



Mikania cordata leaves extract promotes activity against pathogenic bacteria and anticancer activity in EAC cell-bearing swiss albino mice

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ABSTRACT

This study was aimed to screen the activity of the methanolic extract of *Mikania cordata* leaves (MLME) against pathogenic bacteria and Ehrlich ascites carcinoma (EAC)-induced cancer in mice. Antibacterial activity was tested against some Gram-positive (*Bacillus subtilis* IFO 3026 and *Sarcina lutea* IFO 3232) and Gram-negative (*Klebsiella pneumoniae* ATTC 10031, *Proteus vulgaris* MTTC 321, *Pseudomonas denitrificans* KACC 32026, and *Xanthomonas campestris* IAM 1671) bacteria by disk diffusion and liquid microdilution assay. The anticancer activity was assessed by EAC cell death, apoptosis, hematological parameters determination, and 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide test. The MLME exhibited prominent antibacterial activity against the test strains. The minimum inhibitory concentrations were ranged from 1.25 to 20 mg/ml for the bacterial strains that were found ampicillin resistant. The MLME exhibited remarkable anticancer activity on EAC in a dose-dependent manner. Oral intake of MLME at the dosage of 400 mg/kg body weight (b.w) exhibited the highest EAC cell death with remarkable apoptotic features including chromatin condensation, nuclear fragmentation, and accumulation of apoptotic bodies. The MLME-treated EAC-bearing mice showed dose-dependently restored altered hematological parameters toward the normal level. The IC₅₀ value was 6.6 ± 1.91 µg/ml. These findings suggest that the *M. cordata* leaves have strong antibacterial and anticancer properties.

INTRODUCTION

Pathogenic microbes, such as bacteria, viruses, parasites, and fungi are the causal agent of communicable diseases, which are reflected as a significant hazard to human health due to the scarcity of vaccines, inadequate chemotherapy, and an increase in resistant bacteria against antibiotics (Assob *et al.*, 2011). Utmost of the

contemporary antibiotics have extensive limitations regarding lower antimicrobial spectrum and side effects on the host including allergic reactions, immune-suppression, and hypersensitivity (Blumenthal *et al.*, 2019; Londonkar *et al.*, 2013). Moreover, their indiscriminate and inappropriate usage has led to the increasing clinical resistance of formerly sensitive microorganisms (Ventola, 2015). New and reemerging infectious diseases are rising very rapidly. Hence, this warns the looking for novel antimicrobial drugs, which will be natural, safe, and effective without side effects. Cancer is the second foremost cause of death after a cardiac disease that claimed 8.7 million deaths globally in 2015 (Global Burden of Disease Cancer Collaboration *et al.*, 2017). In 2015, about 17.5 million new cases of cancer occurred and this number is predicted to be increased by 70 percent in the next 20 years (Siegel *et al.*, 2015). Cancer occurs when

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cells with non-repaired damaged DNA grow, divide, and spread abnormally instead of self-destruction by programmed cell death. These types of unregulated atypical cells when invading other parts of the body normally initiate the progression that is called metastasis (Hanahan *et al.*, 2011). Currently, the management of this condition is limited to the single and/or combined use of chemotherapy, radiotherapy, and surgery. Although these options are advancing significantly with higher survival rates, they are more or less costly with various side effects likely the reduction of neighboring normal cells, serious post-treatment complications, and in some cases, the resurrection of chemotherapy and radiotherapy resistant secondary malignancies (Al-Mamun *et al.*, 2016; Islam *et al.*, 2018). Therefore, the exploration of safer antitumor candidates with the least side effects and better selectivity is a big interest worldwide today.

Phytochemicals derived from medicinal plants have been reported to possess significant antiproliferative potential over the last two decades (Al-Dabbagh *et al.*, 2019; Islam *et al.*, 2014; Zhong *et al.*, 2018). These studies reported that medicinal plant is a good source of antioxidants such as phenolics, flavonoids, vitamins, and carotenoids, and the intake of these plants either in the provision of chemical components or fresh extracts is mainly related with the lower risk of degenerative diseases including cancer (Demain and Vaishnav, 2011). Polyphenols such as apigenin, luteolin, myricetin, genistein, daidzein, quercetin, epigallocatechin gallate, cyanidin, curcumin, resveratrol, ferulic acid, nobiletin, diosmetin, and ellagitannins have been reported to possess strong anticancer properties (Goh *et al.*, 2019; Tavsana and Kayalia, 2019; Tomeh *et al.*, 2019). Moreover, about 60% of the modern anticancer drugs that are used in pharmaceutical industries are largely extracted from natural origins (Islam *et al.*, 2014). Current anticancer drug targets one specific pathway whereas phytochemicals exhibit their anticancer effect by targeting numerous apoptotic signaling pathways which are reflected as the vital event in the anticancer activity (Demain and Vaishnav, 2011). Phytomolecules are now at the center of attraction in treating different cancer types as they are selective in their actions and specific only for the cancer cells. Several promising modern anticancer drugs of plant origin have been reported in recent times due to their success in managing abnormal cellular proliferation. Vinca alkaloids (vinorelbine, vindesine, vincristine, and vinblastine), taxanes (docetaxel and paclitaxel), camptothecin derivatives, cephalotaxus, capsaicin, gingerol, cyanidin glycosides, lycopene, saffron (crocetin), resveratrol, apigenin, vitamin D and E from plant oil, kaempferol, genipin, lupeol, ellipticin, ecombretastatins, triterpenoid acids, etc., from plant, animal, and aquatic origins are among the many natural substances possessing anticancer activity (Gao *et al.*, 2017; Iqbal *et al.*, 2017; Lichota and Gwozdziński, 2018; Nelius *et al.*, 2016). Most of these phytochemicals are also effective in inhibiting the growth as well as killing a wide range of bacterial cells. Therefore, the demand for plant-based bioactive agents is increasing for chemotherapeutic purposes as they are natural, easily oral consumption as part of a patient's dietary intake, more tolerated, and non-toxic to normal human cells (Desai *et al.*, 2008).

