IJPSR (2016), Vol. 7, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 February, 2016; received in revised form, 22 March, 2016; accepted, 04 May, 2016; published 01 July, 2016

ANTITUMORAL AND APOPTOTIC EFFECTS OF MAGAININ II AGAINST COLO 320 DM CANCER CELL LINE

Poongodi Arasu [1], Nalini Ganesan [*1], Srinivasan Sivasubramanian [2] and Palani Gunasekaran [2]

Department of Biochemistry [1], SRMC & RI, Sri Ramachandra University, Chennai-116, Tamilnadu, India
Department of Virology [2], King Institute of Preventive Medicine & Research, Guindy, Chennai-32, Tamilnadu, India

Keywords:

Antimicrobial Peptide, Magainin II, Colon Cancer, Cytotoxicity, Apoptosis

Correspondence to Author:
Nalini Ganesan

Professor,
Department of Biochemistry,
SRMC&RI, Sri Ramachandra University, Chennai, Tamilnadu, India.

E-mail: nalinisrmc@gmail.com

ABSTRACT: Antimicrobial peptides (AMP) showed a broad spectrum of cytotoxic activity against cancer cells, which could provide a new class of anticancer drugs. In this study, we have evaluated the cytotoxic and apoptotic activity of magainin II, an AMP compared with 5-Fluorouracil (5-FU) in colo320 DM cancer cells, and magainin II cytotoxicity was assessed on vero cell line by MTT. The cells cultured in the presence or absence of magainin II and 5-FU. The magainin II inhibited cell viability in dose dependent manner was quantified by MTT assay. The IC_{50} of magainin II was found to be 89.3nM and at this concentration the cytotoxicity was not observed in vero cell line. The cytotoxicity of the magainin II was further confirmed by lactate dehydrogenase (LDH) assay and neutral red uptake assay (NRU). Apoptosis induction was verified by DNA fragmentation analysis. The morphological changes in colo 320 DM cells was measured by DAPI and acridine orange/ethidium bromide staining, showed nuclear condensation, and this substantiates the apoptotic action of magainin II. The cell cycle analysis by FACS revealed that there was a accumulation of cells at G0/G1 phase in magainin II treated cells. Overall, these results revealed that magainin II has selective cytotoxicity in cancer cells and has the potential to induce apoptosis. Magainin II may offer a novel and effective therapeutic candidate in the treatment of colon cancer with potentially low cytotoxic effects on normal cells. These findings highlight the importance of magainin II as a drug capable of exerting an *in vitro* antitumoral activity by triggering apoptosis.

INTRODUCTION: Antimicrobial peptides (AMP) gain increasing attention as potential candidates in cancer therapy and have various advantages such as selective cytotoxicity against cancer cells, active against multidrug-resistance mechanism as well as effects in combination therapy [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(7).2951-58

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (7).2951-58

These AMPs show relatively lesser cytotoxicity against untransformed proliferating cells [2].

Magainin II is a linear natural peptide belongs to a family of AMP was isolated from the skin of the African frog, *Xenopus laevis*, shows broad antimicrobial spectrum, anti-endotoxin and anticancer activity [3, 4] with no hemolytic effect [5]. Besides their well-known antimicrobial activity, studies have also reported significant cytotoxic effect of magainin against a wide range of cancer cell lines including melanoma, bladder cancer, breast and lung cancers as well as lymphomas and leukemias [6, 7]. *In vivo*, magainin peptides have been shown to improve survival of animals with ascites-

producing tumors [8]. Furthermore, in a subcutaneous xenograft model of melanoma tumor growth in nude mice, local treatment of magainin II completely ablated the tumor [4].

Magainin II shows a synergistic effect with cecropin peptides (cecropin A and B) [9], inhibit cell proliferation and viability in a dose dependent fashion in bladder cancer cells [10]. However, there is no report on the use of magainin peptides for treatment against colorectal cancer (CRC).

CRC is one of the major type of cancer worldwide, in terms of both morbidity and mortality. In India, CRC is the seventh most common cancer in men and the sixth in women [11]. A major limitation inherent to most conventional chemotherapeutic drug is less selectivity against tumor, which results in deleterious side effects and develops multidrug resistance to conventional chemotherapy agents by cellular changes through multimechanisms [12]. The search for new classes of anticancer drugs with new modes of action because of these limitation.

The aim of the present study was to evaluate the antitumor activity of magainin II in comparison with conventional chemotherapeutic drug, 5-FU on colo 320 DM cell line and to assess the toxicity of magainin II on vero cell line. The potential role of this antimicrobial peptide as apoptotic inducer on colo 320 DM cell line were also investigated. Therefore, the findings from this study may serve to evaluate the value of magainin II as a chemotherapeutic option and may offer a new strategy for overcoming multi-drug-resistant drugs, which represent a major problem in cancer therapy.

