained after recrystallized with hot chloroform. IR $\nu_{max}cm^{-1}$ (KBr): 3407 (OH), 2923 (CH), 1642 (C=O) and 1596 (C=C); UV MeOH $\lambda_{max}nm$ (log ε): 374 (1.89), 350 (3.99), 344 (2.19), and 340 (3.29); EIMS *m/z* (% intensity): 424 (53.79), 409 (5.20), 393 (1.19), 3.81 (19.45), 368 (31.93), 353 (100.00), 335 (20.79), 310 (7.59), 299 (23.66) and 169 (8.41); ^{1}H-NMR (500 MHz, acetone-d_6): δ 13.61 (OH-1), 9.62 (OH-6), 6.82 (*s*, 1H, H-5), 6.47 (*s*, 1H, H-4), 5.25 (*t*, *J* = 6.9 Hz, 1H, H-12), 5.18 (*t*, *J* = 6.9 Hz, 1H, H-17), 4.10 (*d*, *J* = 6.9 Hz, 2H, H-11), 3.94 (OMe-3), 3.77 (OMe-7), 3.29 (*d*, *J* = 6.9 Hz, 2H, H-16), 1.80 (*s*, 3H, Me-14), 1.75 (*s*, 3H, Me-19), 1.63 (*s*, 3H, Me-15) and 1.61 (*s*, 3H, Me-20); ^{13}C-NMR (125 MHz, acetone-d_6): δ 186.8 (C-9), 168.5 (C-4a), 164.4 (C-1), 161.5 (C-10a), 160.2 (C-6), 160.1 (C-3), 148.5 (C-7), 142.0 (C-8), 135.4 (C-18 and C-13), 128.6 (C-12), 127.2 (C-17), 115.9 (C-8a), 115.7 (C-2),

108.0 (C-9a), 106.6 (C-5), 93.8 (C-4), 65.2 (OMe-7), 60.4 (OMe-3), 30.8 (C-11), 29.8 (C-15), 29.8 (C-20), 25.8 (C-16), 22.2 (C-14) and 21.7 (C-19).

The structure of β-mangostin was established due to significant correlations in Heteronuclear Multiple Bond Connectivity, Heteronuclear Multiple Quantum Coherence, Distortionless Enhancement by Polarization Transfer together with the signals displayed by ^{1}H and ^{13}C-NMR spectra. The fragmentation pattern and molecular mass of the compound were further confirmed by the Electron Impact Mass Spectrometry and the typical absorption bands of the functional groups were displayed from the Infrared spectroscopic data.

The purity of the compound was analyzed by Shimadzu UFLC system equipped with a PDA UV detector and Ion Trap TOF mass spectrometer. Column: Waters Xbridge 50 × 2.1mm 2.5µM. Mobile phase: H_2O (0.1% formic acid):MeCN (0.1% formic acid). Flow rate: 0.50mL/min. Column temperature: 40°C. Gradient: 10–100% MeCN over 7 min (Figure 2), and the compound was also identified by comparison of its spectral data to those reported in the literature.[8]

Acute Oral Toxicity Study

General observations

There was no mortality, abnormal behaviour or physical signs of toxicity observed after oral administration of β-mangostin at any tested doses. In all cases the faeces of the experimental animals were dry and dark coloured for all groups. No differences were observed in average daily food and water intake among the groups.

Effect of β-mangostin on animal weight and relative organ weight

β-mangostin had no significant ($p>0.05$) effect on the body weight of mice in any group throughout the experiment. Figure 3 suggests that based on differential analysis on body weight, animals of both the sexes showed normal weight gain.

