Asian Journal of Chemistry; Vol. 33, No. 10 (2021), 2465-2471

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23310

Mechanism of Antiangiogenic and Antioxidant Activity of Newly Synthesized CAMBA in Ehrlich Ascites Carcinoma-Bearing Mice

NADA I. ABOU-TALEB[1], OLA A. ELBLASY[1], ESRAA A. ELBESOUMY[1], HAIDY I. BASUNY[1], ESRAA A. ELHAMADI[1], MOHAMED S. NASR ELDIN[1], AHMED A. EMARA[1], ALI A. ALI[2], MOHAMED A. SALEM[3], FAKHER M. AHMED[3] and MOHAMMED A. HUSSEIN[4,*]

[1]Department of Radiology and Medical Imaging, Faculty of Applied Medical Science, October 6 University, 6th of October City, Egypt
[2]Vice President of Post Graduate Studies, October 6 University, 6th of October City, Egypt
[3]Department of Organic Chemistry, Faculty of Pharmacy, October 6 University, 6th of October City, Egypt
[4]Department of Biochemistry, Faculty of Applied Medical Science, October 6 University, 6th of October City, Egypt

*Corresponding author: Tel: +20 124832580; E-mail: prof.husseinma@o6u.edu.eg

Received: 16 May 2021; *Accepted*: 13 August 2021; *Published online*: 20 September 2021; AJC-20521

The aim of present study was to evaluate antiangiogenic activity of newly synthesized caffeic acid methyl benzoate amide (CAMBA) in EAC-bearing mice. The IC_{50} value of CAMBA against the Hep-G2 liver carcinoma cell line was calculated. Adult albino mice weighing 25 ± 5 g was used to assess the antiangiogenic activity of CAMBA (25 and 50 mg/k.b.w.) in EAC-bearing mice. IC_{50} CAMBA against the Hep-G2 cell line equals 52.8 µg/mL. The daily oral administration of CAMBA at concentrations of 25 and 50 mg/kg.b.w. for 30 days to EAC-bearing mice resulted in a significant improvement in tumor volume and tumor weight, ALT, AST, ALP, MMP-2 and -9, TNF-α, NOx, TBARs, GSH, CAT, SOD, GPx and VEGF-C gene expression in EAC-bearing mice. Furthermore, CAMBA almost normalized these effects in liver histoarchitecture. The biochemical, histological and ultrasound examinations of our study suggested that CAMBA have antiangiogenic activity in EAC-bearing mice.

Keywords: CAMBA, Hep-G2 liver carcinoma, Antiangiogenic, Ehrlich ascites carcinoma, Anticancer activity.

INTRODUCTION

Cancer is one of the main causes for world's leading deaths. Although we already have a number of cancer agents, there is still a lack of adequate control of cancer [1]. There is a continued demand for new and more efficient anti-cancer drugs which can contribute to control this problem [2]. The process of cancer and tumors involves the oxidative stress. Because of this, ROS can cause damage, damage or breach in the double chain macromolecules, alterations in guanine and thymine bases and the synthesis of malondialdehyde mutations [3]. This also leads to changes in guanine and thymine bases and to the exchange of sister chromatids [4]. Human beings have developed to protect free radicals and ROS with antioxidant systems. Some antioxidants produced in the body (endogens) and others derive from diet (exogenous) are included in these systems [5]. The main groups are plants, which are part of many standard regimes for anticancer products, such as vinca alkaloids, camptothecins, taxans and epipodophyllotoxins [6]. There have been numerous natural compounds with anti-inflammatory [7], antioxidant [8] and anticarcinogenic [9] activities.

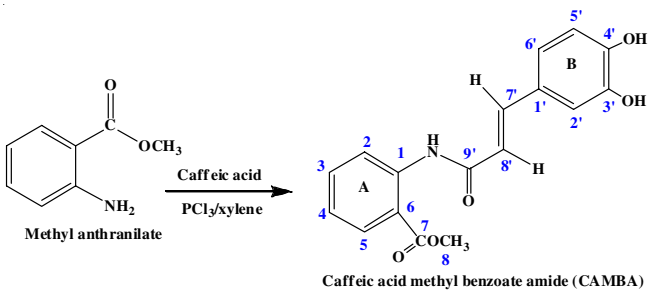
The natural sources (propolis) with a range of biological activities are perfectly illustrated by caffeic acid phenylethyl ester, one of the most active compounds in many natural products [10]. Many studies demonstrated that caffeic acid phenethyl ester (CAPE) has antioxidant [11], hepatoprotective [12] and anti-inflammatory [13] activities. Hussein & Gobba [12] reported the facile route to prepare CAMBA as a promising antidiabetic and antioxidant new drug. But there are no reports about antitumor activity of CAMBA. As an extension of our research plans to synthesis of new drug and evaluate their medicinal importance [17-19]. The antiangiogenic and antioxidant activities of the caffeic acid methyl benzoate amide (CAMBA) [12] in Ehrlich ascites carcinoma bearing mice was evaluated.

This is an open access journal, and articles are distributed under the terms of the Attribution 4.0 International (CC BY 4.0) License. This license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit the author for the original creation. You must give appropriate credit, provide a link to the license, and indicate if changes were made.

EXPERIMENTAL

The melting points were calculated using a Gallenkamp melting point apparatus and are not corrected. Shimadzu MR470 a Shimadzu MR470, Varian EM 360 (^{1}H NMR at 240 MHz and ^{13}C NMR at 75 MHz) and HP Model MS-5988 were used to record infrared IR, ^{1}H & ^{13}C NMR and mass spectra, respectively. At Cairo University's Microanalytical Center, microanalytical data (C, H and N) were calculated.