Mikania is the plant of Asteraceae family, having around 450 species, is found all over the tropical areas of Asia, Africa, South America, and Bangladesh. The *Mikania* species have been demonstrated to comprise numerous bioactive phytocomponents such as polyphenols, flavonoids, alkaloids, steroids, gums, tannins, saponins, and sesquiterpenes (Brigida da Silva *et al.*, 2018). Among the *Mikania* species, *M. cordata*, also known as

Heartleaf Hempvine, is a twining and trailing woody-stemmed perennial herb characterized by its ovate heart-shaped leaves with 4–10 cm long with a pointed tip and truncate base, whitish flowers, capitulum inflorescence, white pappus, and narrowly oblong cypsela type of fruits (Ali *et al.*, 2011). The local name of *M. cordata* is Assamlata or Taralata in Bangladesh and is found all over the country. The traditional use of various parts of the plant in Bangladesh is to treat a snake bite, coughs, diabetes, jaundice, septic sore, muscle spasms, colds, influenza, fever, bronchitis in children, and to stop bleeding from wounds and cuts. It is also used in the preparation of traditional herbal medication for the treatment of pain, inflammation, and infectious diseases (Nayeem *et al.*, 2011). Different pharmacological properties of *M. cordata* tested in experimental animals had been reported. The ethanol extract of leaves has been evaluated for its anti-diarrheal activity and the anti-ulcerogenic effect in Long Evans rats (Brigida da Silva *et al.*, 2018). The essential oil, and ethyl acetate and chloroform extracts from aerial parts have been described for its anti-nociceptive, anti-inflammatory, and antipyretic activities in experimental animals (Siddiqui *et al.*, 2018). Although *in vitro* anticancer potentiality against human breast adenocarcinoma cell line has been demonstrated for ethanol extract of leaves of *M. cordata* (Uy *et al.*, 2015), there are no scientific reports regarding the assessment of *in vivo* anticancer activities in Ehrlich ascites carcinoma (EAC) cell. Therefore, this study tested methanol extract of *M. cordata* leaves for its antibacterial activity against pathogenic bacteria and anticancer activity against EAC cancer cell in mice.

MATERIALS AND METHODS

Reagents and chemicals

Trypan blue, Dimethylsulfoxide (DMSO), Vincristine sulfate, Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Penicillin-streptomycin, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution were purchased from Thermo Fisher Scientific, USA. Standard antibiotic disks and methanol were procured from Bio-Rad, USA and Merck, Germany, respectively. Nutrient agar media and nutrient broth media were obtained from Liofilchem, Italy. All other reagents and chemicals used in this work were of reagent rating and bought from Sigma-Aldrich Corporation, Germany.

Plant material

Mikania cordata leaves were collected from Jashore, Bangladesh and authenticated at Bangladesh National Herbarium, Dhaka (voucher number DACB 42864). The collected leaves were washed with tap water and dried at room temperature (~28°C) in shade. The leaves were ground into fine powder by a blender and stowed in the sealed vessel until subjected to solvent extraction.

Preparation of plant extract

The extraction of plant product was carried out according to the method described earlier with slight modification (Al-Mamun *et al.*, 2016). A total of 1,200 g of leaves powder was distributed in eight 500 ml conical flasks. Each flask was added with 400 ml of absolute methanol and kept for 72 hours at 160 rpm in a shaking incubator at 37°C and thereafter centrifuged for 15

minutes at 8,000 rpm. The resulting pellets were dissolved again in absolute methanol and retained back in shaking incubator for 48 hours. The supernatants were collected and filtered through Whatman filter paper (No.1) and the filtrate was concentrated by a vacuum evaporator (Stuart, UK). After complete evaporation of water, the crude extract was stored at 4°C.

Antibacterial activity assay

The antibacterial potentiality of the crude extract was tested by the disk diffusion method (Bubonja-Sonje *et al.*, 2011). The bacterial pure cultures used in this study were *Bacillus subtilis* IFO 3026, *Sarcina lutea* IFO 3232, *Klebsiella pneumonia* ATCC 10031, *Proteus vulgaris* MTTC 321, *Pseudomonas denitrificans* KACC 32026, and *Xanthomonas campestris* IAM 1671. The crude methanol extract was dissolved into DMSO and each disk (6 mm diameter) of Whatman filter paper (No. 1) was soaked with 10 µl of 20 mg/ml (200 µg/disk) of *M. cordata* leaves (MLME) and air-dried. Blank disks soaked with DMSO and were used as a negative control. The ready disks were employed on a nutrient agar plate spread with 100 µl of tested bacterial broth culture and incubated at 37°C for 24 hours. Standard antibiotics, *i.e.*, Tetracycline at 30 µg/disk, Erythromycin at 15 µg/disk, Ampicillin at 10 µg/disk, and Ciprofloxacin at 5 µg/disk were used as the positive control. After incubation, the culture plates were observed and the inhibition zones formed around each disk were measured in the millimeter scale (Dash *et al.*, 2013). Minimum inhibitory concentration (MIC) of MLME was determined by liquid microdilution assay as previously described by Chandrasekaran and Venkatesalu (2004).

Experimental animal, ethics statement, and EAC cell transplantation

Swiss albino mice (25–30 g) of the male sex were purchased from the Pharmacy Department of Jahangirnagar University, Bangladesh. A total number of 162 mice were obtained and kept in the animal house of the Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh. The handling of mice and the methodology applied in this study was approved by the Animal, Medical Ethics, Biosafety and Biosecurity Committee (IAMEBBC) of University of Rajshahi, Bangladesh (no: 31/320/IAMEBBC/IBSC). EAC cell used in this study was kindly provided by Professor Dr. Md. Abu Reza, Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh. EAC cells were reared by weekly i.p of EAC cells in mice by succeeding transplantation of 6×10^6 cells in 0.2 ml phosphate-buffered saline (PBS)/mice (Al-Mamun *et al.*, 2016).

Selection of appropriate dosages

An acute oral toxicity assessment was performed according to the Organization for Economic Co-operation and Development (OECD) guidelines (Test no. 425) to select the suitable dosage of MLME for the evaluation of the anticancer activity. Briefly, a total of 66 mice were distributed into eleven groups each with six mice. Each group of mice (groups I, II, III, IV, V, VI, VII, VIII, IX, X, and XI) was administered orally with 0.2 ml of MLME at the concentration of 12.5, 25, 50, 100, 200, 400, 800, 1,600, 3,200, 4,800, and 6,400 mg per kg b.w, respectively. Each mouse of group XI which was untreated control

was orally administered with 0.2 ml of 2% DMSO in PBS. After treatment, mice were permitted to feed *ad libitum* and were closely monitored for the next 24 hours for any behavioral, neurological, and autonomic profiles and the 14 days for mortality. From these data, the percent (%) of mice mortality was enumerated for each concentration using the following formula:

$$\% \text{ mortality} = N_d/N_t \times 100$$

(where N_d = Number of the dead mouse in each group; N_t = Number of mice taken in each group for testing, *i.e.*, 6).