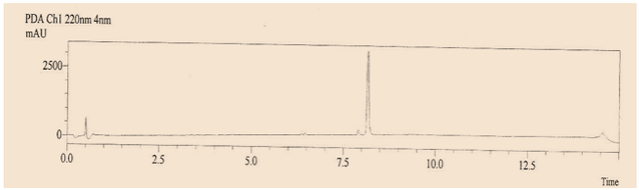

Figure 2. Chromatogram of β-Mangostin at 220nm. Analysis was performed using a Shimadzu UFLC system equipped with a PDA UV detector and Ion Trap TOF mass spectrometer. Column: Waters Xbridge 50 × 2.1mm 2.5µM. Mobile phase: H2O (0.1% formic acid): MeCN (0.1% formic acid). Flow rate: 0.50mL/min. Column temperature: 40°C. Gradient: 10–100% MeCN over 7 min.

It is common to have reduction in organ weight in toxicity conditions; hence, the liver, kidney, lungs, spleen and heart were collected and weighed after the sacrificing animals. It was observed that there was no significant difference in the relative organ weight among all the treated groups as compared to the control (Figure 4).

Serum biochemical parameters

As observed from the serum biochemistry data presented in Table 1, there were no treatment-related significant differences in various parameters including serum electrolytes such as sodium, potassium and chloride. The effect of β-mangostin on liver function parameters such as ALT, AST, ALP, conjugated bilirubin and total bilirubin in serum were also investigated. No significant differences in these markers were noted with treatment of 250 and 500mg/kg β-mangostin. Data in relation to effects of β-mangostin on triglyceride, high density lipoprotein

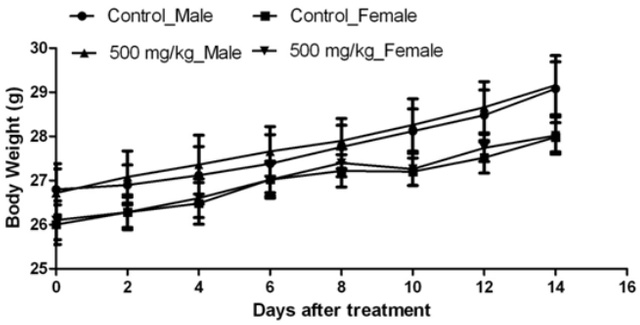

Figure 3. Comparison of the changes in body mass across sexes in control and treatment groups during observation based on acute toxicity test of β-mangostin on ICR mice. β-mangostin was given to the treated group and 10% Tween-20 was given to the control group.

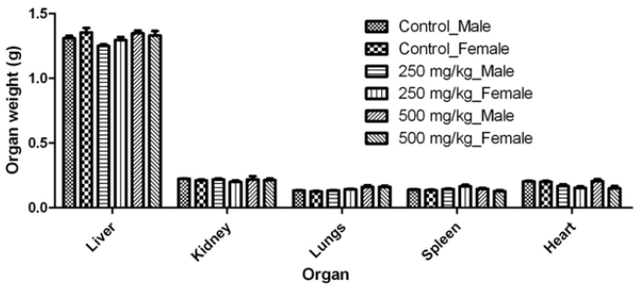

Figure 4. Comparison of organ weight across sexes in control and treatment groups observed during acute toxicity test of β-mangostin on ICR mice. β-mangostin was given to the treated group and 10% Tween-20 was given to the control group.

cholesterol HDL and total cholesterol levels are shown in Table 1. Animals of both sexes that received high dose (500mg/kg) showed significant decrease in triglyceride, HDL cholesterol and total cholesterol levels. This effect was however not evident in low dose treatment group.

Histopathological evaluation

No significant differences were observed in gross examination during autopsy and histopathological evaluations of liver and kidney stained with hematoxylin and eosin. Histological profile of the normal and treated animals revealed normal hepatocytes with well-preserved cytoplasm, prominent nucleus, nucleolus and central vein. No sign of inflammation, fatty change and necrosis were seen in these animals (Data not shown).

Effect of b-mangostin on oxidative stress biomarkers

Oxidative stress in the tissues was evaluated quantitatively using measurements of MDA and GSH (Table 2). β-mangostin (500mg/kg) treated animals showed slightly higher MDA and GSH levels regardless of their gender compared to normal control, but was not significant

Table 1. The effects of β-mangostin on the biochemical parameters in serum of ICR mice.