Synthesis of caffeic acid methyl benzoate amide (CAMBA): Hussein's method was used to synthesize caffeic acid methyl benzoate amide (CAMBA) [12]. Phosphorus trichloride (3 mL) was added to a solution of caffeic acid (0.01 mol) and methyl anthranilate (2.31 g, 0.01 mol) in xylene (50 mL). The reaction mixture was heated under reflux for 3-4 h. The crude product was recrystallized from ethanol to produce CAMBA (**Scheme-I**). Yield: 77%; m.p.: 178-180 ºC. Elemental analysis of $C_{18}H_{17}NO$ calcd. (found) %: C, 65.17 (66.20); H, 4.79 (4.25) N, 4.47 (4.15). IR (KBr, ν_{max}, cm^{-1}): 3332 (OH), 3133 (NH), 2962 (CH-arom.), 2854 (CH-aliph.), 1715, 1685 (2C=O), 1520 (C=N). MS (*m/z*): 313 (M^+, 10.57%), 276 (10.25%), 224 (14.29%), 183 (21.42%), 137 (25.05%), 97 (100%), 55 (46.42%).^{1}H NMR (DMSO-d_6): 2.4 (s, 3H, $COCH_3$), 6.1-6.2 and 7.3-7.4 (d, 2H, CH=CH, two *trans*-olefinic protons), 6.7-8.0 (m, 7H, Ar-H), 10.2 (s, 1H, NH). ^{13}C NMR (75 MHz, CD_3OD): 140.322 (C-1), 121.392 (C-1′), 123.303 (C-2),115.529 (C-2′), 130.500 (C-3), 145.452 (C-3′), 125.521 (C-4), 145.702 (C-4′), 129.859 (C-5), 115.929 (C-5′), 114.663 (C-6), 119.102 (C-6′), 165.021 (C-7), 144.63 (C-7′), 55.520 (C-8), 109.21 (C-8′), 199.47 (C-9′).



Scheme-I: Synthesis of CAMBA

Determination of cytotoxicity on Hep-G2 cells: To form a full monolayer sheet, the 96 well tissue culture plate was inoculated with 1 × 10^5 cells/mL (100 µL/well) and incubated at 37 ºC for 24 h. The growth medium was decanted from 96-well microtiter plates after forming a confluent sheet of cells and washed two times with washed medium by the cell monolayer. The sample was double diluted in the medium of RPMI with 2% serum (maintenance medium). Each dilution (0.1 mL) was tested in each well, with 3 wells as controls and maintenance only. The plate was incubated at 37 ºC.

Physical symptoms of toxicity, such as partial or total monolayer loss, rounding, shrinkage or cell granulation were examined in the cells. The MTT solution (5 mg/mL in PBS) was prepared (Bio Basic, Canada Inc.). Each well received 20 µL of MTT solution. Thoroughly mix the MTT into the media, place on a shaking table for 5 min at 150 rpm. To allow the MTT to be metabolized, incubate for 1 to 5 h at 37 ºC and 5% CO_2. In 200 µL DMSO, resuspend formazan (MTT metabolic product). Thoroughly mix the formazan into the solvent, place on a shaking table at 150 rpm for 5 min. At 560 nm, read the optical density and deduct the history at 620 nm. The optical density should be proportional to the number of cells.

Animal ethics: The Research Ethics Committee at the Faculty of Applied Medical Sciences, October 6 University in Egypt, granted ethical approval for data collection (No. 2020-1008).

Mice: The Animal Care and Use Committee of October 6 University developed guidelines for this experiment, which were followed. Adult mice weighing about 25 ± 2 g were purchased from Cairo University's Faculty of Veterinary Medicine. They were held in cages in an air-conditioned environment at 22 ºC, a relative humidity of 60% and a light period of 8:00 to 20:00. Each animal was fed a daily diet *ad libitum* during the acclimatization period.

Experimental design: EAC cells were provided from Cairo's Cancer Institute. The cells were maintained *in vivo* in Swiss albino mice *via* intraperitoneal transplantation (2 × 10^6 cells per mice) into all groups except the first [20].

Experimental design: The animals were divided into 5 groups consisting of 6 animals, two controls groups and three treatment groups:

Group I (Normal control A): Distilled water (3 mL) was orally administered for 30 days.

Group II (EAC control): Subcutaneous injection of 2 × 10^6 cells/mice in water was administered.

Group III: (EAC + CAMBA): Oral suspension of 25 mg/kg b.w. CAMBA in water for 30 days in a single daily dose was administered [12].

Group IV: (EAC + CAMBA): Oral suspension of 50 mg/kg b.w. CAMBA in water for 30 days in a single daily dose was administered [12].

Group V: (EAC + 5-FU): Intraperitoneal injection of 20 mg/kg b.w. 5-fluorouracil (5-FU) on alternate days for 4 weeks for 30 days in a single daily dose [21].

Six mice from each group were dissected on the 31st day, 24 h after the injection all mice were sacrificed at the end of the experiment. Blood was collected, centrifuged and plasma transaminases (L-alanine and L-aspartate) [22], alkaline phosphatase (ALP) [23], MMP-2 [25] and MMP-9 [25] were determined by enzyme-linked immunosorbent assay (ELISA) kits specific for mice (Abnova, Taipei City, Taiwan). The tumour mass was removed from each mice in groups (II-V) to estimate its weight. Also, ascites fluid was extracted from the peritoneal cavity and measured using a graduated centrifuge tube [21,26].

Hepatic TBARS [27], nitric oxide (NOx) [28], tumour necrosis factor (TNF-α) [29], GSH [30], catalase (CAT) [31], superoxide dismutase (SOD) [32] and glutathione peroxidase (GPx) [33] levels were estimated using rat ELISA kits in accordance with the supplier's protocol (Rapid, Bio. Laboratories, Inc.).