Determination of EAC cell death *in vivo*

The assessment of *in vivo* cancer cell death was performed according to the previously described method with slight modification (Rahman *et al.*, 2017). In brief, a total of 36 mice were distributed into six groups (six in each group) and provided food and water *ad libitum*. All mice in each group were i.p injected with a volume of 0.2 ml of EAC cells (1×10^6 cells/mouse) suspended in PBS. Following 24 hours of post-EAC transplantation, each mouse of groups I, II, and III were treated with 0.2 ml of MLME at the concentration of 100, 200, and 400 mg per kg b.w (p.o), respectively, for therapeutic evaluation and continued for 6 days. Every mouse of group IV (positive control group) was injected with 0.2 ml of vincristine sulfate (VS) i.p at the concentration of 300 µg per kg b.w. Group V (negative control group) was orally administered with the same volume of 2% DMSO in PBS. The group VI was untreated and served as the EAC control. After 6 days of treatment, three mice from each group were sacrificed on day 7. The appropriate amount of EAC cells were collected by the repeated i.p wash with PBS and harvested in cold PBS. Viable EAC cell counts by Trypan blue dye were made with a hemocytometer. The total number of viable EAC cells per mice of the treatment group was compared with those of the EAC control group. The EAC cell death was enumerated using the following formula:

$$\text{Cells/ml} = \frac{\text{the cells count in the central square} \times \text{the dilution factor}}{10^4}$$

The number of viable EAC cells of the treatment group was compared with those of the EAC control group. The cell death was enumerated using the following formula:

$$\% \text{ Cell death} = (C_w - T_w)/C_w \times 100$$

where C_w = average number of viable EAC cells in the EAC control group and T_w = average number of viable EAC cells in the treated group.

Apoptosis evaluation by 4', 6-diamidino-2-phenylindole (DAPI) staining

The apoptosis was determined by the previously described method with minor modifications (Al-Mamun *et al.*, 2016). One milliliter (1 ml) EAC cells from each group (groups I to VI) of mice were centrifuged for 3 minutes at 1,200 rpm. After that, the cell pellet was washed with PBS three times each with 3-minute centrifugation at 1,200 rpm. The cells were mixed with 5 µl of 4', 6-diamidino-2-phenylindole (DAPI) solution and incubated in dark. After 10 minutes incubation, PBS was added to the DAPI containing pellet and centrifuged for 3 minutes at 1,200 rpm. Last, the cell pellet was dissolved in 200 µl of PBS and

10 μ l cell suspensions were mounted on a glass slide to observe morphological changes of EAC cells by fluorescence microscope (XDS-2FL, Optika, Italy).

Hematological studies of blood parameters

To see the effect of MLME on the hematology of EAC cell-bearing mice, they were separated into six groups. Groups II to VI except for group I (control group) had been injected i.p on day zero with 0.2 ml of EAC cells (1×10^6 cells/mouse). Group II was marked as the EAC control group. One day after EAC cell inoculation, MLME at the dosages of 100, 200, and 400 mg per kg b.w was administered orally into the groups III, IV, and V, respectively, and group VI was considered as positive control received vincristine sulfate at the concentration of 300 μ g per kg b.w. 2% DMSO in normal PBS at a volume of 5 ml/kg b.w was administered to the group I (untreated control group). Treatments were continued for 12 days and in each group, six male mice were handled. On the day of 13, blood was collected from the freely flowing tail vein and the total numbers of white blood cell (WBC) and red blood cell (RBC), and the percentage of hemoglobin and differential WBC (monocyte, lymphocyte, and neutrophil) counts were determined by previously described methods (Kathiriya *et al.*, 2010).

Determination of cytotoxicity by MTT assay

To assess the cytotoxicity of MLME against EAC cell *in vitro*, MTT assay was performed by the previously described method with minor modification (Ramos-Silva *et al.*, 2017). The EAC cells were inoculated at a density of 1×10^6 cells per well into a 96 well plate containing DMEM supplemented with 10% FBS and 100 U/ml penicillin-streptomycin and incubated at 37°C with 5% CO₂ and 95% air. After 75% of the confluence of the cells, they were added with 3.125, 6.25, 12.5, 25, 50, and 100 μ g/ml concentrations of MLME separately. The control cells were treated with 2% DMSO in normal PBS. After 24 hours incubation, the medium was aspirated carefully, and the 96 well plates were gently washed with PBS. Twenty microliters of 5 mg/ml of MTT solution were dispensed into each well of both the MLME-treated and DMSO-treated cells and allowed to react at 37°C in the dark. The supernatant was discarded after 8 hours incubation and 150 μ l of DMSO was used to dissolve the formazan. The optical density of every well was determined at 570 nm by using the Multiskan EX Microplate Reader (Thermo Fisher Scientific, USA). The percent of cell viability and death were enumerated by the following equation:

$$\text{Cell viability (\%)} = \left[\frac{\text{optical density (OD) of treated cells}}{\text{OD of control cells}} \right] \times 100$$

$$\text{Cell death (\%)} = 100 - \text{Cell viability (\%)}$$

Cytotoxicity was represented as the concentration of MLME inhibiting cell growth by 50% (IC₅₀ value).

Statistical analysis

Values are represented as mean \pm standard deviation (STDEV) of the average of triplicate experiments is only for antibacterial activity and MTT assay. The statistical test was

performed using one-way analysis of variance (ANOVA) using Origin Lab version 7. The post-hoc tests after one-way ANOVA used in this study were Bonferroni and Tukey Tests. Regression line analysis for the determination of lethal dosage (LD₅₀) and IC₅₀ values were done by Microsoft Office Excel 2010. $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***) were considered to be statistically significant, highly significant, and very highly significant, respectively, when treatment effects were compared with the control values.

RESULTS AND DISCUSSION

The plants are the natural reservoirs of biologically active secondary metabolites that have the potentials to combat different diseases (Dash *et al.*, 2013). The metabolites derived from the green leaves of *M. cordata* comprise polyphenols, terpenoids, alkaloids, steroids, gums, and phytosterols (Iriti and Faoro 2009; Rufatto *et al.*, 2012). These metabolites arise from the main secondary metabolic itineraries have wide-ranging antibiotic, powerful antioxidant, and anticancer activities (Iriti and Faoro, 2009). Therefore, we investigated the pharmacological effects of *M. cordata* leaves extract for its antibacterial and anticancer activities with the prospect of advancement in herbal medicinal plant research which will enable the development of newer antimicrobial and anticancer drugs and explore novel entities for specific targeting.

