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IN VITRO ASSESSMENT OF GARLIC EXTRACT AND PURIFIED ALLICIN AGAINST LIVER (HEPG2) AND COLORECTAL (HCT-116) CANCER CELL LINES

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ABSTRACT

Cancer is one of the major public burden worldwide. It is a multicellular disease that can arise from all cell type. In the recent decades, the number of cancer related showed a clear elevation, in turn creating huge health and economic problems. Garlic has been used as folk medicine approach for over 4000 years, and was the most widely researched medicinal plant. The present study showed that garlic extract and Allicin can induce inflammatory responses, inducing apoptosis. Liver and colorectal cancer cell lines were tested for the anti-cancer activity of test materials regarding the up-regulation of pro-apoptotic gene, Bax and P53, also anti-apoptosis gene, Bcl-2 down regulation. Toxicity of garlic extract and Allicin to HCT-116 was cell type and concentration dependent and the IC_{50} value recorded was 4.16µg/ml for Allicin and 16.6µg/ml for garlic extract. While it was 48 µg and 2.49µg/ml for HEPG-2 using garlic and Allicin it was respectively. Regarding the anticancer potential it was noted that pro and anti-apoptotic genes showed a remarked up and down regulation respectively compared to the genetic profile of untreated cell control. On the genetic level anti-apoptotic gene Bcl-2 and pro-apoptotic gen Bax and p53 were tested to prove the postulation that the garlic extract and Allicin can be used in dealing with cancer cells. In the meantime cellular DNA distribution showed cell arrest during the G2/ M phase of cell division.

KEYWORDS: Garlic, Allicin, Cancer cell lines, Apoptosis, cell cycle.

INTRODUCTION

Cancer is a group of more than two hundreds neoplastic diseases, all of which are caused by deregulation of multiple cell signaling pathways.[1] characterized by uncontrolled growth and spread of abnormal cells. If the spread of abnormal cells is not controlled, it can result in death.[2] Cancer involves genetics and epigenetic changes associated with molecular alterations involving certain types of genes, such as proto-oncogenes and tumor suppressor genes, as a result of genetic predisposition.[3] Various therapies have been used for treating cancer such as surgery, radiotherapy, chemotherapy, and hormone therapy.[4] But despite these therapeutic options, cancer remains associated with high mortality. Natural and some synthetic compounds can prevent, suppress, or reverse the progression of cancer.[5] Several anticancer agents are naturally produced by a wide range of different organisms including microorganisms, plants, and animals. These natural products have an effect on cellular signaling and gene expression.[6] Natural products have proven to be the most reliable single source of new and effective anticancer agents.[7] There is increasing evidence that garlic and compounds isolated from garlic have significant antiproliferative effects on human cancer cells. Much of this work has recently been reviewed by Pinto and Rivlin.[8] and Knowles and Milner.[9-10] The effects shown by garlic derivatives include induction of apoptosis, regulation of cell cycle progression and modification of pathways of signal transduction. Additionally, they reported that garlic derivatives appear to regulate nuclear factors associated with immune function and inflammation. Some of the pertinent reports will be summarized here. In 1997, Zheng and co-workers reported that the inhibitory effects of Allicin on proliferation of leukemia cells were associated with the cell cycle blockage at the S/G2M boundary phase and induction of apoptosis.[11] This effect was exhibited on neoplastic (leukemia) cells, but not nonneoplastic cells. In the last few years, a number of reports have appeared concerning the antiproliferative effects of several compounds derived from garlic. Hong and co-workers studied the effects of DAS, DADS and garlic extract on p53-wild type H460 and p53-null type H1299 non-small cell lung cancer (NSCLC) cells.[12]

They reported that DAS or DADS treatment, but not garlic treatment, of both cell types resulted in the highest number of cells in an apoptotic state. DADS was found to be more effective in inducing apoptosis in NSCLC cells. In H460 cells, the level of p53 protein, which is involved in the activation of apoptosis by DNA damage, was increased following DADS treatment. DAS and garlic treatment of H460 cells induced a rise in the level of Bax (a cell death agonist) and a fall in the levels of Bcl-2 (a cell death antagonist). It is well known that p53 activates the transcription of Bax and represses the expression of Bcl-2.[13,14] Thus, this study demonstrated that DAS, DADS and garlic extract are effective in reduction of an antiproliferative gene in NSCLC and suggested that modulation of apoptosis-associated cellular proteins by DAS, DADS and garlic extract may be the mechanism for induction of apoptosis.