Quantitative real-time PCR: The total RNA extracted was extracted from the liver of the mice and portions of (10-15 µg) of the isolated RNA were subjected to quantitative PCR analysis

in real time, using Sepasol-RNA1 Super according to instructions of the manufacturer. The two-step RT-PCR gene expression has been measured. The level of VEGF-C was quantified with the previously described quantitative real-time PCR [34]. The tests in 50 mL single-plex reaction mixture were conducted. Conditions of reaction were a pre-incubation at 50 ºC in 2 min, followed by 10 min by 40 cycles of 95 ºC in 15 s and 60 ºC in 1 min, respectively. The primer sequences were VEGF-C: F 5′-AACGTGTCCAAGAAATCAGCC-3′, R: 5′-AGTCCTCT-CCCGCAGTAATCC-3′. The internal control used GAPDH-F: 5′-CTCAACTACATGGTCTACATGTTCCA-3′ and -R: 5′-CCATTCTCGGCCTTGA-CTGT-3′.

Histological assessment: The liver is sliced and parts have been fixed in histologic solution of 10 % formaldehyde buffered. 5 µm thick was stained with hematoxylin eosin (HE) and examined by light microscopic according to the reported method [35].

Ultrasound protocol

Experimental mice were evaluated at Smart Scan Radiology Center, Cairo, Egypt. All experimental ethics procedures were achieved. Once placed on the handling platform, each mouse was fixed in a supine recumbence position, the abdominal area was shaved to reduce imaging artifact. A conducting gel was applied to the area and the procedure were done using a multi-frequencies linear transducer (7-12 MHz).

A gel helps the transducer makes close contact with the body eliminating air pockets between the transducer and the skin that can block the sound waves to pass into the body. The probe was used on the abdomen and moved back and front over the abdomen until the interested images are captured.

Doppler study was also performed using the same transducer to get more details about the lesions vascularization to predict the diagnosis. Liver Images were stored on the ultrasound machine including images of all groups.

Statistical analysis: With SPSS-18 programme, all of the resulted data was statistically evaluated. One-way analysis of variance (ANOVA) was used to evaluate hypotheses, followed by the least significant difference (LSD) test. Statistical significance was determined by $p < 0.05$.

RESULTS AND DISCUSSION

Fig. 1 shows the IC_{50} of CAMBA against liver carcinoma (Hep-G2) cell line = 52.8 µg/mL. Oral administration of CAMBA at 25 and 50 mg/kg and i.p. injection of 5-fluorouracil showed a significant decrease ($p < 0.05$) in tumour volume and weight compared to the mice bearing EAC (Table-1). The decrease in tumour volume and weight in group of mice which supplemented CAMBA more pronounced than 5-fluorouracil.

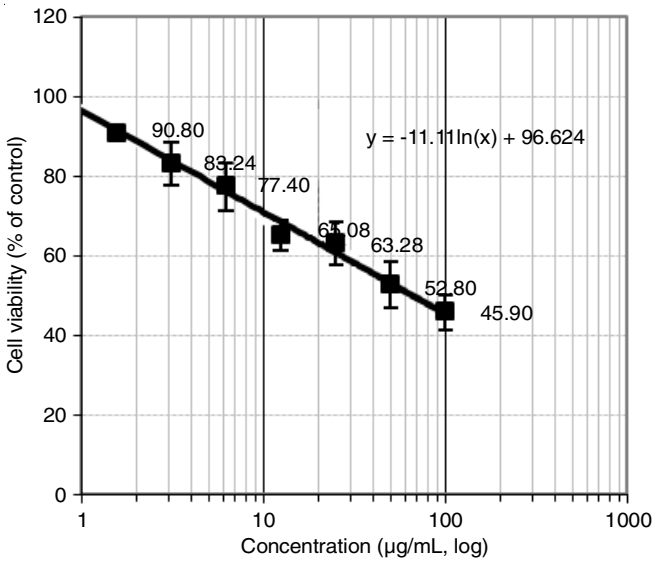


Fig. 1. IC_{50} of CAMPA against liver carcinoma (Hep-G2) cell line

Tables 2 and 3 display a substantial increase in AST, ALT, ALP, TNF-α, NOx and TBARs, as well as a decrease in MMP-2, MMP-9 and GSH in plasma and hepatic tissue of mice with EAC compared to the standard nonbearing EAC group ($p < 0.05$). As compared to mice bearing Ehrlich ascites carcinoma, CAMBA at 25 and 50 mg/kg and i.p. injection of 5-fluorouracil resulted in a substantial decrease in AST, ALT, ALP, TNF-α, NOx and TBARs, as well as a rise in MMP-2, MMP-9 and GSH levels ($p < 0.05$).

When compared to the normal control group, subcutaneous EAC implantation resulted in a substantial decrease in liver CAT, SOD and GPx activity ($p < 0.05$). As compared to mice with Ehrlich ascites carcinoma, mice given 25 and 50 mg/kg CAMBA and i.p. injections of 5-fluorouracil showed a substantial improvement in CAT, SOD and GPx activity ($p < 0.05$).

In mice with Ehrlich ascites carcinoma, levels of liver vascular endothelial growth factor C (VEGF-C) gene expression were significantly lower ($p < 0.05$) as compared to the normal control group. When compared to Ehrlich ascites carcinoma bearing mice, CAMBA at 25 and 50 mg/kg and i.p. injection of 5-fluorouracil resulted in a substantial increase in the levels of VEGF-C gene expression ($p < 0.05$) (Table-4).

TABLE-1
EFFECT OF CAMBA AND 5-FLUOROURACIL (5FU) ON TUMOR VOLUME AND TUMOR WEIGHT

No.	Groups	Tumor volume (mL)	Tumor weight (g)
I	Normal group (non-tumor bearing mice (NTB)	0.0 ± 0.0^{a}	0.0 ± 0.00^{a}
II	EAC control (tumor bearing mice (TB)	2.1 ± 0.11^{e}	1.7 ± 0.25^{e}
III	EAC + CAMBA (25 mg/kg b.w)	1.10 ± 0.31^{d}	0.96 ± 0.09^{d}
IV	EAC + CAMBA (50 mg/kg b.w.)	0.67 ± 0.14^{b}	0.48 ± 0.16^{b}
V	EAC + 5-fluorourcil (20 mg/kg b.w.)	0.78 ± 0.20^{c}	0.65 ± 0.22^{c}

5-Flourourasil was given i.p. as a daily dose of 20 mg/kg b.w. It was given to all groups except the normal and control one. The tested CAMBA was orally given daily for 4 weeks at 25 and 50 mg/kg.b.w. Values are given as mean ± SD for groups of six animals each. Data followed by the same letter are not significantly different at $p \leq 0.05$.