MATERIALS AND METHODS:

Materials:

MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (sigma), India. 100U/ml penicillin and 2mM glutamine (Sigma-Aldrich). Propidium iodide (PI) from sigma, DAPI (Sigma Aldrich, USA), All reagents were of analytical grade.

Cell line and culture condition:

The human colon cancer cell line, colo 320 DM, was obtained from the National centre for Cell Science Pune (NCCS). The cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco) containing 10% fetal bovine serum, 100U/ml penicillin and 100U/ml streptomycin at 37°C in a humidified atmosphere with 5% CO_2 and vero cell line was cultured in Dulbecco modified Eagle medium (DMEM).

Drugs:

Lyophilized magainin II was purchased from Genicbio (Shanghai, China) and 5-FU (sigma-Aldrich) were reconstituted in serum free RPMI-1640 (Sigma) and phosphate buffered saline (PBS).

MTT assay for the determination of cell viability:

Cell viability was analyzed by MTT assay, as described by Mosmann [13]. Briefly, colo 320 DM and vero cells (1×10^5 cells/ml) were seeded in 96-well plates and were treated with different concentrations of 5-FU (10-100µM) and magainin II (10-100nM) 89.34nM for 24h with FCS free complete media. 100µl of MTT (5 mg/ml) was added to 24h treated wells. After the plates were incubated at 37°C for 4h, 100µl of di-methyl sulfoxide (DMSO) was added to each well to dissolve the formosan crystals after the supernatant was aspirated, and absorbance was measured at 620nm using a 96-well microplate reader (Thermo Multiskan, USA). The percentage of surviving cells was calculated with the following equation:

$$\text{The ratio of cell viability (\%)} = \frac{\text{Mean absorbance of treated cells}}{\text{Mean absorbance of untreated cells}} \times 100$$

The IC_{50} values were calculated using Graph Pad Prism 5, Software, San Diego, CA, USA.

Lactate dehydrogenase (LDH) assay:

Colo 320 DM cells (1×10^5 cells/ml) were seeded in 6 well plates and were treated with IC_{25} and IC_{50} of 5-FU and magainin II with FCS free complete media for 24h. The maximal LDH release of cells was determined by addition of lysis solution (2% Triton X-100). The released LDH is measured colorimetrically with maximum absorbance read at 440nm [14]. The LDH activity released by cells cultured in the absence or presence of 5-FU and magainin II was expressed as a percentage of the total LDH released by cells cultured in the presence of 2% Triton X-100.

Neutral red uptake assay:
The viable cell was determined by NRU assay by Borenfreund [15]. The colo 320 DM cells (1×10^5 cells/ml) were cultured for 24h and were treated with IC_{25} and IC_{50} of 5-FU and magainin II. 20µl of 3% neutral red in PBS were added to each well including untreated cells after 24h for 30 min. 0.1ml of 10% acetic acid and 40% ethanol solution was added to solublize neutral red by a 30-min incubation. Finally, cells were washed twice with PBS and absorbance at 550nm was measured using a microplate reader (FluOstar OPTIMA BMG Labtec).

Measurement of DNA damage by DNA fragmentation:
DNA damage by apoptosis was evaluated by genomic DNA fragmentation as described by Bossú [16]. After 24-h treatment 1×10^5 colo 320 DM cells were centrifuged at 10,000rpm, the pellet was suspended in 0.5ml lysis buffer, then centrifuged at 10,000rpm/4°C/10min. The supernatants were transferred into new tubes and 100µl of ice-cold 5M NaCl and 700µl of ice-cold absolute isopropanol were added and vigorously vortexed.

The tubes were incubated overnight at -20°C then centrifuged again at 10,000rpm/4°C/15min. The supernatants were discarded and the pellets were rinsed by adding 500µl of ice-cold 70% ethanol, centrifuged at 10,000rpm/4°C/15min.The resulting DNA was dissolved by adding 50µl of TE solution. The DNA concentration was measured spectrophotometrically and 10µl DNA/lane were run on 1% agarose gel to determine the DNA fragmentation levels.

Morphological characterization of colo 320 cancer cell apoptosis determined by DAPI staining by fluorescent microscopy:
The colo 320 DM cells were seeded in 6 well plates and maintained at 37°C with 5% CO_2 for 48h. Subsequently, the cells were treated with IC_{25}, IC_{50} 5-FU and magainin II. After 24h the medium was removed gently, and the cells were washed twice with PBS, fixed in 4% para-formaldehyde for 20min, re-washed, and stained with DAPI (0.5 g/ml) at 37°C for 20min in the dark. Stains were then washed with methanol followed by PBS. The apoptotic nuclei (intensely stained, fragmented nuclei, and condensed chromatin) were viewed under a fluorescent microscope (Nikon Eclipse-80i, Japan) with an excitation at 359nm and emission at 461nm wavelengths.