MATERIALS AND METHODS

Garlic was obtained from the Egyptian market and was thermally dried in oven at 60oC for two days and grinded into a fine powder .powder was suspended in 1:1 water-ethanol mix for extraction.

Allicin 95%was kindly supplied from the national organization for drug control and research (NODCAR).

Cancer Cell Line

Human Hepatocellular carcinoma (HepG2) ATCC®HB-8065 and Human colorectal cancer (HCT-116) ATCC®HTB-8065 cell lines were supplied from Research and Development Sector, The Holding Company for production of Vaccines, Sera and Drugs (VACSERA), Cairo, EGYPT. Cells were grown in RPMI-1640 containing L-glutamine, non-essential amino acids. Sodium bicarbonate Sodium Pyruvate, 10% fetal bovine serum (FBS) and antibiotics (100U/mL penicillin and 100 mg/mL streptomycin) at 37°C in a humidified atmosphere of 5% CO2.

Preparation of garlic extract

Dried garlic (10 g) was grinded and soaked in 25mL mixture of distilled water and ethanol (1:1 v / v) and left for 72 hrs. The extract was cold centrifuged at 10000 rpm (Jouan , Ki-22 France) and supernatant was filtered through 0.2 µm Millipore filter||| –disc (Millipore-USA).[15]

Maintenance of cells was carried as follows

Cells were grown at 37°C in 5% CO2 incubator until 90% confluent, then cells, were removed from flask by trypsinization as the medium was aspirated from the culture flask. Cells were washed by gentle agitation to remove any remaining serum that might inhibit the action of the trypsin and the washing solution was discarded and the cell monolayer was trypsinized using 0.25% trypsin-EDTA solution. Complete growth medium with 10% FBS to the flask and the cells were dispersed by pipetting up and down to obtain single cell suspension .Then, 1mL of cell suspension was removed for total cell count and viability. Cells were centrifuged at approximately 1800 rpm for 5 min to 10 min at 4°C. The medium was removed and pellet was re-suspended in 9 mL complete growth medium and cell suspension was transferred into new pre-labeled flask as 2x105 viable cells/ml.

Cytotoxicity

MTT assay is a sensitive, quantitative and reliable colorimetric method that measures viability of cells. The assay is based on the ability of mitochondrial lactate dehydrogenase enzyme (LDH) in living cells to convert the water soluble substrate 3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyltetrazolium bromide (MTT) into a dark blue formazan which is water insoluble. Solubilization solution (dimethyl sulfoxide) was added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring it using spectrophotometer at a wave length usually 570 nm.[16] The cytotoxicity of Allicin and garlic extract was performed on two cancer cell lines, HepG2,HCT116 cell lines, and one normal cell line ,Vero cell. The cell survival was evaluated according to,[17] as the cell suspention (2 x105 cells/ml) was dispensed as 100µl in a 96-well plate and the plates were incubated for 24 hours at 37oC in humidified air atmosphere enriched with 5% Co2. The growth medium was removed and 100µl of fresh media was added to each well of the plate except the first well of first six rows. 200 µl of Allicin (1000µg/ml) were added to first column. Test extract was two-fold serially diluted. Negative cell control was considered. The plates were incubated at 37oC for 24-48 hours.