TABLE-2
EFFECT OF CAMPA AND 5FU ON PLASMA TRANSAMINASES (L-ALANINE AND L-ASPARTATE), ALKALINE PHOSPHATASE (ALP), MATRIX METALLOPROTEINASE-2 AND -9 (MMP-2 AND -9) IN MICE BEARING EHRLICH ASCITES CARCINOMA

Groups	Treatment description	ALT (U/L)	AST (U/L)	ALP (U/L)	MMP-2 (ng/dl)	MMP-9 (ng/dl)
I	Normal group (non-tumor bearing mice (NTB)	11.70 ± 1.30[a]	14.87 ± 1.08[a]	64.00 ± 5.11[a]	21.85 ± 2.17[e]	13.43 ± 1.43[e]
II	EAC control (tumor bearing mice (TB)	31.64 ± 3.70[d]	38.44 ± 4.06[d]	115.53 ± 9.63[e]	6.70 ± 0.45[a]	3.25 ± 0.58[a]
III	EAC + CAMBA (25 mg/kg.b.w)	18.38 ± 2.16[b]	19.72 ± 3.72[b]	85.22 ± 7.55[c]	13.99 ± 1.06[c]	8.40 ± 0.71[b]
IV	EAC + CAMBA (50 mg/kg.b.w.)	14.90 ± 1.28[a]	15.08 ± 2.84[a]	78.64 ± 9.16[b]	16.48 ± 2.09[d]	12.56 ± 0.91[d]
V	EAC + 5-fluorourcil (20 mg/kg.b.w.)	27.55 ± 3.00[c]	25.64 ± 3.78[c]	96.30 ± 8.25[d]	10.53 ± 1.44[b]	6.27 ± 0.55[c]

Data shown are mean ± standard deviation of number of observations within each treatment. Data followed by the same letter are not significantly different at $p \leq 0.05$.

TABLE-3
EFFECT OF CAMBA AND 5-FLUOROURCIL ON LEVELS OF LIVER TUMOUR NECROSIS FACTOR (TNF-α), NITRIC OXIDE (NOx), THIOBARBATURIC ACID REACTIVE SUBSTANCES (TBARs) AND REDUCED GLUTATHIONE (GSH) IN MICE BEARING EHRLICH ASCITES CARCINOMA

Groups	Treatment description	TNF-α (pg/mg protein)	INOs (pg/mg protein)	TBARs (nmol/mg protein)	GSH (mg%)
I	Normal group (non-tumor bearing mice (NTB)	175.08 ± 18.76[a]	85.43 ± 5.44[a]	2.65 ± 0.32[a]	4.23 ± 0.65[b]
II	EAC control (tumor bearing mice (TB)	398.45 ± 14.80[e]	195.33 ± 11.08[e]	7.05 ± 0.54[e]	1.67 ± 0.17[a]
III	EAC + CAMBA (25 mg/kg b.w)	210.87 ± 15.86[c]	123.55 ± 10.22[c]	3.50 ± 0.25[c]	3.85 ± 0.36[b]
IV	EAC + CAMBA (50 mg/kg b.w.)	194.38 ± 13.75[b]	101.65 ± 9.83[b]	3.11 ± 0.18[c]	4.05 ± 0.21[c]
V	EAC + 5-fluorourcil (20 mg/kg b.w.)	252.60 ± 17.54[d]	138.90 ± 19.34[d]	6.08 ± 0.74[d]	2.17 ± 0.36[b]

Data shown are mean ± standard deviation of number of observations within each treatment.
Data followed by the same letter are not significantly different at $p \leq 0.05$.

TABLE-4
EFFECT OF CAMBA AND 5FU ON LEVELS OF LIVER CATALASE (CAT), SUPEROXIDE DISMUTASE (SOD) AND GLUTATHIONE PEROXIDASE (GPx) IN MICE BEARING EHRLICH ASCITES CARCINOMA

Groups	Treatment description	CAT (Umol H_2O_2 consume/mg tissue)	SOD	GPx
I	Normal group (non-tumor bearing mice (NTB)	43.87 ± 4.87[d]	31.76 ± 3.06[e]	26.38 ± 2.69[e]
II	EAC control (tumor bearing mice (TB)	31.00 ± 2.65[a]	9.84 ± 0.90[a]	6.73 ± 0.94[a]
III	EAC + CAMBA (25 mg/kg b.w)	31.76 ± 3.40[b]	20.11 ± 2.51[c]	19.80 ± 1.41[c]
IV	EAC + CAMBA (50 mg/kg b.w.)	38.66 ± 4.11[c]	25.87 ± 3.05[d]	24.00 ± 1.33[d]
V	EAC + 5-fluorourcil (20 mg/kg b.w.)	22.07 ± 3.08[b]	14.90 ± 1.65[b]	10.52 ± 1.02[b]

Values are given as mean ± SD for groups of six animals each. Values Data followed by the same letter are not significantly different at $p \leq 0.05$. SOD: one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1min/mg protein; GPx: µg of GSH consumed/min mg protein.