Determination of apoptosis induction and cell death by acridine orange/ethidium bromide (AO/EtBr):
1µl of a dye mixture (10mg/ml acridine orange (AO) and 100mg/ml ethidium bromide (EtBr), in distilled water) was directly stained with IC_{25} and IC_{50} of 5-FU and magainin II treated cells grown on clean microscope cover slips. After staining the cancer cells were washed with PBS (pH 7.2) and incubated for 1min, the cells were then visualized under fluorescence microscope (Nikon Eclipse, Inc., Japan) at 400× magnification with an excitation filter at 480nm.

Cell cycle distribution by flow cytometry:
Cells were plated into 100mm dishes (1×10^6 cells/dish) for 48h.The cells were treated with IC_{50} 5-FU and magainin II for 24h then subjected to FACS analysis. Briefly, cells were trypsinized, washed with PBS/0.5% FCS and fixed in 70% ethanol at -20 ºC. After subsequent washes cells were resuspended in 1ml of PBS/0.5% FCS, containing 40U/ml of RNAse and stained with PI (25mg/ml) for 30 min at 37°C. The DNA content was analysed by FACScan flow cytometer (Becton-Dickinson, San Jose, CA) and CellQuest acquisition/analysis software (Becton-Dickinson).

Statistical analysis:
Statistical analysis was carried out with a statistical program Graph Pad Prism 5 (Graph Pad prism Inc., Software, San Diego, CA, USA). All the experiments were carried out in triplicates and the data were expressed as mean ± SD. The difference between means was analyzed by one-way ANOVA followed by Dunnett's post test for comparision. The values are expressed as mean ± SD. $P \leq 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION: In this study, magainin II was evaluated for its cytotoxicity on colon cancer colo 320 DM cells, normal cell toxicity in vero cells by MTT assay. Magainin II

and 5-FU both inhibit the viability of colo 320 DM cells for 24h in a dose-dependent fashion (**Fig. 1a**). The average IC_{50} values of magainin II and 5-FU against colo320 DM cell lines were found to be 89.34nm and 5-FU at 24.06µM respectively. The present result showed that magainin II strongly reduces the viability of colo 320 DM cells. Consistent with these results, previous reports indicated that magainin II inhibits cell proliferation in different cancer cell lines [6, 17].

Hence, for further studies IC_{25} and IC_{50} values of 12µM and 24.06 µM of 5-FU and 44nM and 89.34nM of magainin II for colo 320 DM cells were considered. Conversely, the vero cell lines showed significantly higher IC_{50} values in MTT assays as compared to colo 320 DM cells. The IC_{50} of magainin II for these normal cells was within the range of 800-1000µM, which is about 80-100 times higher than the IC_{50} of magainin II for colo 320 DM cells (**Fig. 1b and 1c**). In summary, magainin II showed a selective inhibitory effect on colon cancer cell viability versus normal vero cells.

In agreement with our findings, studies from Anghel et al.demonstrated the cytotoxicity of magainin II with average IC_{50} concentration 120 µM against MDA-MB-231 and M14K tumour Cell lines for MTT assay[18]. A significantly increased LDH release was observed in IC_{50} of magainin II on colo 320 DM cells. When compared with control, IC_{25} of magainin II, 5-FU and IC_{50} of 5-FU, indicating that the treatment with magainin II showed loss of membrane of plasma membrane in colo 320 DM cells (**Fig.1d**).

This might be due to increased permeability of plasma membrane coupled with excessive leakage of LDH from the cells to the medium. We also quantified the cytotoxic activity of magainin II using neutral red. The results showed a modest reduction of viability in colo320 DM cells after the treatment with IC_{50} magainin II when compared with control, IC_{25} of magainin II, 5-FU and IC_{50} of 5-FU. This indicates that magainin II alter the endosomal and lysosomal integrity of the cell line (**Fig.1e**).