The treatment medium was decanted and cells were washed three times using phosphate buffer saline (PBS). MTT (5mg/mL in PBS) solution was added to each well as 50µl /well. The plates were incubated at 37oC in a humidified air atmosphere enriched with 5% Co2 for 3-4 hours. The resulting MTT formazan complex was dissolved by addition of 50µl of DMSO. The "absorbance was measured at 570 nm using ELISA plate reader (Biotek-Elx-800-USA). Cytotoxicity assay was performed in triplicate and the percentage of cell survival was calculated using following formula:

%cell Survival = [Mean Optical Density of Test /Mean Optical Density] x 100

The mean percentage cell survival was plotted against the corresponding concentration of Allicin or garlic and the IC50 (50%inhibitory concentration) value was determined using Masterplex-2010 reader program.

Histopathology

HepG2 and HCT-116 cell lines, were treated using the calculated IC50 concentration of test extracts (Allicin and garlic) for 24 h. Cisplatin (1 mg/ml) as positive control was included and untreated flask was kept as negative control. Detached cells were harvested and the remaining adhered cells were collected by trypsinization

and both were cold centrifuged for 10 minutes at 1800 rpm. Pellets were re-suspended in 200µl medium, 25µl of suspended cells were dispensed on clean glass slide and spread as blood film, dried and fixed using 70% methanol.

Staining of slide

Each slide was stained with Hematoxylin and Eosin dye and the slide were left for 24 h. After 24 h each slides were examined under light microscope (Leica-Germany) Microsystems Wetzlar GmbH type DMLB2 with software Leica Qwin 500, Germany.

Quantitative Real Time RT PCR (qRT-PCR)

Anticancer activity of Allicin and garlic extract compared to cisplatin was investigated through the expression of Bax, P53 and BCL2 genes using real time RT-PCR.

HepG2 and HCT-116 cell lines were treated using the calculated IC50 concentration of test extracts (Allicin and garlic) for 24 h post decanting of growth media. Cisplatin (1mg/ml) as positive control was included and untreated flask was kept as negative control. Detached cells and residual adhered cells were collected and prepared for rt-PCR and cell cycle analysis.

Cell cycle analysis

HCT-116 and HEPG-2 cancer cell lines in 25 Cm2 cell culture flasks were treated with the IC50 values of test extracts dissolved in RPMI-1640 medium, for 24h. For cell cycle analyses, the cells were harvested and fixed gently with 70% (v/v) ethanol in PBS, maintained at a temperature of 4˚C overnight and then re-suspended in PBS containing 40 µg/ml PI and 0.1 mg/ml RNase and 0.1% (v/v) Triton X-100 in a dark room. After 30 min at 37˚C, the cells were analyzed using a flow-cytometer (Becton-Dickinson, San Jose, CA, USA) equipped with an argon ion laser at a wavelength of 488 nm. The cell cycle and sub-G1 group were determined and analyzed, as described previously.[18]

mRNA Expression levels of cell apoptosis-related genes

Total RNA was extracted from control and treated HCT-116 and HEPG-2 cancer cell lines using GeneJET RNA Purification kit (Fermantus-UK) according to the manufacturer's protocol. The concentration and the integrity of RNA were assessed spectrophotometrically at 260/280 nm ratio and by gel electrophoresis, respectively. First-strand cDNA was synthesized with 1 μg of total RNA using a Quantitect Reverse Transcription kit (Qiagen, Germany) in accordance with the manufacturer's instructions. These samples were subsequently frozen at a temperature of -80∘C until use for determination of the expression levels of P53, Bax and Bcl-2 genes using real-time PCR. Quantitative real-time PCR was performed on a Rotor-Gene Q cycler (Qiagen, Germany) using QuantiTect SYBR Green PCR kits (Qiagen, Germany) and forward and reverse primers for each gene. The nucleic acid sequences of the primers were as follows:P53(F: 5'-TCA GAT CCT AGC GTC GAG CCC-3' & R: 5'-GGG TGT GGA ATC AAC CCA CAG-3') &Bax(F: 5'-ATG GAC GGG TCC GGG GAG CA-3' & R: 5'-CCC AGT TGA AGT TGC CGT CA-3') as well as anti-apoptotic gene Bcl-2 (F: 5'-GTG AAC TGG GGG AGG ATT GT-3' & R: 5'-GGA GAA ATC AAA CAG AGG CC-3') compared to β-actin as a housekeeping gene (F: 5'-CAA GGT CAT CCA TGA CAA CTT TG-3' & R: 5'-GTC CAC CAC CCT GTT GCT GTA G-3'). Real-time PCR mixture consisted of 12.5 μL 2x SYBR Green PCR Master Mix, 1 μL of each primer (10 pmol/μL), 2 μL cDNA, and 8.5 μL Rnase-free water in a total volume of 25 μL. Amplification conditions and cycle counts were at temperature of 95∘ C for 15 min for the initial activation, followed by 40 cycles of denaturation at 94∘ C for 15 s, annealing at 60∘ C for 30 s, and elongation at 72∘ C for 30 s. Melting curves were performed after real-time PCR to demonstrate the specific amplification of single products of interest. A standard curve assay was performed to determine the amplification efficiency of the primers used. Relative fold changes in the expression of target genes (P53, Bax and Bcl-2) were accomplished using the comparative $2^{-\Delta\Delta Ct}$ method,[22] with the β-actin gene as an internal control to normalize the level of target gene expression. ΔΔCT is the difference between the mean ΔCT (treatment group) and mean ΔCT (control group), where ΔCT is the difference between the mean CT gene of interest and the mean CT internal control gene in each sample. Logarithmic transformation was performed on fold change values before being statistically analyzed, using the fold change values of three replicates for each gene measured.