Histological studies: Histopathological examination of liver sections of the normal group I showed normal liver architecture and no inflammation or fibrosis (Fig. 2a). On the other hand, in the liver of EAC-bearing control group II, histological examination complete liver showed severe portal inflammation (black arrow), with piece meal necrosis (spillage of the inflammatory cells into the liver lobules), congested veins (blue arrow), foci of spotty necrosis (the circle), steatosis vacuoles (red arrow) (H&E ×200) (Fig. 2b). Histopathological examination also showed moderate portal inflammation (black arrow), scattered steatosis vacuoles (red arrows), some degenerated hepatocytes (green arrows) and foci of spotty necrosis (circles)) ×200 H&E (of EAC-bearing mice treated with CAMBA at 25 mg/kg.b.w. as compared with the EAC-bearing control group. Also, liver from EAC-bearing mice treated with CAMBA 50 mg/kg.b.w. group IV showed marked improvement with no inflammation, no venous congestion, no spotty necrosis (Fig. 2c). The hepatocytes appear vacuolated with hydropic degeneration the black arrows (×200 H&E) (Fig. 2d). In addition, all samples of EAC-bearing mice showed moderate lobular inflammation (black arrow), congested veins (blue arrow), few steatosis vacuoles (red arrow) (×200H&E) by treatment with 5-FU 20 mg/kg.b.w. group (V) (Fig. 2e).

Ultrasound studies: Ultrasound examination of liver of normal group I showed that the liver parenchyma was regular, homogenous with normal echogenicity (Fig. 3a). While for the liver of EAC-bearing control group (II), ultrasound imaging showed a liver mass which appear as irregular, heterogenous, ill-defined with mixed contents, also ascites can be evaluated in some captions (Fig. 3b).

Ultrasound examination also showed coarse texture and the abnormal focal lesion was regressed of EAC-bearing mice treated with CAMBA at 25 mg/kg.b.w. as compared with the EAC-bearing control group III (Fig. 3c). In addition, liver examination by ultrasound imaging from EAC-bearing mice treated with CAMBA 50 mg/kg.b.w. group (IV) showed marked improvement with no ascites can be evaluated in some captions (Fig. 3d). In addition, all samples of EAC-bearing mice showed moderate focal lesion was regressed by treatment with 5-FU 20 mg/kg.b.w. group V (Fig. 3e).

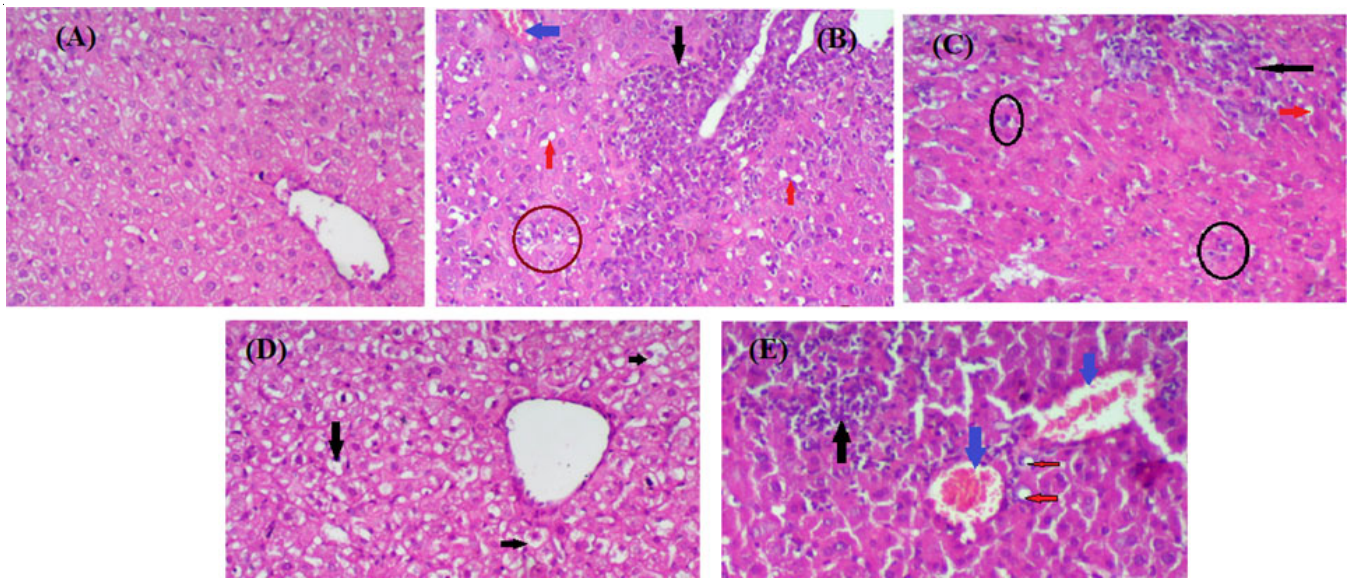


Fig. 2. Sections stained with hematoxylin and eosin (H&E; 400 X) histological examination of mice' liver of different groups compared to control group; (A), Group I: Normal control; (B), Group II: EAC group; (C), Group III: Was administrate EAC + CAMBA (25 mg/kg.b.w); (D), Group IV: Was administrate EAC + CAMBA (50 mg/kg.b.w); (E), Group V: Was administrate 5FU(20 mg/kg b.w.) + EAC