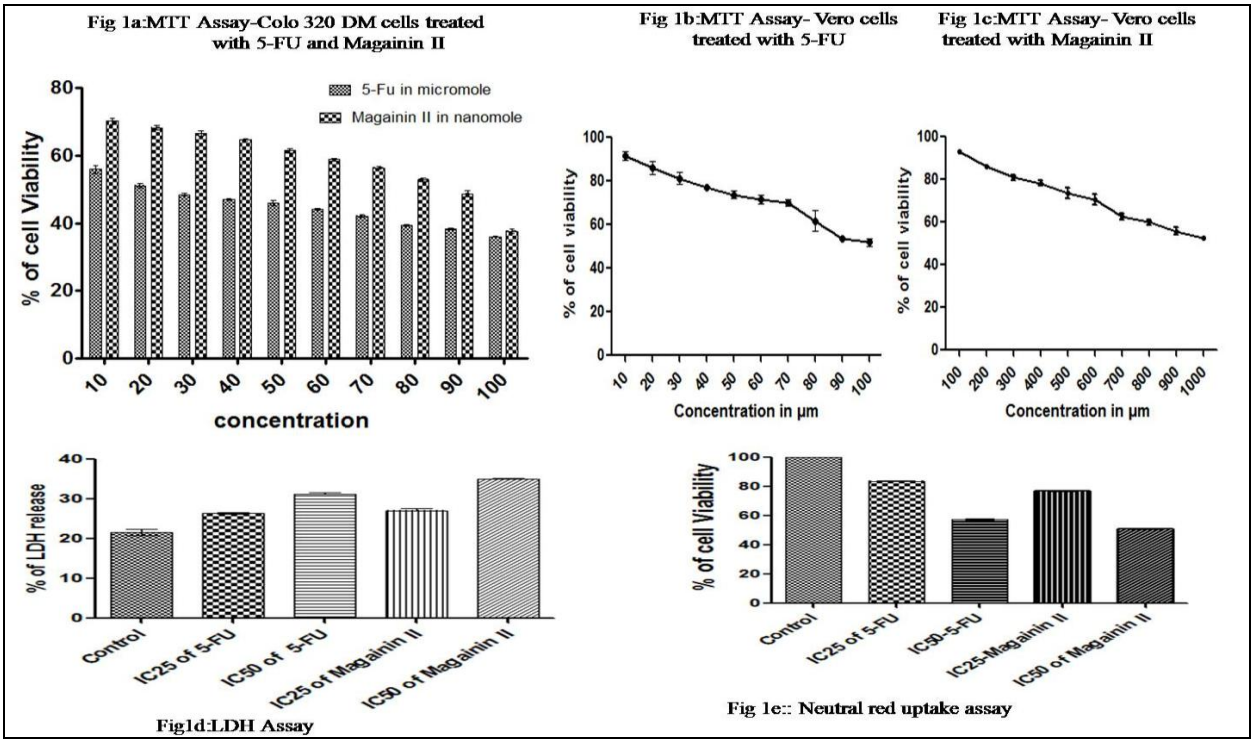

FIG. 1: DETERMINATION OF CYTOTOXICITY, (a) DOSE-DEPENDENT CYTOTOXIC EFFECT ON COLO 320 DM CELLS TREATED WITH 5-FU (10-100µM) AND MAGAININ II (10-100nM) MEASURED BY MTT ASSAY. (b) CYTOTOXICITY OF VERO CELLS MEASURED BY MTT ASSAY.(c) PERCENTAGE OF LDH RELEASE AFTER TREATMENT OF COLO 320 DM CELLS IN THE PRESENCE AND ABSENCE (CONTROL) OF 5-FU AND MAGAININ II AT A IC_{25} AND IC_{50} CONCENTRATION (d) NEUTRAL RED UPTAKE AFTER TREATMENT OF COLO 320 DM CELLS IN THE PRESENCE AND ABSENCE (CONTROL) OF 5-FU AND MAGAININ II AT A IC_{25} AND IC_{50} CONCENTRATION.

Data are mean±SD from three independent determinations in triplicate. *P < 0.05 was considered to be statistically significant, compared with values from cells incubated in the absence of 5-FU and magainin II (controls)

Based on its spectrum of activity, magainin II is highly potent against cancer cells, but not against normal mammalian cells [19]. The lytic action of magainin II is due to the presence of net positive charges of magainin that interacts with the negative charges located in the outer leaflet of the cancer cell membrane [20]. O-glycosylated mucin, a type of glycoprotein, exists in the cancer cell membranes that contribute to the negative charges on the cancer cell surfaces [21].

However, normal mammalian cell membranes are mainly composed of neutral zwitterionic phospholipids and sterols [22]. The net positive charge and hydrophobicity, which have the ability to adopt an amphipathic conformation, are critical structural parameters for antitumor activity [23, 24]. Therefore cationic AMP of magainin II is resistant to cancer cells.

Apoptosis is considered to be the main cell death mechanism that occurs in response to cytotoxicity of the cells. Apoptosis is a process of programmed cellular suicide that is characterized by several morphological and cellular changes including chromatin condensation, membrane blebbing, DNA fragmentation and cleavage of key cellular proteins [25, 26]. We have examined the extent of nuclear DNA fragmentation by agarose gel electrophoresis and the results are shown in **Fig. 2**. DNA ladder pattern was observed after the treatment of colo 320 DM cells with IC_{25} and IC_{50} of 5-FU and magainin II. A significant increase of DNA fragmentation has been detected in IC_{50} of magainin II.