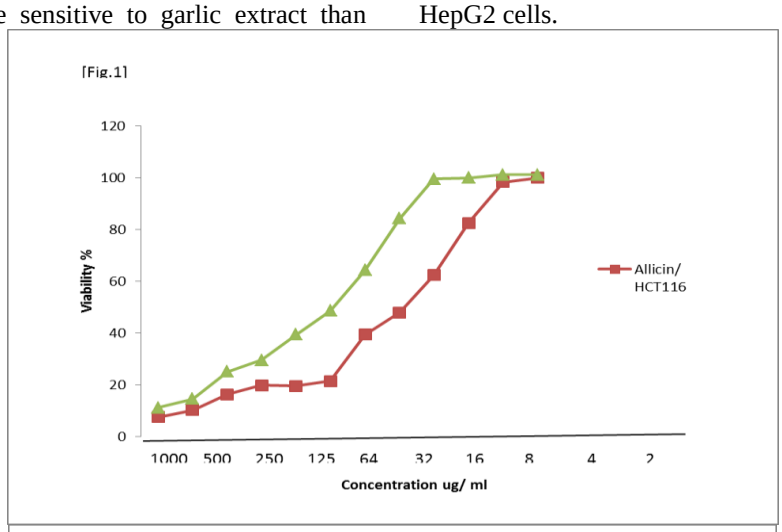
Statistical analysis

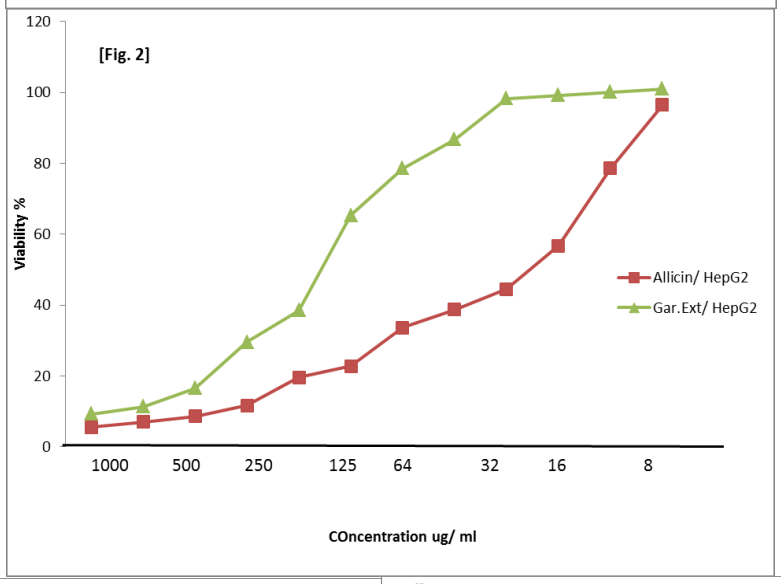
All experiments were carried out in three independent tests. Data were expressed as the mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA).The results were considered statistically significant at probability< 0.05.