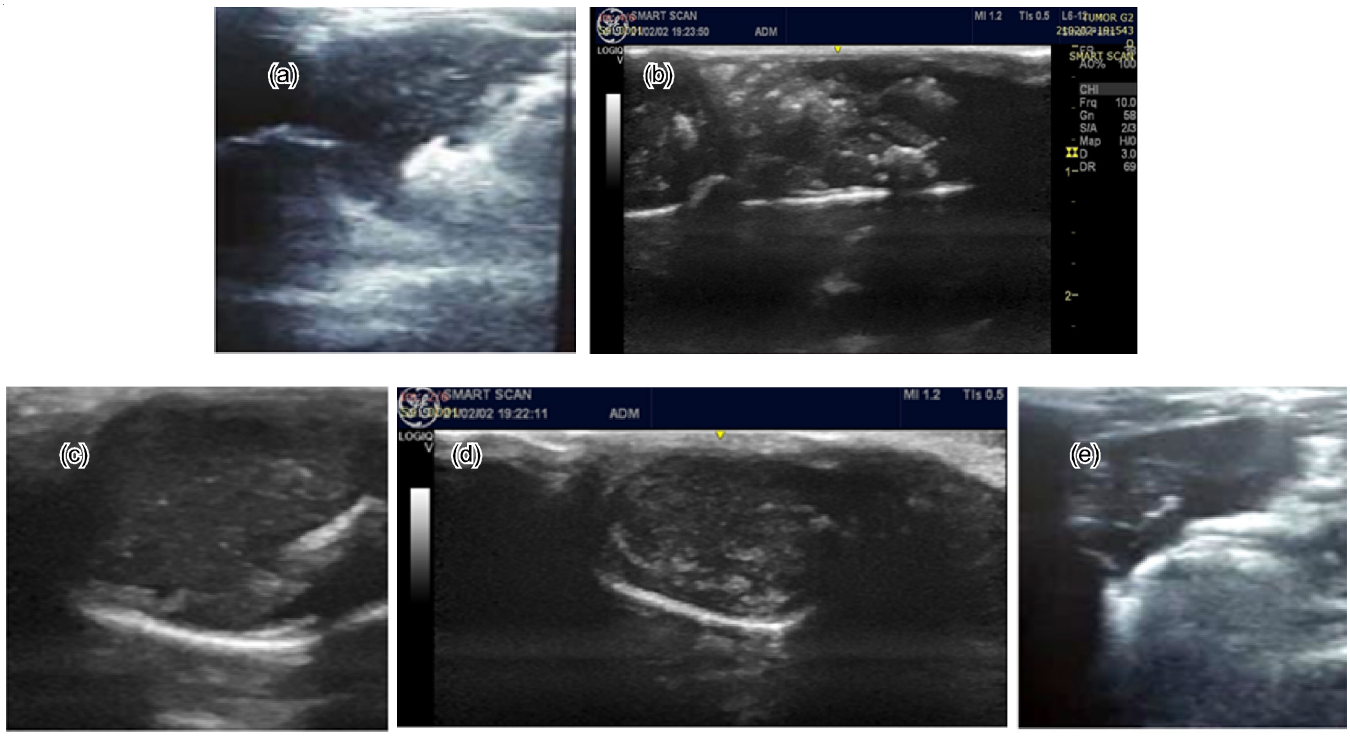


Fig. 3. Ultrasound examination using a multi-frequencies linear transducer (7-12 MHZ) of mice' liver of different groups compared to control group; (a), Group I: Normal control; (b), Group II: EAC group; (c), Group III: Was administrate EAC + CAMBA (25 mg/kg.b.w); (d), Group IV: Was administrate EAC + CAMBA (50 mg/kg.b.w); (e), Group V: Was administrate 5FU(20 mg/kg b.w.) + EAC

Cytotoxic activity: In Ehrlich ascites carcinoma bearing mice, the antiangiogenic activity of CAMBA was investigated. The cytotoxic activity of CAMBA against liver carcinoma (Hep-G2) cell line was determined using formazan assay. Cell line was incubated with different concentrations (0-100 µg/mL) and used to create CAMBA concentration *versus* cell viability curve. The response parameter (IC_{50}) was calculated for each cell line (Fig. 1) at the end of the incubation period (48 h) [36]. The best cytotoxic results were obtained with CAMBA due to bearing benzyl moiety with *p*-dihydroxyl groups of the quinazoline nucleus. Also, an electron withdrawing carbonyl groups at the positions 7 and 9. The presence of methoxy group

probably, these active group makes CAMBA more expected to work as DNA alkylators and produces its antiangiogenic activity [37]. The *in vivo* results proved that the administration of CAMBA decreases the tumor volume and weight as compared to that of the EAC control group. On the other hand, reduction of tumor volume and weight indicates a decrease in abnormal cell divisions, *i.e.* tumor proliferation [38,39]. In this study, it is observed that CAMBA can revert or inhibit EAC induced tumor [40], which may be due to free radical scavenging properties [12,13].

The liver is the primary organ for drug detoxification and plasma levels of liver marker enzymes were measured. AST, ALT, ALP, NO, TNF-α and TBARs levels were increased in EAC controlled mice, whereas MMP-2, MMP-9, GSH, GPx, SOD and CAT levels were decreased. Compared to the standard control group, subcutaneous implantation of EAC into mice resulted in a substantial increase in AST, ALT, ALP, NO, TNF-α and TBARs, as well as a significant decrease in MMP-2, MMP-9, GSH, GPx, SOD and CAT. These findings are well correlated with the Borik & Hussein [41] findings that the consumption of free amino acids for the protein synthesis of rapidly dividing tumour cells can disrupt liver enzyme activity [42]. The altered liver enzyme level was returned to that of the normal community after the treatment with CAMBA.

Reactive oxygen species (ROS) have been implicated in a range of cellular processes, ranging from apoptosis and necrosis to cell proliferation and carcinogenesis, according to a number of studies [43]. Polyphenolic natural antioxidants are common antitumor and anti-inflammatory agents [12-19] and their impact on signal transduction in cell proliferation and angiogenesis [44] has a chemopreventive function in cancer. CAMBA's antitumor activity against EAC *in vivo* may be due to this essential property.

Furthermore, CAMBA inhibited the expression of VEGF and reduced tumor angiogenesis *in vivo*, which was associated with a decrease in NO, TNF-α, in liver tissues [45]. Similarly, CAMBA also induced apoptosis and inhibited tumor cells' invasiveness by increasing MMP-2 and MMP-9 levels activity through suppressing the constitutively active STAT3 [46]. According to the structure activity relationship of CAMBA and their anti-inflammatory effects, Hussein [12] demonstrated that the antioxidant and antitumor activity of CAMBA is dependent on its structure function. The presence of *p*-dihydroxyl groups in the B-ring and conjugated double bond is considered important for successful radical scavenging by CAMBA. The presence of a double bond between the B ring and the carbonyl group in CAMBA causes electrons to become more delocalized, resulting in the formation of a quinone structure that has electron donating properties [14]. Hussein's findings [17-19] also suggested that CAMBA's antioxidant activity is due to the two mechanisms (i) electron transfer (ET) and hydrogen atom transfer (HAT). However, with CAMBA, where the carbonyl is indirectly connected to the aromatic ring, a consistent increase in antioxidant activity has been identified [44].