MLME exhibited antibacterial activity

The antibacterial potentiality of MLME against the test bacteria was examined by the occurrence of the clear zone of inhibition (Fig. 1A). The leaves extract at 200 μ g/disk concentration showed significant antibacterial effects against two Gram-positive bacteria (*B. subtilis* and *S. lutea*) and three Gram-negative bacteria (*K. pneumoniae*, *P. vulgaris*, and *P. denitrificans*) with the zone of inhibition ranging from 11 ± 0.816 to 13 ± 0.816 mm. This result indicates the broad-spectrum antibacterial activity of MLME. As shown in Figure 1B, the highest zone of inhibition was found as 13 ± 0.816 mm against *S. lutea*, *P. vulgaris*, and *P. denitrificans*. The inhibition zone was observed as 11 ± 0.816 mm against *K. pneumoniae* whereas the zone of inhibition was 12 ± 0.816 mm against *B. subtilis*. Though all the test bacteria showed resistance against ampicillin, the test plant extract exhibited potent antibacterial activity. However, the reference antibiotics: tetracycline, erythromycin, and ciprofloxacin as positive control showed antibacterial activity, which is higher than that of the plant leaves extract exhibited. The previous study revealed that *M. cordata* leaves have various bioactive compounds, which might show antibacterial activity due to either individual or combined penetration of the outer phospholipidic layer of Gram-negative bacteria and peptidoglycan layer of Gram-positive bacteria (Ali *et al.*, 2011). However, this antibacterial activity exhibited by the methanol extract may be attributed to the presence of some polar and non-polar bioactive constituents persisting in the methanol extract as this solvent is an amphiphilic compound that can extract more of the extractives of polar molecules and also non-polar ones (Londonkar *et al.*, 2013). As shown in Table 1, the results of the minimum inhibitory concentration revealed that the antibacterial activity of the MLME is concentration-dependent. The MIC values for tested Gram-positive bacteria were 5 to 20 mg/ml while for Gram-negative bacteria this range was 1.25 to 10 mg/ml (Table 1). The best MIC was 1.25 mg/ml against *P. vulgaris*. The least efficacy

was shown against *B. subtilis*. It is noticeable that all the test bacteria in this study showed antibiotic resistance against ampicillin while MLME showed potent antibacterial effect against all the tested bacteria. The necessity of searching natural plant products is justified to combat the growing resistance pattern of bacteria.

Acute toxicity study to standardize dosage of MLME

Acute toxicity study is related to the median LD₅₀ was performed to select the suitable dosage of MLME for evaluation of the anticancer activity. As shown in Figure 2A, two-third of the mice died at the dosage of 3,200 mg per kg b.w after 3 hours and only one-third died at the dosage of 1,600 mg per kg b.w after

11 hours. None of the mice died at the dosage of 800 mg per kg b.w. However, after 11 hours, no behavioral change symptoms, diarrhea, neither weight loss, nor any mortality was recorded during the next 13 hours and all along for 14 days in all MLME-treated groups. The oral LD₅₀ value of the extract was 2836.851 ± 61.781 mg per kg b.w, which is above 2,000 mg per kg b.w is a recommended nontoxic LD₅₀ value by the OECD (Walum, 1998), suggesting that the lethal dosage is far greater than the effective anticancer dosage (i.e., 400 mg per kg b.w). Therefore, three dosages 100, 200, and 400 mg per kg b.w were selected for the present study. A similar study was performed by previous research (Debaprotim *et al.*, 2014).

MLME killed EAC cancer cells

Generally, the cell count is determined by trypan blue dye staining. To assess the EAC tumor cell killing effect of MLME, the total number of viable EAC cell of the treatment group was compared with those of the control group (EAC untreated group) by hemocytometer counting of trypan blue dye staining. The cell count result showed that the viability of the EAC cell was decreased extensively by all dosages (100, 200, and 400 mg per kg b.w) of MLME in comparison with the control group (Fig. 3A). Microscopic observation of EAC cells from MLME-treated group and untreated control group showed that the viable EAC cells exclude trypan-blue dye and appear as white whereas dead EAC cells retained trypan-blue dye and viewed as blue, which indicates that MLME has EAC cancer cell killing effect. The percentage of EAC cancer cell death at the dosages of 100, 200, and 400 mg per kg b.w of MLME was 46.536% ± 1.82%, 54.942% ± 1.559%, and 66.386% ± 1.287%, respectively (Fig. 3B). The reference anticancer drug, vincristine sulfate at the dosage of 300 µg per kg b.w showed 59.806% ± 0.366% EAC cell death. Consistent with this result, it was reported that methanol extract of *Eucalyptus camaldulensis* (Islam *et al.*, 2014) and *Solanum schimperianum* leaf (Almoulah *et al.*, 2017) decreased the growth kinetics of EAC cancer cell. Thus, the MLME would be the potential source of the anticancer agent.

MLME caused apoptosis in EAC cancer cell

If natural products possess anticancer properties, they should have the apoptotic cell death mechanism. To test whether the anticancer effect of MLME was mediated by apoptosis, the morphological changes of EAC cells were microscopically observed by DAPI staining after 6 days of treatment of MLME and vincristine sulfate. As shown in Figure 4A, the critical morphological characteristics of apoptosis (chromatin condensation, nuclear fragmentation, and accumulation of

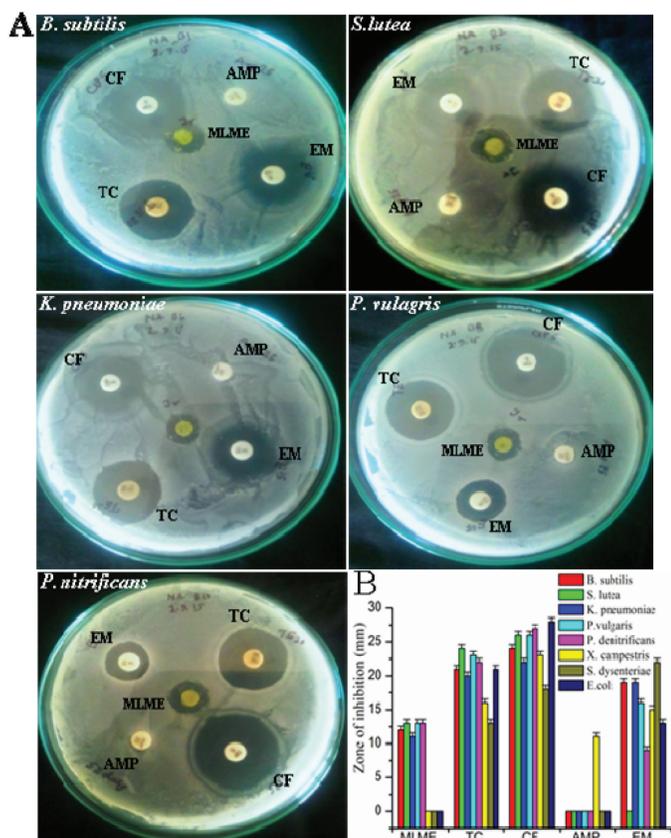


Figure 1. Antibacterial effect of MLME against Gram-positive and Gram-negative bacteria. (A) The zones of inhibition of bacterial growth by MLME and commercial antibiotics. (B) The ratios of zones of inhibition in millimeter (mm). Tetracycline (TC), Ciprofloxacin (CF), Ampicillin (AMP) and Erythromycin (EM) were used as positive control. Values are represented as mean ± STDEV ($n = 3$).