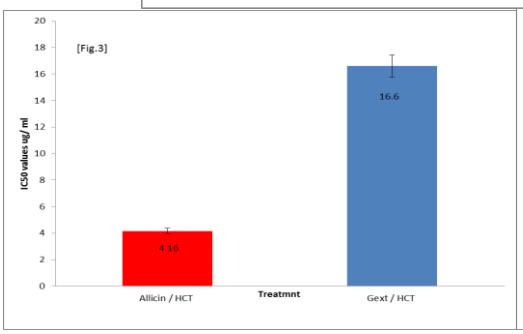
RESULT

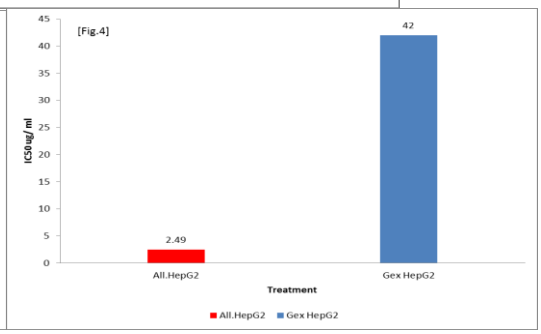
Allicin as a purified garlic derivative and crowed garlic extract were tested for their in Vitro cytotoxicity against colorectal and hepatic cancer cell lines. Data revealed that cytotoxicity was concentration and cell type dependent, as lower concentration enhanced the higher viability profile, oppositely the higher doses significantly decreased viability [Fig.1-2]. In the meantime the related IC50 values were cell type and test product dependent and Allicin showed a significantly decreased values ($p < 0.01$) than those induced post cell treatment with garlic extract recording 2.49 and 4.16 ug/ml for HCT-116 and HepG2 respectively [Fig.3-4], Also, the IC50 value of garlic extract was significantly effective on HCT than HEPG2 recording 16.6µgm/ml and 42µgm/ml post HCT-116 and HepG2 cell treatment respectively ($p < 0.01$) [**Error! Reference source not found.**. That mean HCT-

16 cell line was more sensitive to garlic extract than HepG2 cells.