As shown by CAMBA, increasing the distance between the carbonyl group and the catechol aromatic ring (B) increases potency, while decreasing the distance between the amide group and the non-catechol aromatic ring (A) increases antioxidant activity. Thus, it can be concluded that CAMBA has antiangiogenic and antioxidant potential in Ehrlich ascites carcinoma bearing albino mice, which can be attributed to its structure-activity relationship.

Conclusion

The current study found that caffeic acid methyl benzoate amide (CAMBA) have potent biochemical, histological and ultrasound imaging findings of present study suggested that CAMBA have antiangiogenic activity of CAMBA in Ehrlich ascites carcinoma-bearing mice by normalizing the levels of inflammatory mediator and VEGF gene expression. To the best of our knowledge, the prophylactic effect of CAMBA against Ehrlich ascites carcinoma-induced liver toxicity has never been reported earlier. This could pave the way for the development of newer, safer and more powerful antitumor drugs.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre and A. Jemal, *CA Cancer J. Clin.*, **68**, 394 (2018); https://doi.org/10.3322/caac.21492
2. Z. Xiao, G.E. Lester, Y. Luo and Q. Wang, *J. Agric. Food Chem.*, **60**, 7644 (2012); https://doi.org/10.1021/jf300459b
3. S.A. Mir, M.A. Shah and M.M. Mir, *Crit. Rev. Food Sci. Nutr.*, **13**, 2730 (2017); https://doi.org/10.1080/10408398.2016.1144557
4. A.J. Young and G.M. Lowe, *Arch. Biochem. Biophys*., **385**, 20 (2001); https://doi.org/10.1006/abbi.2000.2149
5. L. Chen, J.Y. Hu and S.Q. Wang, *J. Am. Acad. Dermatol.*, **23**, 231 (2012); https://doi.org/10.1016/j.jaad.2012.02.009
6. T.A. Yap, A. Omlin and J.S. de Bono, *J. Clin. Oncol.*, **31**, 1592 (2013); https://doi.org/10.1200/JCO.2011.37.6418
7. W. Guo, E. Kong and M. Meydani, *Nutr. Cancer*, **61**, 807 (2009); https://doi.org/10.1080/01635580903285098
8. X.-J. Chao, Z.-W. Chen, A.-M. Liu, X.-X. He, S.-G. Wang, Y.-T. Wang, P.-Q. Liu, C. Ramassamy, S.-H. Mak, W. Cui, A.-N. Kong, Z.-L. Yu, Y.-F. Han and R.-B. Pi, *CNS Neurosci. Ther*., **20**, 840 (2014); https://doi.org/10.1111/cns.12286
9. T. Wan, Z. Wang, Y. Luo, Y. Zhang, W. He, Y. Mei, J. Xue, M. Li, H. Pan, W. Li, Q. Wang and Y. Huang, *Oxid. Med. Cell Longev.*, **2019**, 8239642 (2019); https://doi.org/10.1155/2019/8239642
10. R. Rezaei, K. Baghaei, S.M. Hashemi, M.R. Zali, H. Ghanbarian and D. Amani, *Front. Med*., **8**, 619939 (2021); https://doi.org/10.3389/fmed.2021.619939
11. H.F. Liao, Y.Y. Chen, J.J. Liu, M.L. Hsu, H.J. Shieh, H.J. Liao, C.J. Shieh, M.S. Shiao and Y.J. Chen, *J. Agric. Food Chem.*, **51**, 7907 (2003); https://doi.org/10.1021/jf034729d
12. M.A. Hussein and N.A. Gobba, *Med. Chem.*, **3**, 286 (2013); https://doi.org/10.4172/2161-0444.1000153
13. G.-W. Rao, G.-J. Xu, J. Wang, X.-L. Jiang and H.-B. Li, *Chem. Med. Chem*., **8**, 928 (2013); https://doi.org/10.1002/cmdc.201300120
14. A.M. Alafeefy, S.I. Alqasoumi, A.E. Ashour and M.M. Alshebly, *J. Enzyme Inhib. Med. Chem.*, **28**, 375 (2013); https://doi.org/10.3109/14756366.2012.668541