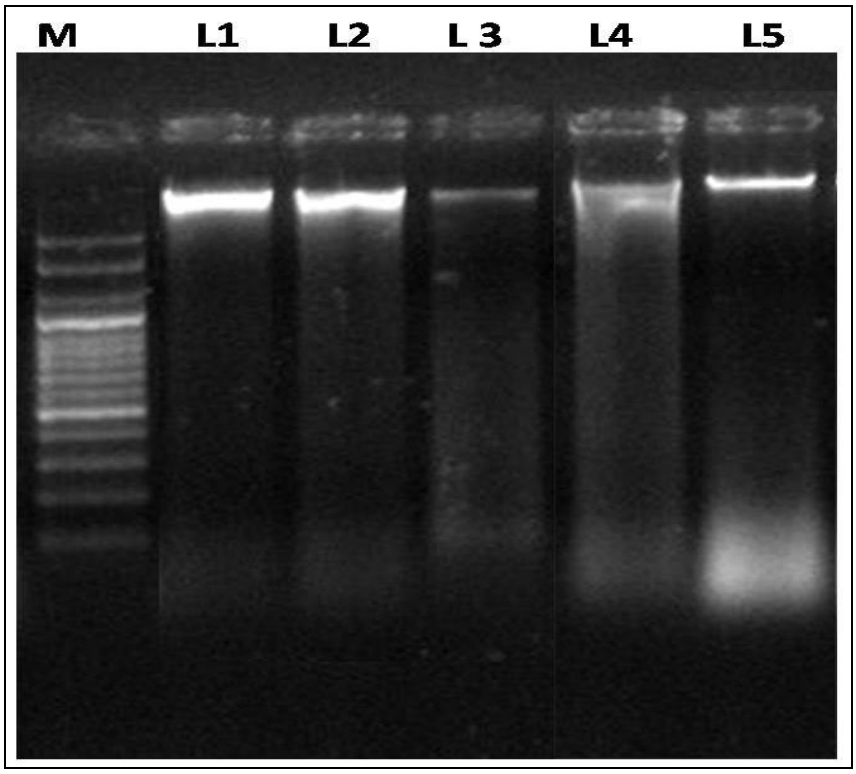

FIG. 2: A PHOTOGRAPH OF AN UV LIGHT-ILLUMINATED AGAROSE GEL CONTAINING TOTAL CELLULAR DNA; LANES WERE LOADED WITH DNA PREPARATIONS MADE FROM 24H 5-FU AND MAGAININ II TREATED COLO 320 DM CELLS.

Lane M contains 100bp ladder; lanes 1–5 contain DNA from colo 320 DM cells treated with 5-FU and magainin II (L1: Control, L2: IC_{25} 5-FU(12.3µM), L3: IC_{50} 5-FU(24.06 µM), L4: IC_{25} magainin II (44nM) and L5: IC_{50} magainin II (89.3nM). Results are representative of three independent experiments.

This results suggested that these magainin II caused DNA fragmentation characteristic of apoptotic process with the generation of multiple DNA fragments. A biochemical hallmark of apoptosis was the chromatin cleavage including oligonucleosomes, which were depicted as DNA ladders in the electrophoresed gel [27]. Similar results were observed in cecropin treated human promyelocytic leukemia HL-60 cell line [28]. Magainin I or BMAP-28 (bovine cathelicidin-derived AMP), cationic peptides which also induced DNA fragmentation [17, 29].

In addition, magainin II effectively triggers apoptosis on colo320 DM cells treated in the absence and in the presence of IC_{25} and IC_{50} of 5-FU and magainin II for 24h were assessed using fluorescence microscopy following DAPI staining. The results revealed that apoptotic morphological changes were exhibited in colo 320 DM cells after incubation with IC_{25} and IC_{50} of 5-FU and magainin II. As shown in (**Fig. 3a–c**), untreated cells exhibited unchanged morphology, characteristic for colon cancer cells. After 24 h of treatment at IC_{50} magainin II, a two-fold increase in apoptotic cells was visible, and these cells exhibited apoptotic features such as morphological changes, cell shrinkage, chromatin condensation and fragmentation into discrete bodies. By contrast, only a few apoptotic colo 320 cells were observed following the treatment at IC_{25} of magainin II, 5-FU and which is of less magnitude than IC_{50} of 5-FU. On the basis of overall cell morphology and cell membrane integrity, necrotic and apoptotic cells can be distinguished using fluorescence microscopy. The morphological changes in cells were observed using AO/EtBr fluorescence staining after treating them with 5-FU and magainin II at their IC_{25} and IC_{50} concentrations for 24h (**Fig. 3d**).

The images revealed that the apoptotic cells containing the condensed form of nuclei and apoptotic bodies were stained orange whereas the necrotic cells were stained red and the untreated colo 320 DM cells were stained uniform green. The significant differences in apoptosis induction were observed between the control and cancer cells after treatment with 5-FU and magainin II for 24h. The higher apoptosis induction was observed in cancer cells treated with IC_{50} magainin II compared with IC_{25} of 5-FU and magainin II and IC_{50} of 5-FU. Therefore, these results corroborate the involvement of this magainin II in an apoptotic induction rather than in a necrotic action.