Parameter	Sex	Normal control	β-Mangostin 250mg/kg (b.w)	β-Mangostin 500mg/kg (b.w)
Sodium (mmol/l)	Male	146.4±11.2	141.2±10.3	144.2±10.0
	Female	147.2±13.5	149.1±11.2	146.2±11.5
Potassium (mmol/l)	Male	5.1±0.4	6.2±0.3	6.2±1.0
	Female	4.8±0.5	3.6±0.2	5.9±1.1
Chloride (mmol/l)	Male	111.0±10.2	114.3±11.0	119.1±11.5
	Female	110.7±13.0	120.1±12.4	114.2±12.2
Carbon Dioxide (mmol/l)	Male	15.1±1.1	14.2±3.1	16.7±3.1
	Female	13.9±1.4	16.1±2.0	15.0±0.8
Anion Gap (mmol/l)	Male	24.8±1.6	29.8±2.9	26.0±0.7
	Female	26.3±1.1	21.2±2.8	24.1±2.2
Urea Nitrogen (mmol/l)	Male	12.9±2.0	14.1±3.1	13.1±3.1
	Female	9.9±1.3	10.3±1.2	11.1±2.0
Creatinine (µmol/l)	Male	21.7±2.6	22.1±3.4	24.0±1.3
	Female	28.3±2.8	29.1±1.8	30.0±1.2
Total Protien (g/l)	Male	65.2±3.8	64.2±1.9	66.2±7.8
	Female	56.25±4.1	60.21±2.4	55.11±1.1
Albumin (g/l)	Male	17.1±1.9	18.1±2.1	18.3±1.1
	Female	14.5±2.7	15.0±1.8	15.1±9.0
Total Protein (g/l)	Male	58.2±2.1	62.4±4.2	56.6±4.0
	Female	59.5±3.2	64.3±3.0	61.0±4.1
Albumin (g/l)	Male	14.0±1.0	13.2±2.1	12.2±2.7
	Female	11.1±1.0	10.2±2.5	16.6±3.3
Globulin (g/l)	Male	44.7±1.5	49.6±1.4	49.2±3.0
	Female	49.0±3.1	45.5±2.7	55.5±4.6
Total Bilirubin (µmol/l)	Male	2.0±0.6	2.4±0.1	2.5±0.2
	Female	1.5±0.9	1.1±0.5	1.4±0.01
Conjugated Bilirubin (µmol/l)	Male	0.7±0.01	1.1±0.4	0.6±0.02
	Female	1.0±0.2	1.3±0.7	1.1±0.09
Alkaline Phosphatase (IU/l)	Male	48.4±1.2	45.7±1.3	39.7±2.8
	Female	52.6±2.5	59.0±2.1	61.2±1.7
Alanine Aminotransferase (IU/l)	Male	75.0±2.5	81.1±2.2	68.8±1.8
	Female	70.0±1.9	76.1±1.0	81.1±4.1
AST (IU/l)	Male	404.2±13.1	396.3±21.2	421.5±16.0
	Female	411.8±19.9	410.4±19.4	401.9±30.1
G-Glutamyal Transferase IU/l	Male	2.6±0.2	2.0±0.4	3.1±0.8
	Female	1.5±0.3	1.7±0.3	2.2±0.9
Triglyceride (mmol/l)	Male	0.7±0.01	0.9±0.01	0.1±0.01*
	Female	1.1±0.5	1.8±0.01	0.2±0.01*
Total Cholestrol (mmol/l)	Male	1.5±0.07	1.1±0.01	0.3±0.03*
	Female	1.3±0.02	1.2±0.03	0.5±0.01*
HDL Cholestrol (mmol/l)	Male	1.59±0.5	1.09±0.2	0.19±0.6*
	Female	1.8±0.1	1.1±0.9	0.2±0.3*

Data are expressed as mean ± SD of five mice for each group. '*' indicates statistical difference at $p<0.05$.

Table 2. Tissue malondialdehyde levels and glutathione content of β-mangostin on the ICR mice.