Table 1. Minimum inhibitory concentration of methanol extract of *Mikania cordata* leaves.

Bacteria	Minimum inhibitory concentration (mg/mL)						
	20	10	5	2.5	1.25	0.625	0.312
<i>B. subtilis</i>	0	4 ± 0.816	9.333 ± 1.247	18.333 ± 1.247	22 ± 1.632	34.333 ± 1.247	42.666 ± 1.247
<i>S. lutea</i>	0	0	0	1.333 ± 0.471	3 ± 0.816	4.333 ± 0.471	11.333 ± 1.247
<i>K. pneumoniae</i>	0	0	8 ± 0.816	20.666 ± 1.247	52.666 ± 2.054	75.666 ± 2.867	90 ± 2.160
<i>P. vulgaris</i>	0	0	0	0	0	2.333 ± 1.247	3 ± 0.816
<i>P. denitrificans</i>	0	0	2 ± 0.816	11 ± 1.632	25 ± 0.546	39.333 ± 2.867	94.666 ± 3.681

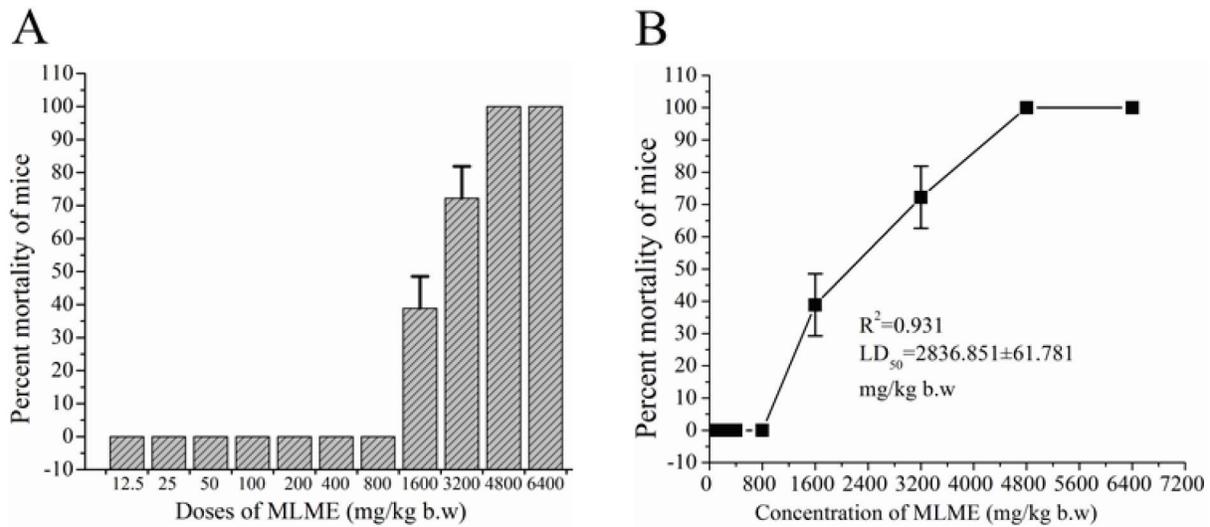


Figure 2. Acute toxicity study carried out by the oral administration of MLME in Swiss albino mice. (A) The selection of dosages of MLME upon oral administration of MLME. (B) The LD_{50} value of MLME was enumerated from the regression of MLME concentration versus percent mortality. The dosage, 2836.851 ± 61.781 mg per kg b.w was required to kill half the members of mice during 24 hours test duration.

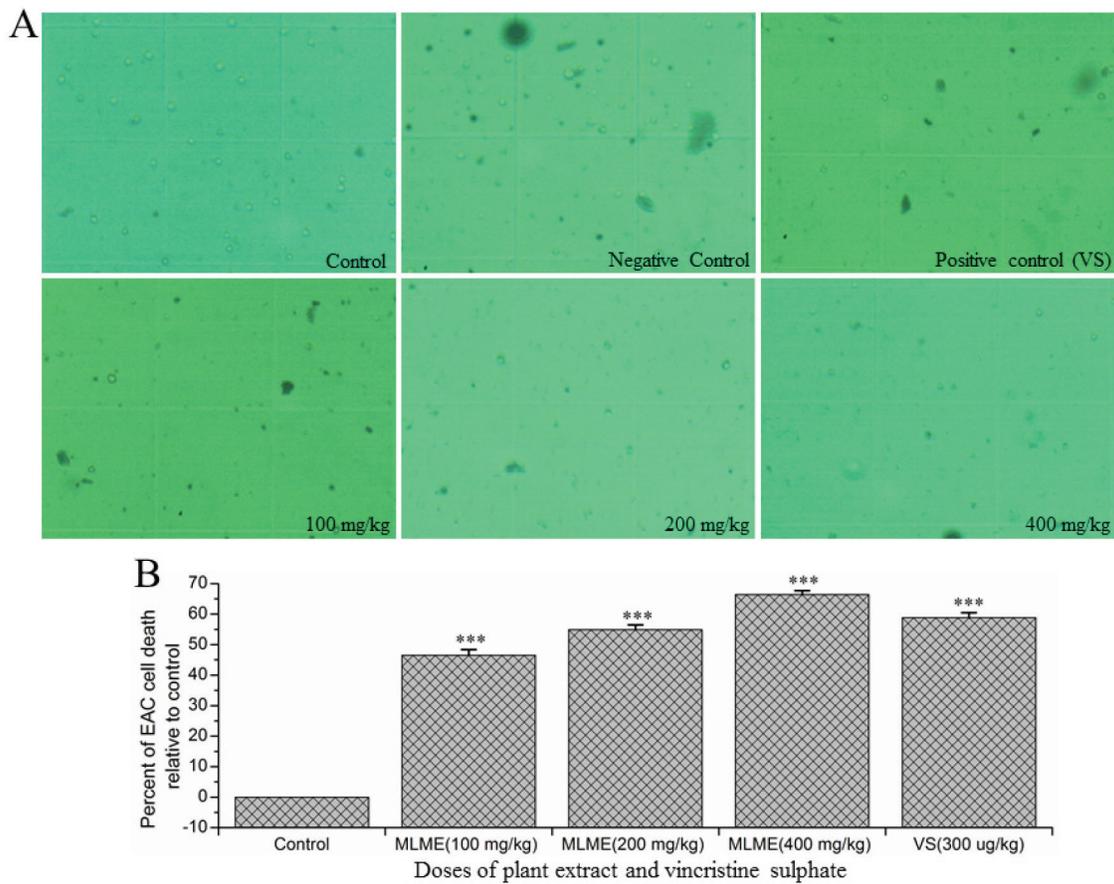


Figure 3. *In vivo* EAC tumor cell death by MLME at different dosages. (A) Hemocytometer counting of EAC tumor cells collected from both controls and treated groups of mice was determined using trypan blue dye after 6 days of EAC tumor cells transplantation. (B) Percentage of EAC tumor cell death on each dosage of MLME. Each value represents a mean \pm STDEV ($n = 6$). The level of significance *** = $p < 0.001$ is very highly significantly different from untreated control.