15. M.M. Ghorab, Z.H. Ismail and M. Abdalla, *Arzneimittelforschung*, **60**, 87 (2010); https://doi.org/10.1055/s-0031-1296254
16. V.B. Konkimalla, V.L. Suhas, N.R. Chandra, E. Gebhart and T. Efferth, *Expert Rev. Anticancer Ther.*, **7**, 317 (2007); https://doi.org/10.1586/14737140.7.3.317
17. H.A.E.H. El Gizawy, M.A. Hussein and E. Abdel-Sattar, *Pharm. Biol.*, **57**, 485 (2019); https://doi.org/10.1080/13880209.2019.1643378
18. M.A. Hussein, *Med. Chem. Res.*, **21**, 1876 (2012); https://doi.org/10.1007/s00044-011-9707-0
19. M.A. Hussein, Med Chem Res., **22**, 4641 (2013); https://doi.org/10.1007/s00044-013-0468-9
20. C.N. Kavitha, K.D. Raja and S.K. Rao, *J. Cancer Res. Ther.*, **17**, 491 (2021); https://doi.org/10.4103/jcrt.JCRT_454_19
21. B. Choudhury, R. Kandimalla, R. Bharali, J. Monisha, A.B. Kunnumakara, K. Kalita and J. Kotoky, *Front. Pharmacol.*, **7**, 3 (2016); https://doi.org/10.3389/fphar.2016.00003
22. G. Schumann and R. Klauke, *Clin. Chim. Acta*, **327**, 69 (2003); https://doi.org/10.1016/s0009-8981(02)00341-8
23. A.L. Caúla, R. Lira-Junior, E.M. Tinoco and R.G. Fischer, *J. Periodontal Res.*, **50**, 793 (2015); https://doi.org/10.1111/jre.12266
24. J.G. Lee, S. Dahi, R. Mahimkar, N.L. Tulloch, M.A. Alfonso-Jaume, D.H. Lovett and R. Sarkar, *Proc. Natl. Acad. Sci. USA*, **102**, 16345 (2005); https://doi.org/10.1073/pnas.0508085102
25. H.E. Turner, Z. Nagy, M.M. Esiri, A. Harris and J.A.H. Wass, *J. Clin. Endocrinol. Metab.*, **85**, 2931 (2000); https://doi.org/10.1210/jcem.85.8.6754
26. F. Feriyani, H. Maulanza, R.R. Lubis, U. Balqis and D. Darmawi, *Scient. World J.*, **2021**, 6617292 (2021); https://doi.org/10.1155/2021/6617292
27. A. Bhattacharjee, A. Basu, J. Biswas, T. Sen and S. Bhattacharya, *Mol. Cell Biochem.*, **424**, 13 (2017); https://doi.org/10.1007/s11010-016-2839-2
28. K.M. Miranda, M.G. Espey and D.A. Wink, *Nitric Oxide*, **5**, 62 (2001); https://doi.org/10.1006/niox.2000.0319
29. M.E. Dominiecki, G.L. Beatty, Z.K. Pan, P. Neeson and Y. Paterson, *Cancer Immunol. Immunother.*, **54**, 477 (2005); https://doi.org/10.1007/s00262-004-0610-0
30. M. Schulz, S. Iwersen-Bergmann, H. Andresen and A. Schmoldt, *Crit. Care*, **16**, R136 (2012); https://doi.org/10.1186/cc11441
31. M.H. Hadwan and S.K. Ali, *Anal. Biochem.*, **542**, 29 (2018); https://doi.org/10.1016/j.ab.2017.11.013
32. T. Ramasarma, A.V. Rao, M.M. Devi, R.V. Omkumar, K.S. Bhagyashree and S.V. Bhat, *Mol. Cell Biochem.*, **400**, 277 (2015); https://doi.org/10.1007/s11010-014-2284-z
33. Y. Wang, Y. Liu, F. Ding, X. Zhu, L. Yang, P. Zou, H. Rao, Q. Zhao and X. Wang, *Anal. Bioanal. Chem.*, **410**, 4805 (2018); https://doi.org/10.1007/s00216-018-1117-4
34. D. Feng, W.H. Ling and R.D. Duan, *Inflamm. Res.*, **59**, 115 (2010); https://doi.org/10.1007/s00011-009-0077-8
35. H. Yamaguchi and J. Shen, Eds.: K. McCall and C. Klein, Histological Analysis of Neurodegeneration in the Mouse Brain; Necrosis, Methods in Molecular Biology, Humana Press, Totowa, NJ, vol. 1004 (2013).
36. A. Aragón-Muriel, Y. Liscano, Y. Upegui, S.M. Robledo, M.T. Ramírez-Apan, D. Morales-Morales, J. Oñate-Garzón and D. Polo-Cerón, *Antibiotics*, **10**, 728 (2021); https://doi.org/10.3390/antibiotics10060728
37. A. Bala, B. Kar, P.K. Haldar, U.K. Mazumdar and S. Bera, *J. Ethnopharmacol.*, **129**, 131 (2010); https://doi.org/10.1016/j.jep.2010.03.010
38. J. Kamarauskaite, R. Banienem, D. Trumbeckasm, A. Strazdauskasm and S. Trumbeckaitem, *Antioxidants*, **10**, 747 (2021); https://doi.org/10.3390/antiox10050747
39. H. Ozyurt, B. Ozyurt, K. Koca and S. Ozgocmen, *Vascul. Pharmacol.*, **47**, 108 (2007); https://doi.org/10.1016/j.vph.2007.04.008
40. M.F. Tolba, H.A. Omar, S.S. Azab, A.E. Khalifa, A.B. Abdel-Naim and S.Z. Abdel-Rahman, *Crit. Rev. Food Sci. Nutr.*, **56**, 2183 (2016); https://doi.org/10.1080/10408398.2013.821967
41. R.M. Borik and M.A. Hussein, *Curr. Pharm. Biotechnol.*, (2021); https://doi.org/10.2174/1389201022666210601170650
42. A.N. Choudhary, A. Kumar and V. Juyal, *Med. Chem.*, **14**, 172 (2015); https://doi.org/10.2174/1871523015666160114092144
43. H. Göçer and I. Gülçin, *Int. J. Food Sci. Nutr.*, **62**, 821 (2011); https://doi.org/10.3109/09637486.2011.585963
44. J. Fang, Q. Zhou, L.Z. Liu, C. Xia, X. Hu, X. Shi and B.-H. Jiang, *Carcinogenesis*, **28**, 858 (2006); https://doi.org/10.1093/carcin/bgl205
45. W. Fernando, H.P. Rupasinghe and D.W. Hoskin, *Mini Rev. Med. Chem.*, **15**, 479 (2015); https://doi.org/10.2174/1389557515666150414152933
46. H.H. Cao, J.H. Chu, H.Y. Kwan, T. Su, H. Yu, C.-Y. Cheng, X.-Q. Fu, H. Guo, T. Li, A.K.-W. Tse, G.-X. Chou, H.-B. Mo and Z.-L. Yu, *Sci. Rep.*, **6**, 21731 (2016); https://doi.org/10.1038/srep21731