Parameter	Organ	Sex	Normal control	β-Mangostin 250mg/kg (b.w)	β-Mangostin 500 mg/kg (b.w)
MDA (nmol/g)	Liver	Male	0.05±0.005	0.06±0.010	0.07±0.000
	Kidney	Female	0.04±0.007	0.05±0.000	0.06±0.008
GSH (nmol/g)	Liver	Male	0.05±0.003	0.05±0.001	0.06±0.001
	Kidney	Female	0.05±0.005	0.05±0.007	0.06±0.006

Data are expressed as mean ± SD of five mice for each group.

($p>0.05$) while treatment with the dose of 250mg/kg of β-mangostin did not show any abnormal levels of MDA and GSH in male and female treated animals.

In vitro cytotoxicity

To evaluate the cytotoxic activity, β-mangostin was tested with a series of different concentrations on A549, PC-3, HepG2, MCF-7 and MDA-MB-231 cells (Fig. 5). Cell viability was determined using MTT assay. As shown in Fig. 5, β-mangostin induced cytotoxicity in a concentration dependent manner to both breast cancer cells with low IC_{50} value. The data from MTT assay presents that β-mangostin has different degrees of cytotoxicity on different cell types. Amongst the cells, the liver cells, HepG2 showed had very low cytotoxicity with an IC_{50} of 43.5μg/ml.

In the MCF-7 and MDA-MB-231 cells, the morphological features as observed under normal inverted microscope was having slight alterations in cellular components after 24h post-treatment (Fig. 6 A and C) as compared to non-treated cells (Fig. 6 B and D). Cells exposed to β-mangostin showed more prominent growth inhibition and shrinkage of the cells. On the contrary, untreated cells remained confluent throughout the incubation period.

DISCUSSION

A survey by Word Health Organization stated that 70–80% of the people around the world count on non-conventional medicine for primary health care, which is predominantly of herbal source.[20] Especially in the developing nations, where most people who cannot afford a consultation with western style doctor or the cost of medication, this becomes a common practice. This knowledge of man's dependency on traditional remedies is the sole backbone of journey from ethnomedicine to modern medicine.[21] The novel drug development process begins from the secondary metabolite that the plants produce; often ending up to produce successful medications based on new compounds. This becomes a boon where these new compounds are proven clinically useful for deadly diseases including cancer or sometimes, simply inflammation or other diseases for which the scientific community is constantly in hunt for new targets to harness such diseases. However, always not all active secondary metabolites reach the final stage of drug development due to their adverse effects or toxicity.[22]

In the preclinical stage of drug investigation, it is mandatory to have the data showing the safety of that drug using preclinical animal toxicology studies. The *in vivo* toxicity studies using animal models are much reliable because of the similarity in mechanisms of toxicity across species, which are often based on similarity in anatomical, physiological, metabolic, pathological and pharmacological characteristics.[23] A good correlation has been reported between toxicological insults in mice and humans[24] and hence, the vast number of studies demonstrating the acute effects of different test substance (compounds/drugs) in mice; sometimes, with the doses potentially usable in humans. In the mice model, various organ level toxicities and biochemical parameters including the overall condition of experimental animals are studied post-treatment to the compound.[25,26] A common toxic effect noted is the reduction in body weight, which could be primarily attributed to diminished food intake which may be the consequence of toxins released and absorbed in the gastrointestinal tract. As such, the body weight of all experimental animals was noted and there were no significant changes in body weight throughout the study across all groups and both sexes. Accompanied by variation in body weight due to toxicity, is very common that various body organ weights also decrease. β-mangostin could be said safe with this aspect as no significant changes in the organ weight was observed with the mice being treated with this compound.