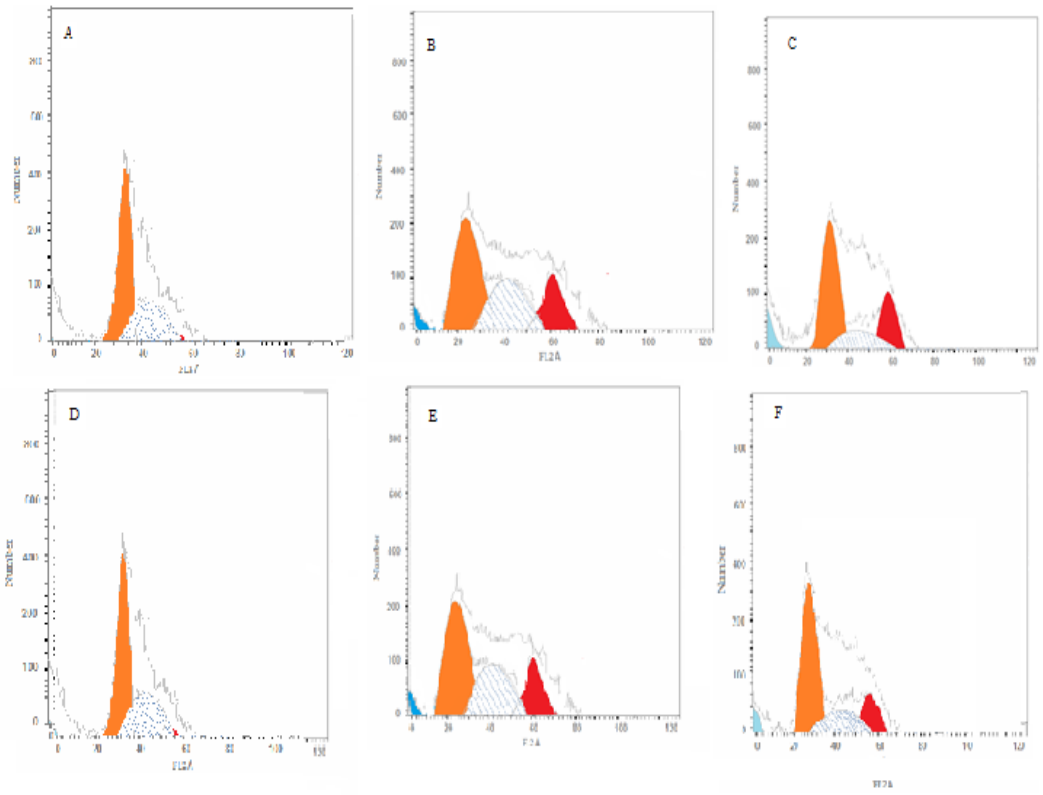




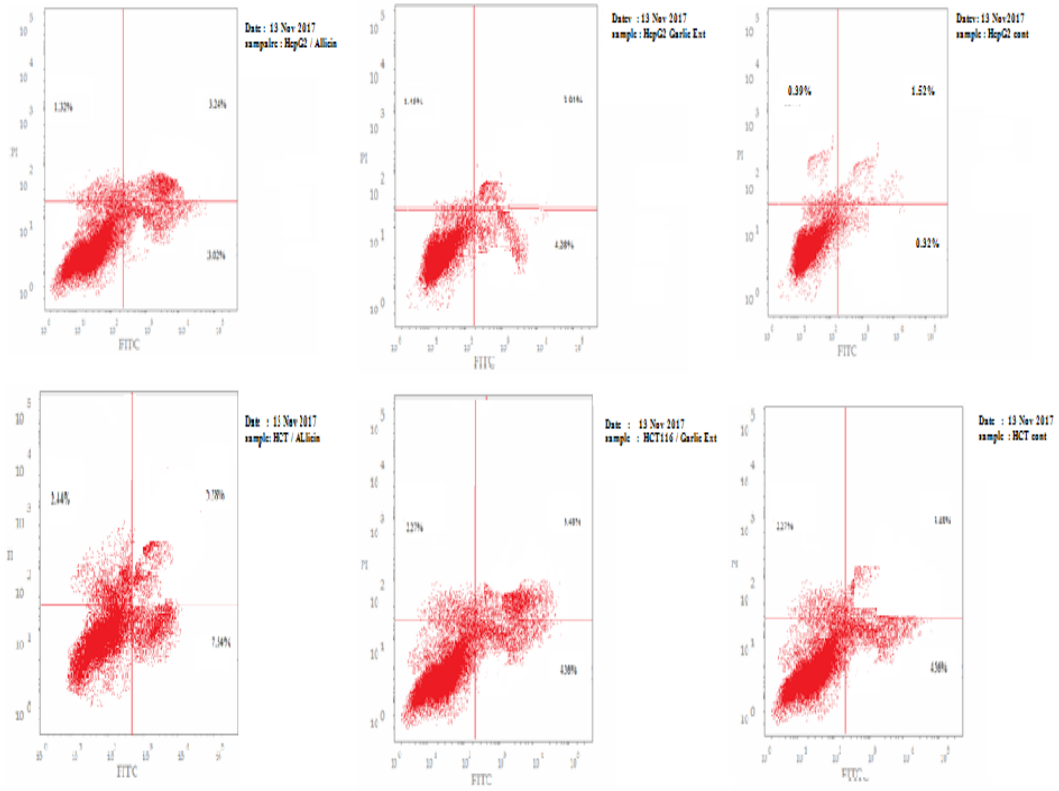




The apoptotic genes profile was traced, where pro-apoptotic genes; P53 and Bax were significantly elevated ($P<0.05$) compared with its values in non-treated cell control, Also, it was noticed that up regulation of pro-apoptotic genes was cell type and extract dependent ,as Allicin was significantly enhancer to up regulation than garlic extract and cisplatin in test cell lines ($P<0.05$). In the meantime the antiapoptotic gene (Bcl-2) was significantly down regulated post cell treatment with garlic extract and Allicin. In the same way Allicin showed higher level of down regulation post cell treatment and garlic extract as well [Fig. 4-6 A-B].