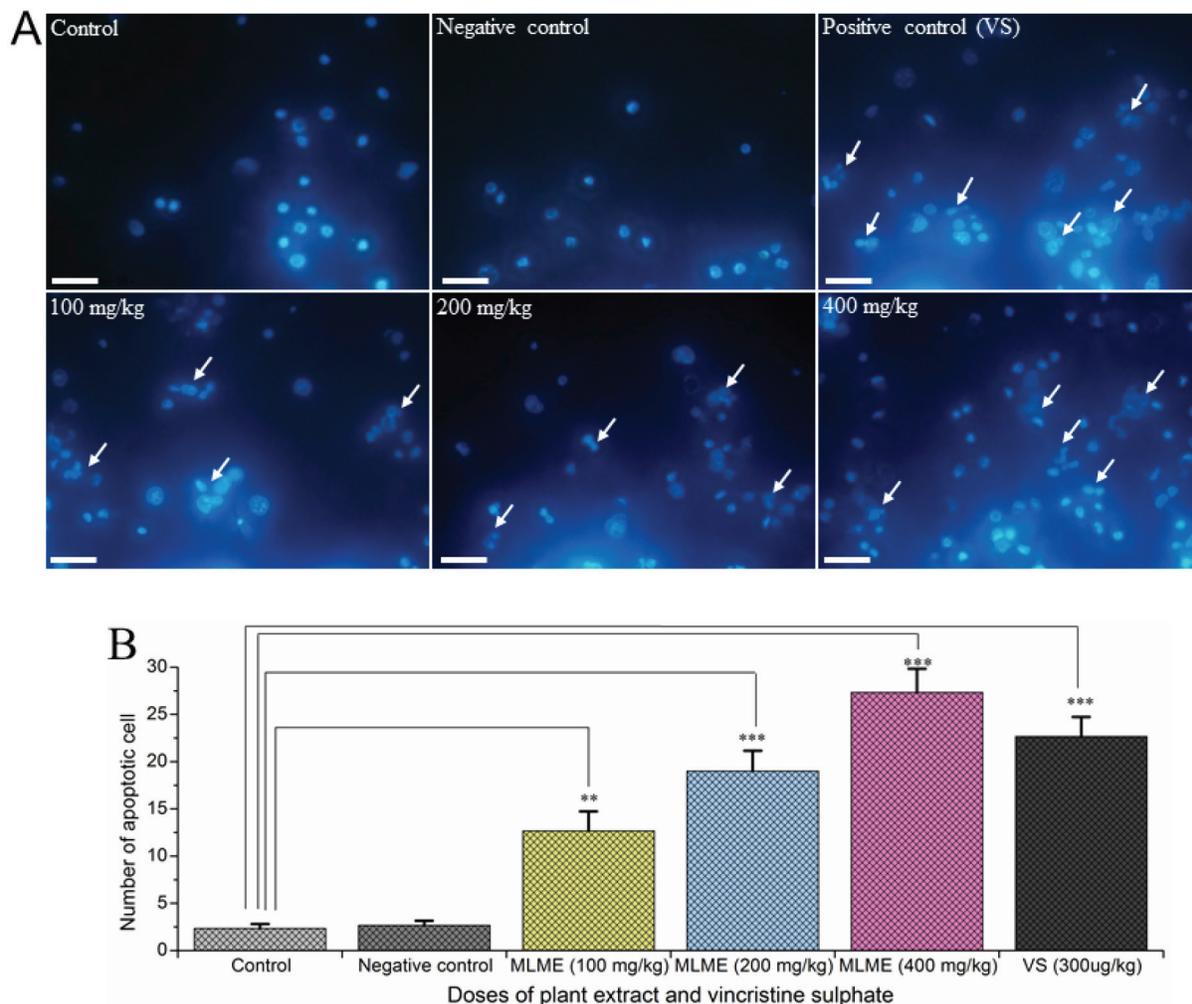


Figure 4. MLME induced apoptosis in EAC tumor cell. EAC cell bearing mice were treated for 5 days followed by collection of EAC tumor cells from MLME treated and non-treated EAC cell-bearing mice and subjected to stain with DAPI and observed by fluorescence microscope. (A) Marked apoptotic features such as nuclear condensation and fragmentation, and aggregation of apoptotic bodies were observed in the EAC tumor cells from treated groups in contrast to round-shaped control cells from the non-treated group. Arrows indicate apoptotic bodies of nuclear fragmentation observed at 40× magnification under an inverted fluorescence microscope. (B) The number of apoptotic cells per slide was estimated by counting apoptotic cells in five different fields. The values are expressed as mean ± STDEV ($n = 6$). The level of significance *** = $p < 0.001$ is very highly significant and ** = $p < 0.01$ is highly significantly different from untreated control.

apoptotic bodies) were observed in the MLME-treated group and VS-treated group. Inhibition of the apoptotic pathway is a key event in tumor development, which allows the abnormal multiplication and growth of cells and promoting to the development of cancer. Thus, triggering apoptosis is deemed as the main approach and a useful indicator of almost every type of cancer treatment and prevention (Khan *et al.*, 2015). The mean number of apoptotic cells is shown in Figure 4B. The results showed that the dose-dependent significant ($p < 0.05$) increase of the apoptotic EAC cells at the dosages of 100, 200, and 400 mg per kg b.w of MLME-treated groups compared to control. The negative control did not show any apoptotic effect on EAC cell whereas positive control showed marked apoptotic features. Consistently, it was reported that methanol extract of *E. camaldulensis* caused EAC cell death by apoptosis (Islam *et al.*, 2014).