Similarly, exposure of various groups of mice to β-mangostin had no adverse effect on the usual markers of liver and kidney toxicity (the plasma levels of liver enzymes, ALT and AST, bilirubin and creatinine). The transaminases (AST and ALT) are well-known enzymes which are good indicators of liver function as biomarkers in predicting toxicity.[27] Any kind of insult to the parenchymal liver cells results elevated level of both transaminases in the blood.[28] The serum AST is of both mitochondrial and cytoplasmic origin and hence any rise of its level

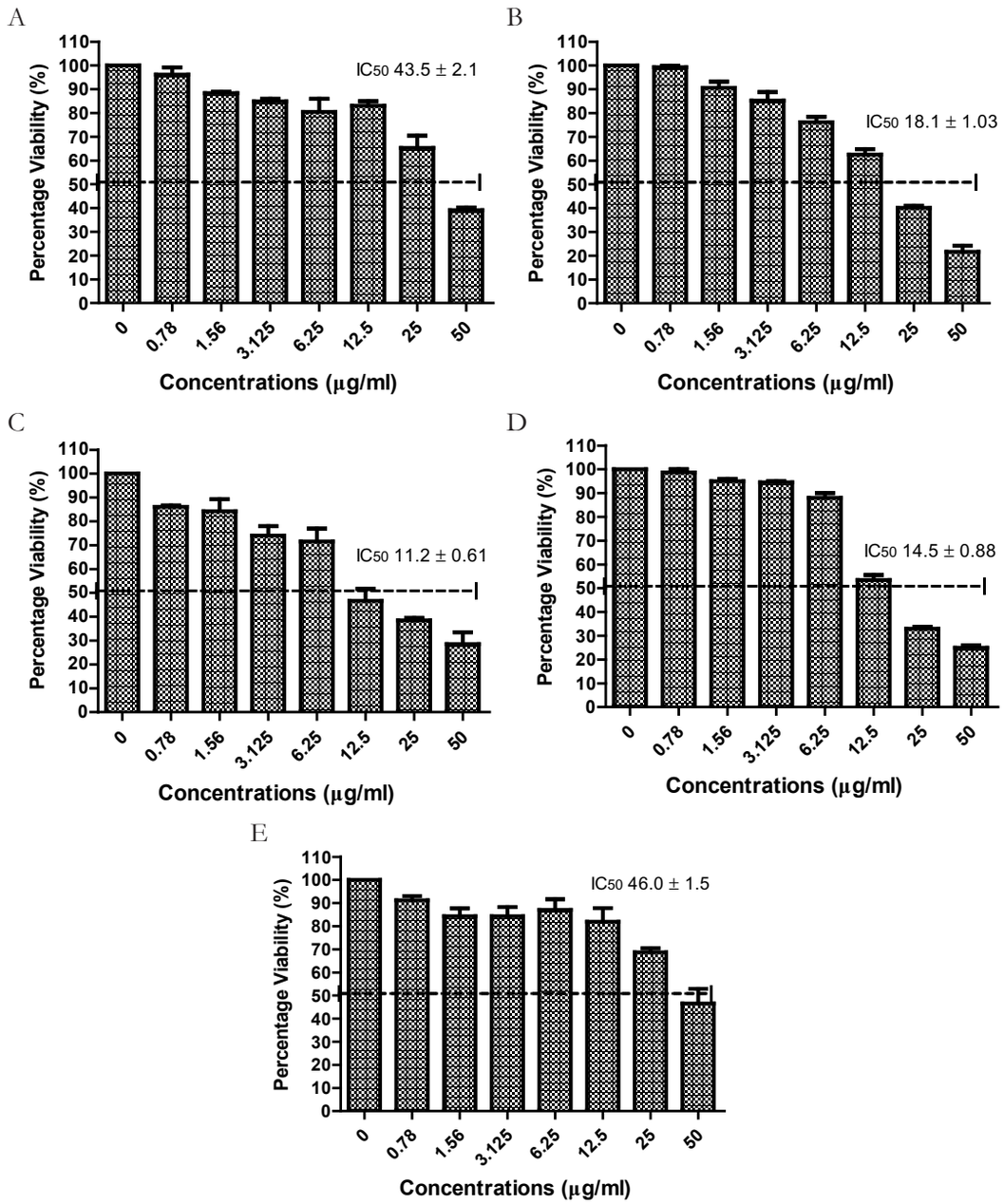

Figure 5. Effect of β-mangostin on different cells (A: HepG2 cells, B: A549 cells, C: MCF-7 cells, D: MDA-MB-231 cells and E: PC3 cells) type expressed as IC50 values in MTT assay.