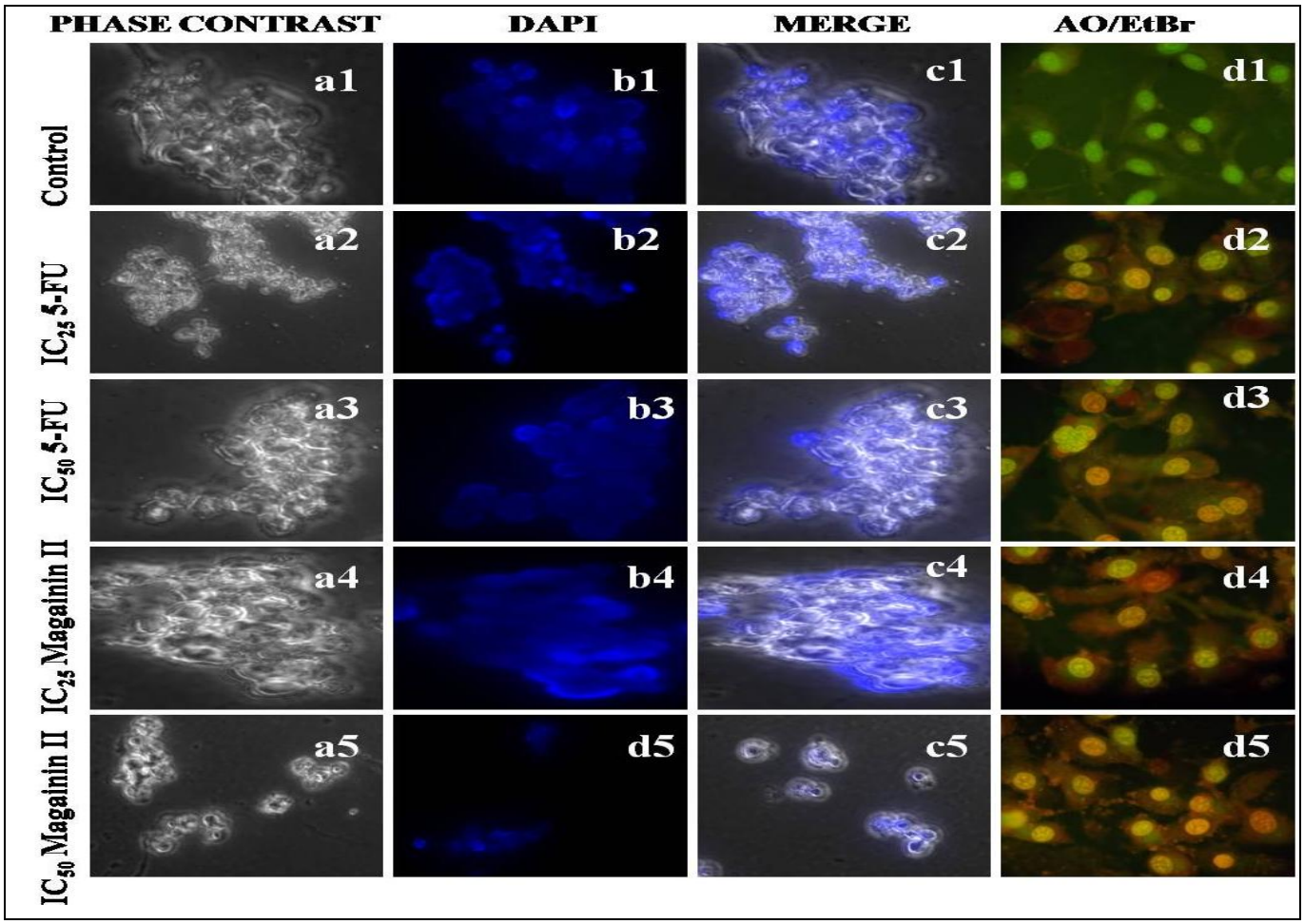

FIG. 3: FLUORESCENCE MICROSCOPY IMAGES OF 5-FU AND MAGAININ. THE PANEL OF IMAGES SHOWS DAPI STAINING SHOWING THE NUCLEAR FRAGMENTATION; AO/EtBr STAINING FOR INDUCTION OF APOPTOSIS, AND VISUALIZED AND PHOTOGRAPHED AT 40× MAGNIFICATION UNDER FLUORESCENCE MICROSCOPE.