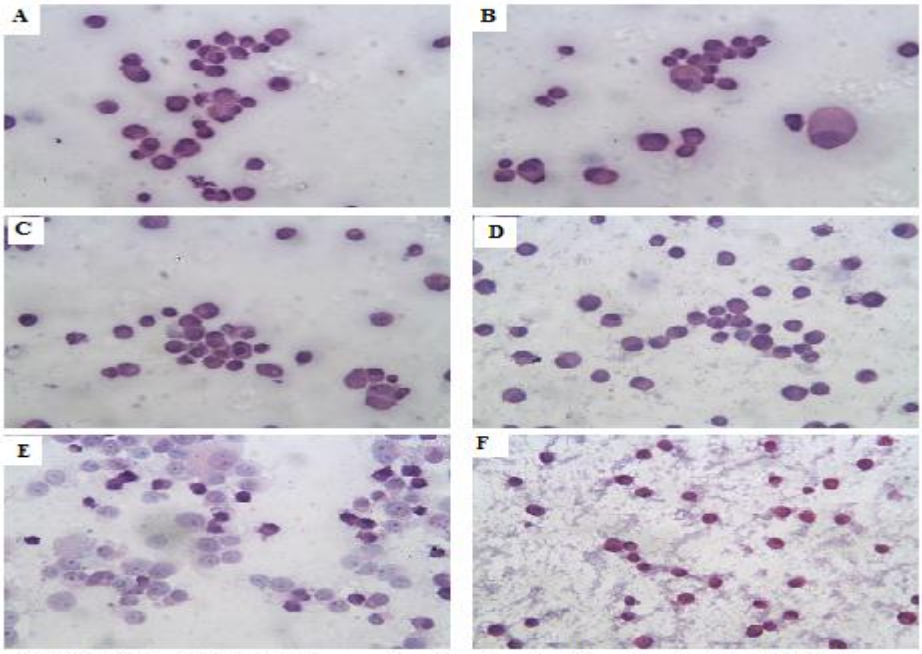


[A] HepG2 cont , [B] HepG2 / Garlic extract, [C] HepG2 / Allicin ,[D] HCT Control , [E] HCT / GaRlic extract and [F] HCT/ Allicin



Histopathological alterations detected in cell lines post treatment with Allicin and garlic extract compared with negative control were grown firmly adherent, fully stretched, with intact membrane, uniform cytoplasmic distribution, polymorphism, hyperchromatism and karyomegaly. On the contrary, cisplatin, garlic extract

and Allicin-treated HEPG- and HCT-116 cells showed a variable morphology recording rounding, cell membrane blebbing, decreased karyomegaly, hyperchromatism and polymorphism. Reduced intercellular junction and cell shrinkage were also observed. Most cells were detached; chromatins were condensed, compacted and split into clumps and typical apoptotic characteristics such as apoptotic bodies emerged [**Error! Reference source not found.**]. Cells were fully stretched, with intact membrane, uniform cytoplasmic distribution, differentiation in cell size (polymorphism), dark nucleus (hyperchromatism) and large nuclei (karyomegaly) [**Error! Reference source not found.**]. Adjacent cells were grown and fused into pieces. In comparison, garlic extract and Allicin-treated HepG2 cells [**Error! Reference source not found.**] were rounded, cell membrane and nucleolar fragmentation, decreased karyomegaly, hyperchromatism and polymorphism. Reduced intercellular junction and cell shrinkage were also observed.