is considered first sign of cell damage that leads to the outflow of the enzymes into the serum. Therefore, the acute administration of β-mangostin did not affect the hepatocytes function and metabolism. Further, there was also no remarkable change in creatinine level upon administration of any of the doses of β-mangostin in the tested animals when compared to the control. As increased creatinine level in serum is characterized in association to renal damage, this indicator is used to identify renal toxicity during drug administration.[29,30] Thus, the results recorded in this study suggest that β-mangostin did not affect the renal function.

Other biochemical parameters analyzed in this study such as the total protein and albumin levels also had no significant changes in the treated groups when compared to control. Even though the aforementioned parameters of treatment group were at par with that of the control group, the cholesterol levels were observed to have decreased with administration of high dose of β-mangostin. Thus

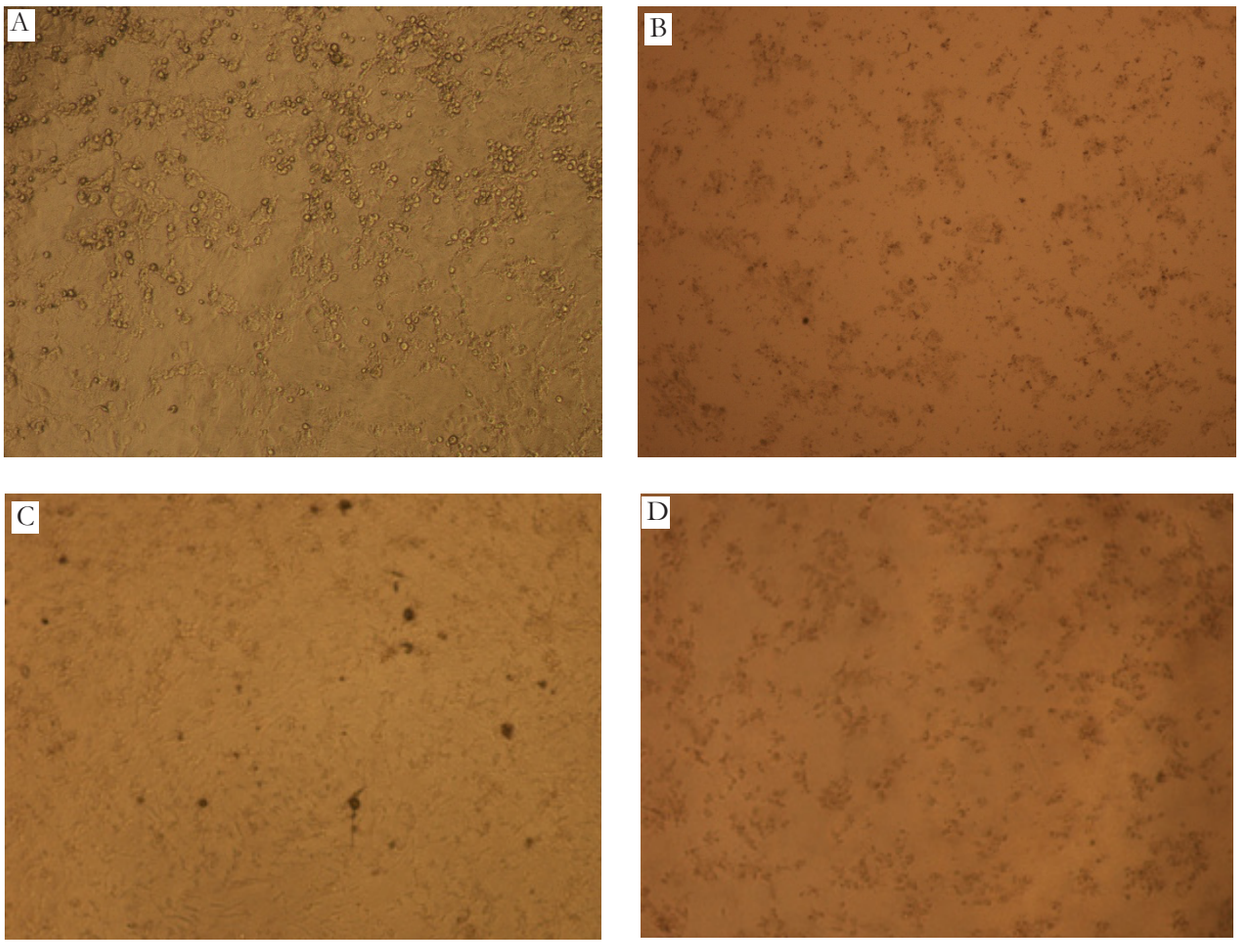

Figure 6. Morphological features of MCF-7 and MDA-MB-231 at IC50 concentrations of β-mangostin. Phase contrast micrographs of MCF-7 and MDA-MB-231cells treated at IC50 of β-mangostin 24h, where (A) MCF-7 control, (B) MCF-7 treated, (C) MDA-MB-231 control and (D) MDA-MB-231 treated (10 × magnifications).

β-mangostin had some effect on the cholesterol metabolism of the mice. The levels of electrolytes that maintain the body fluid equilibrium; however, had no significant changes. This suggests that β-mangostin had not affected the function and equilibrium of body fluids. Except for a significant decrease in the levels of triglycerides and cholesterol, there was no noticeable changes in hematological and biochemical profile upon administration of β-mangostin. Thus it is observed that β-mangostin is relatively low or non-toxic natural compound under the experimental conditions of this study. This is, so far, the first study to report the suitability of *in vivo* administration of β-mangostin, a major phytochemical present in the fuctional food. In support to these findings the gross examination during autopsy and histopathological evaluations of the various organs stained with hematoxylin and eosin revealed no significant differences in the kidney and liver. Toxicity of compounds normally induces the cellular stress by increasing the oxidative stress.