Anti-cancer agents exhibit control of cell cycle, and their effects may be cytostatic or cytotoxic, relying on the cell cycle status of the target cells. Further, the inhibition of cell proliferation and induction of apoptosis by anticancer agents through various pathways arrest the cell cycle in the G1, S and G2/M phases [30, 31]. Our results showed an increased G1 phase at IC_{50} magainin II for 24h on colo 320 DM cells. In addition, treatment with magainin II significantly decrease the cells in S and G2/M phases (**Fig. 4**). In contrast, increased accumulation was observed in S-phase at 5-FU treated cells. The present results showed that 5-FU and magainin II induced S-phase and G1 phase arrest in cancer cell lines respectively. This indicates that magainin II and 5-FU elicit different cellular responses with respect to cell cycle arrest and cell death activation.

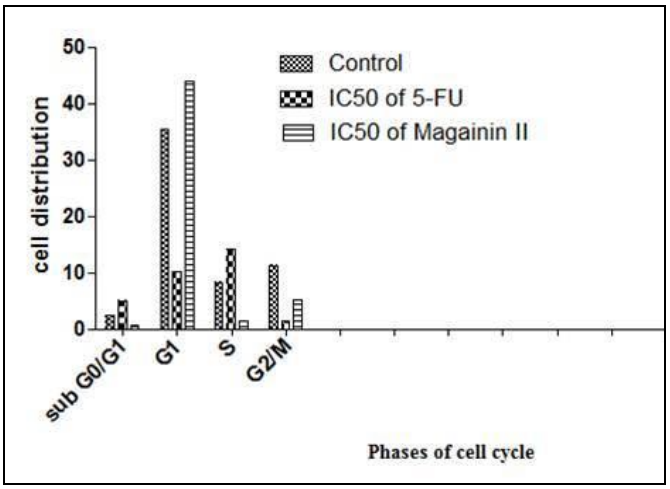

FIG. 4: EFFECT OF 5-FU AND MAGAININ II ON THE CELL CYCLE ARREST. CELLS WERE TREATED WITH 5-FU AND MAGAININ II FOR 24H AND ANALYZED BY FLOW CYTOMETRY

CONCLUSION: In the present study, the antimicrobial peptide magainin II showed a selective antitumor activity in colon cancer colo 320 DM cells but did not affect normal cell line. Our findings showed that magainin II inhibited cell growth, and induces cell cycle arrest and early apoptosis in human colorectal cancer cells. The present study give more insights into the apoptosis of magainin induced cell death, when compared to 5-FU. However, the antitumor mechanisms of magainin II still need further clarification.

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest concerning this study.

ACKNOWLEDGMENT: The authors wish to acknowledge the Chancellor, Sri Ramachandra University for providing the financial support under Chancellor's Research Fellowship.

REFERENCES:

1. Papo N and Shai Y: Host defense peptides as new weapons in cancer treatment. Cellular and Molecular Life Sciences, 2005; 62:784–790.
2. Wang C, Li HB, Li S Tian LL and Shang DJ: Antitumor effects and cell selectivity of temporin-1CEa, an antimicrobial peptide from the skin secretions of the Chinese brown frog (*Rana chensinensis*). Biochimie 2012; 94:434–441.
3. Scott MG, Yan H and Hancock RE: Biological properties of structurally related a-helical cationic antimicrobial peptides. Infection and Immunity 1999; 67:2005–2009.
4. Soballe PW, Maloy WL, Myrga ML, Jacob LS and Herlyn M: Experimental local therapy of human melanoma with lytic magainin peptides. International Journal of Cancer. 1995; 60:280–284.
5. Shin SY, Lee MK, Kim KL and Hahm KS: Structure-antitumor and hemolytic activity relationships of synthetic peptides derived from cecropin A-magainin 2 and cecropin A-melittin hybrid peptides Journal of Peptide Research 1997, 50:279–285.
6. Lehmann J, Retz M, Sidhu SS, Suttmann H, Sell M, Paulsen F, Harder J, Unteregger G and Stöckle M: Antitumor activity of the antimicrobial peptide magainin II against bladder cancer cell lines. European Urology 2006; 50:141–147.
7. Ohsaki Y, Gazdar AF, Chen HC, Johnson BE: Antitumor activity of magainin analogues against human lung cancer cell lines. Cancer Research 1992; 52:3534–3538.
8. Baker MA, Maloy WL, Zasloff M and Jacob LS: Anticancer efficacy of magainin2 and analogue peptides. Cancer Research 1993; 53:3052–3057.
9. Cirioni O, Silvestri C, Ghiselli R, Orlando F, Riva A, Mocchegiani F, Chiodi L, Castelletti S, Gabrielli E, Saba V, Scalise G and Giacometti A: Protective effects of the combination of alpha-helical antimicrobial peptides and rifampicin in three rat models of *Pseudomonas aeruginosa* infection. Journal of Antimicrobial Chemotherapy 2008; 62(6):1332–1338.
10. Suttmann H, Retz M, Paulsen F, Harder J, Zwergel U, Kamradt J, Wullich B, Unteregger G, Stöckle M and Lehmann J: Antimicrobial peptides of the Cecropin-family show potent antitumor activity against bladder cancer cells. BMC Urology 2008; 8:5.
11. Globocan 2012, http://globocan.iarc.fr/
12. Gatti L and Zunino F: Overview of tumor cell chemoresistance mechanisms, Methods in Molecular Medicine. 2005; 111:127–148.
13. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983; 65:55–63.
14. King J: The dehydrogenases or oxidoreductases — Lactate dehydrogenase. In: King J, Editor. Practical Clinical Enzymology. Van Nostrand Company Ltd, London, 1965: 83–93.
15. Borenfreund E and Puerner JA: Toxicity determined in vitro by morphological alterations and neutral red absorption. Toxicology Letters 1985; 24:119–124.