MLME prevent the alteration of hematological parameters in EAC cell-bearing mice

As shown in Table 2 and Figure 5, the total numbers of WBC were significantly increased in the EAC control group ($p < 0.01$) compared with the untreated control group. Upon treated with MLME, WBC count was significantly decreased at the dosages of 200 ($p < 0.05$) and 400 ($p < 0.01$) mg per kg b.w, respectively, in comparison with the untreated control group. Consistent with this result, anticancer drugs have also prolonged the lifespan and decrease the number of elevated WBC from the blood (Gayatri *et al.*, 2015; Kathiriya *et al.*, 2010). In a differential count, the presence of neutrophils was increased by $53.306\% \pm 0.63\%$ ($p < 0.001$) and monocytes and lymphocyte count were decreased significantly by $0.742\% \pm 0.08\%$ ($p < 0.05$) and $41.24\% \pm 0.91\%$ ($p < 0.001$), respectively, in EAC control group in comparison with the untreated control group. In contrast, the treatment groups

Table 2. Effect of *Mikania cordata* leaves extract on hematological parameters of EAC cell-bearing mice.

Treatment groups	WBC (Cells/ml) × 10 ⁶	RBC (Cells/ml) × 10 ⁹	% of Hb (gm/dl)	Monocytes (%)	Lymphocytes (%)	Neutrophils (%)
Normal Mice (GI)	8.373 ± 0.793	6.92 ± 0.139	14.896 ± 0.898	1.653 ± 0.413	69.233 ± 0.816	22.523 ± 0.717
EAC bearing mice (GII)	15.16 ± 0.870 ^{##}	4.443 ± 0.675 ^{##}	7.31 ± 0.543 ^{###}	0.742 ± 0.084 [#]	41.24 ± 0.914 ^{###}	53.306 ± 0.637 ^{###}
EAC + MLME (100mg/kg)	14.84 ± 0.286 ^{NS}	5.303 ± 0.408 ^{NS}	8.733 ± 0.597 ^{NS}	1.17 ± 0.183 [*]	52.583 ± 0.939 ^{***}	38.49 ± 0.694 ^{***}
EAC + MLME (200 mg/kg)	12.21 ± 0.592 [*]	6.1 ± 0.163 [*]	9.99 ± 0.408 ^{**}	1.27 ± 0.254 [*]	59.41 ± 1.061 ^{***}	28.456 ± 0.612 ^{***}
EAC + MLME (400mg/kg)	10.186 ± 0.571 ^{**}	6.783 ± 0.184 ^{**}	12.563 ± 0.449 ^{***}	1.32 ± 0.306 ^{**}	61.823 ± 1.551 ^{***}	24.456 ± 0.530 ^{***}
EAC + VS (300 µg/kg)	10.16 ± 0.796 ^{**}	6.9 ± 0.163 ^{**}	12.876 ± 0.396 ^{***}	1.443 ± 0.408 ^{**}	61.93 ± 1.261 ^{***}	24.1 ± 1.512 ^{***}

Values are expressed as mean ± STDEV (n = 6). $p < 0.05$ (#), $p < 0.01$ (##), $p < 0.001$ (###), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), NS = non-significant from cancer control. *Values are significantly different from untreated control, *Values are significantly different from EAC control.

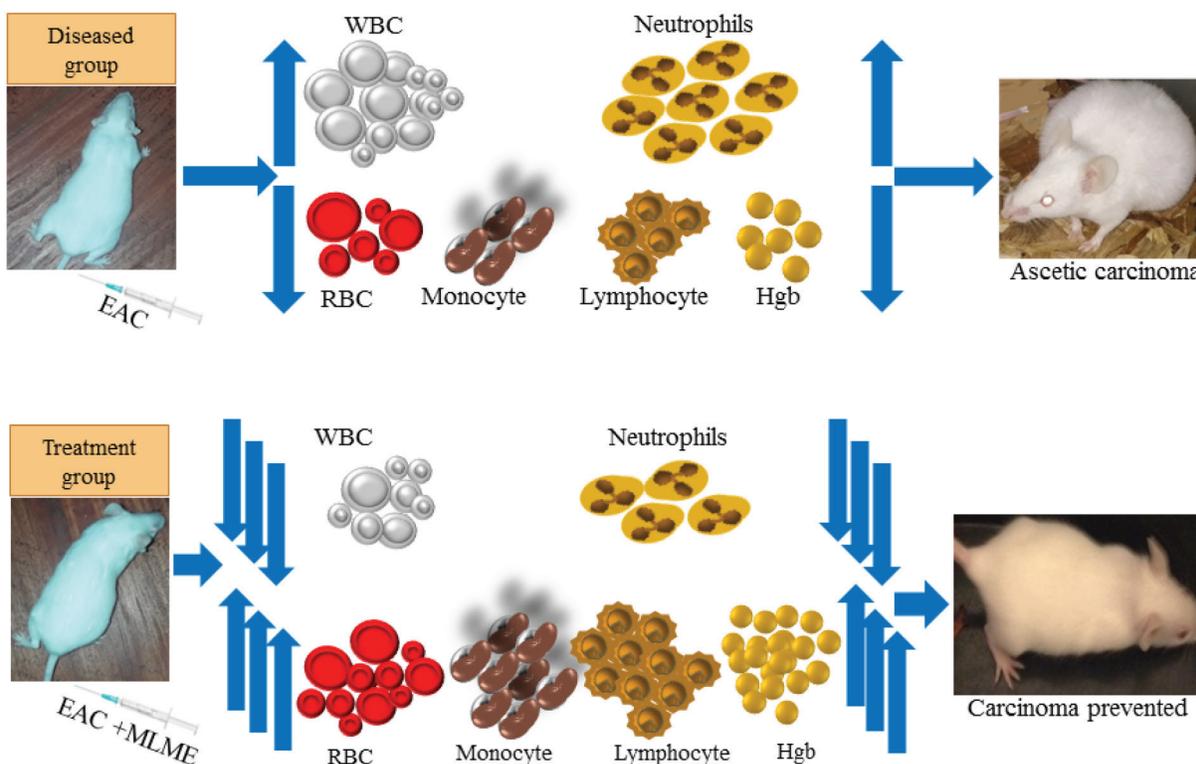


Figure 5. Schematic representation of the prevention of EAC tumor cell-induced altered hematological parameters by MLME. (A) Upon i.p. injection of EAC cell into Swiss albino mice, WBC count and neutrophil percent were increased and RBC count and Lymphocytes, monocyte and hemoglobin percent were decreased and mice acquired ascetic carcinoma. (B) Treatment with MLME, the altered hematological parameters (rising of WBC and neutrophil count and reduction of RBC, Lymphocytes, monocyte, and hemoglobin) were restored towards normal level and mice recovered from ascetic carcinoma.