[31,32] This may attribute to generation of free radicals and later kill the cells. In the current study, we also intended to confirm the possibility of evoking any cellular stress associated to the administration of β-mangostin. Therefore, we have measure the two main biomarkers of oxidative stress: MDA and GSH. The non-significant levels of these biomarkers post-treatment of β-mangostin ruled out the involvement of oxidative stress of the compound at the mentioned doses.

In vitro behavior of cells in response to various compounds are little different than that of the *in vivo* environment. Since we observed no sign of toxicity in animal model, then we focus to study the behavior of different cancer cells upon exposure to β-mangostin *in vitro*. Subsequently, our intention is to study the effect of β-mangostin in treating cancer *in vitro* and *in vivo*. Herein, we explore its anti-proliferative capacity towards various cancer cell lines. As shown in Fig. 5, β-mangostin exhibited different

level of cytotoxicity in the selected cell lines. HepG2 is a well-known *in vitro* model for assessing toxicity of compounds. Previously, many researchers have used this cell line to establish the safety of compounds[33–35]. Comparable to *in vivo* effect, there was no significant cytotoxicity in HepG2 cells produced by β-mangostin. As per the results obtained in the current study, the compound was significantly toxic to two types of breast cancer cells used (MCF-7 and MDA-MB-231). The antipoliferative result thus highlights the need to study the compound for its anticancer action against breast cancer cells.

CONCLUSIONS

In conclusion, the present evaluation had showed the potential of β-mangostin to be selected for further studies. There was no significant toxicity observed in the studied parameters; moreover, the compound showed cell specific cytotoxicity towards breast cancer cells *in vitro*. Hence, currently the mechanism of cytotoxicity of β-mangostin on breast cancer *in vitro* an *in vivo* is being studied in our lab.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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