16. Bossú P: Qualitative analysis of DNA fragmentation by agarose gel electrophoresis. In: Apoptosis: A laboratory Manual of Experimental Methods A. Cossarizza & D. Boraschi, (Eds). Purdue cytometry CD-ROM, Vol IV, 1999; On line http://www.cyto.purdue
17. Cruz-Chamorro L, Puertollano MA, Puertollano E, de Cienfuegos GA and de Pablo MA: *In vitro* biological activities of magainin 1 alone or in combination with nisin. Peptides 2006; 27:1201–1209.
18. Anghel R, Jitaru D, Bădescu L, Bădescu M, Ciocoiu M. The Cytotoxic Effect of Magainin II on the MDA-MB-231 and M14K Tumour Cell Lines. BioMed Research International 2013; 2013: 831709. doi:10.1155/2013/831709.
19. Witjes JA: Bladder carcinoma in situ in 2003: state of the art. European Urology 2004; 45:142–16.
20. Dobrzyńska I, Szachowicz-Petelska B, Sulkowski S and Figaszewski Z: Changes in electric charge and phospholipids composition in human colorectal cancer cells. Molecular and Cellular Biochemistry 2005; 276:113–119.
21. Yoon WH, Park HD, Lim K and Hwang BD: Effect of O-glycosylated mucin on invasion and metastasis of HM7 human colon cancer cells. Biochemical and Biophysical Research Communications 1996; 222: 694–699.
22. Cruciani RA, Barker JL, Zasloff M, Chen HC and Colamonici O: Antibiotic magainins exert cytolytic activity against transformed cell lines through channel formation. Proceedings of the National Academy of Sciences of the United States of America 1991; 88:3792–3796.
23. Eliassen LT, Haug BE, Berge G and Rekdal O: Enhanced antitumor activity of 15-residue bovine lactoferricin derivatives containing bulky aromatic amino acids and lipophilic N-terminal modifications. Journal of Peptide Science 2003; 9:510–517.
24. Yang N, Strøm MB, Mekonnen SM, Svendsen JS and Rekdal O: The effects of shortening lactoferrin derived peptides against tumour cells, bacteria and normal human cells. Journal of Peptide Science 2004; 10:37–46.
25. Hickman JA: Apoptosis induced by anticancer drugs, Cancer and Metastasis Reviews. 1992; 11:121–139.
26. Kerr JFR, Winterford CM and Harmon BV: Apoptosis: Its significance in cancer and cancer therapy. Cancer 1994; 73: 2013–2016.
27. Hu TJ, Wei X, Zhang X, Cheng FS, Shuai XH, Zhang L and Kang L: Protective effect of *Potentillaanserine* polysaccharide (PAP) on hydrogen peroxide induced apoptosis in murine splenic lymphocytes. Carbohydrate Polymers 2010; 79:356–361.
28. Cerón JM, Contreras-Moreno J, Puertollano E: The antimicrobial peptide cecropin A induces caspase-independent cell death in human promyelocytic leukemia cells. Peptides 2010; 31:1494–503.
29. Risso A, Zanetti M and Gennaro R: Cytotoxicity and apoptosis mediated by two peptides of innate immunity. Cellular Immunology 1998; 189:107–115.
30. Kumar N, Afeyan R, Kim HD and Lauffenburger DA: Multipathway model enables prediction of kinase inhibitor cross-talk effects on migration of Her2-overexpressing mammary epithelial cells. Molecular Pharmacology 2008; 73:1668–1678.
31. Shapiro GI and Harper JW: Anticancer drug targets: cell cycle and checkpoint control, Journal of Clinical Investigation 1999; 104:1645–1653

How to cite this article:
Arasu P, Ganesan N, Sivasubramanian S and Gunasekaran P: Antitumoral and Apoptotic Effects of Magainin II against Colo 320 DM Cancer Cell Line. Int J Pharm Sci Res 2016; 7(7): 2951-58.doi: 10.13040/IJPSR.0975-8232.7(7).2951-58.