at the dosages of 100, 200, and 400 mg per kg b.w, the percent of neutrophil was decreased ($p < 0.001$) by 38.49% ± 0.69%, 28.456% ± 0.61%, and 24.456% ± 0.53%, respectively. The percentage of monocytes at the dosages of 100, 200, and 400 mg per kg b.w was increased by 1.17% ± 0.18%, 1.27% ± 0.25% ($p < 0.05$), and 1.32% ± 0.30% ($p < 0.01$) and lymphocyte by 52.583% ± 0.93%, 59.41% ± 1.06%, and 61.823% ± 1.55% ($p < 0.001$), respectively. The plant-based standard drug, vincristine sulphate at the dosage of 300 µg per kg b.w decreased the percent of a neutrophil by 24.1% ± 1.51% ($p < 0.001$) and increased the monocyte and lymphocyte by 1.443% ± 0.40% ($p < 0.01$) and 61.93% ± 1.26% ($p < 0.001$), respectively. From the results, it was observed that neutrophil, monocyte, and lymphocyte count was restored towards the normal level, which indicates that MLME might have a direct

protective effect against alteration of hematological parameters in EAC cell-bearing mice. The similar results were also observed by Nakajima *et al.* (2011). The percentage of hemoglobin ($p < 0.001$) and RBC count ($p < 0.01$) was significantly decreased in the EAC control group in comparison with the untreated control group. The reduction of RBC and the level of hemoglobin in EAC cell-bearing mice implicate the anemic condition. Upon treatment with MLME at the dosages of 100, 200, and 400 mg per kg b.w, the percent of hemoglobin was increased by 8.733% ± 0.59%, 9.99% ± 0.40% ($p < 0.01$), and 12.563% ± 0.44% ($p < 0.001$), respectively, and RBC count was also increased significantly compared to EAC control group. Collectively, the above-mentioned hematological parameters revealed that MLME possesses protective action against cancer-induced altered effects on the hemopoietic system.

MLME caused cytotoxicity in EAC cell in Swiss albino mice

As shown in Figure 6A, upon treatment with MLME at the dosages of 3.125, 6.25, 12.5, 25, 50, and 100 $\mu\text{g/ml}$, it was observed that EAC tumor cell death was increased in a dose-dependent manner. The result showed that the number of viable EAC cells was found to be significantly decreased as the MLME concentration increased. The IC_{50} value was 6.6 ± 1.91 $\mu\text{g/ml}$ (Fig. 6B). The IC_{50} value indicates the amount of the extract which prevents the growth of 50% of the cell population. In MTT assay, the succinate dehydrogenase converts yellow MTT dye to formazan (purple crystals) in the mitochondria of viable cells. The purple formazan crystals were quantified by spectrophotometry to evaluate the magnitude of cytotoxicity and cell viability (Tiwary *et al.*, 2015). Estimation of the half-maximal (50%) inhibitory concentration (IC_{50}) of the anticancer agent is essential for understanding the magnitude of cytotoxic activity against

the cancer cell. The standards of cytotoxicity customized by the U.S. National Cancer Institute (NCI) plant screening program established a crude extract is inactive, moderately active, and active against cancer cell, if IC_{50} values are higher than 100 $\mu\text{g/ml}$, from 20 to 100 $\mu\text{g/ml}$, and lower than 20 $\mu\text{g/ml}$, respectively (Ramos-Silva *et al.*, 2017). MLME exhibited the cytotoxic effect with an IC_{50} value of 6.6 ± 1.91 $\mu\text{g/ml}$, which is less than 20 $\mu\text{g/ml}$. Accordingly, it supports the notion that the test extract is strongly cytotoxic to the EAC cell, which indicates its anticancer potential for further investigation.

CONCLUSION

The present study confirmed that the *Mikania cordata* leave extract has potent antibacterial and anticancer properties. MTT study results also supported the anticancer properties of *Mikania cordata* leave extract. These findings warrant advance studies for isolation and identification of the liable bioactive compounds and to explicate the mechanism(s) linking with the antibacterial and anticancer effects.

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CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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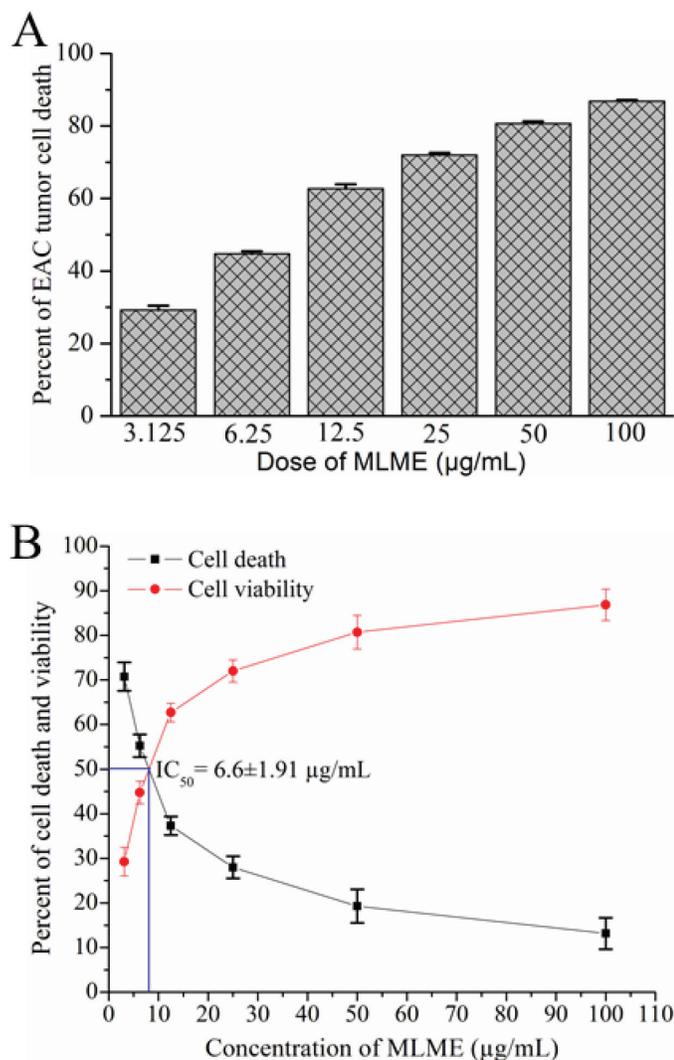


Figure 6. MLME caused cytotoxicity on EAC tumor cell determined by MTT assay *in vitro*. (A) Growth inhibition of EAC tumor cell upon treatment with different dosages of MLME for 24 hours. (B) The IC_{50} value of MLME was enumerated from the MLME concentration versus percent mortality. The values are represented as mean \pm STDEV ($n = 3$).

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