[Fig.7] **Histopathological changes developed post cell treatment with both Allicin and Garlic extract using H&E stain**

Regarding the apoptotic genes profile it was noticed that pro-apoptotic gene Bax was significantly elevated (P<0.05) compared with its value in nontreated cell control, while on the contrary there was a significant down regulation of P53 gene compared with that of cell control and its down regulation was more in case of HEPG2 than HCT-116. Also, it was noticed that up regulation of pro-apoptotic gene were cell type and extract dependent, as Allicin was significantly enhancer to up regulation than garlic extract and cisplatin in the test cell lines (P<0.05). In the mean time the antiapoptotic gene(Bcl-2) was significantly down regulated post cell treatment with garlic extract and Allicin than in case of treatment with cisplatin (P<0.05), in the same way Allicin showed higher level of down regulation post cell treatment and garlic extract as well.

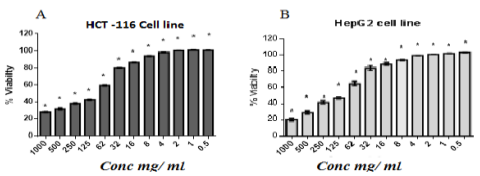


Figure .1 Cytotoxicity of garlic exrtract treated cells by MTT assay

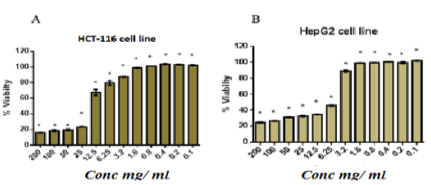


Figure. 2 Cytotoxicity of Allicin treaterd cells by MTT assay

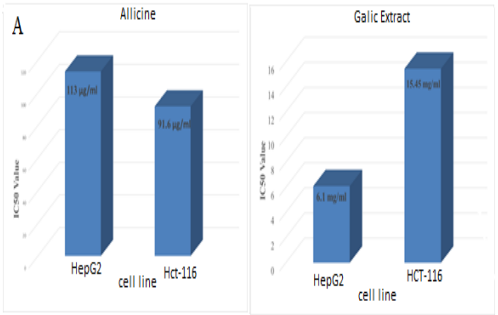


Figure 3 Comparative evaluation of IC50 values of Allicin and garlic extract to HepG2 and HCT-116 cell lines using MTT assay

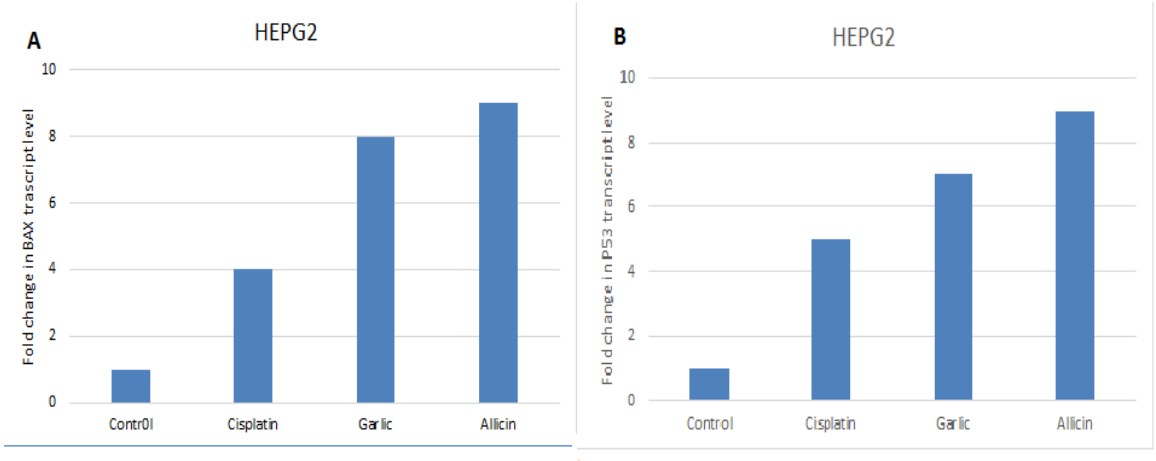


Figure 4 Quantitative determination of regulatory gene expression upon Allicin and Garlic extract treatment

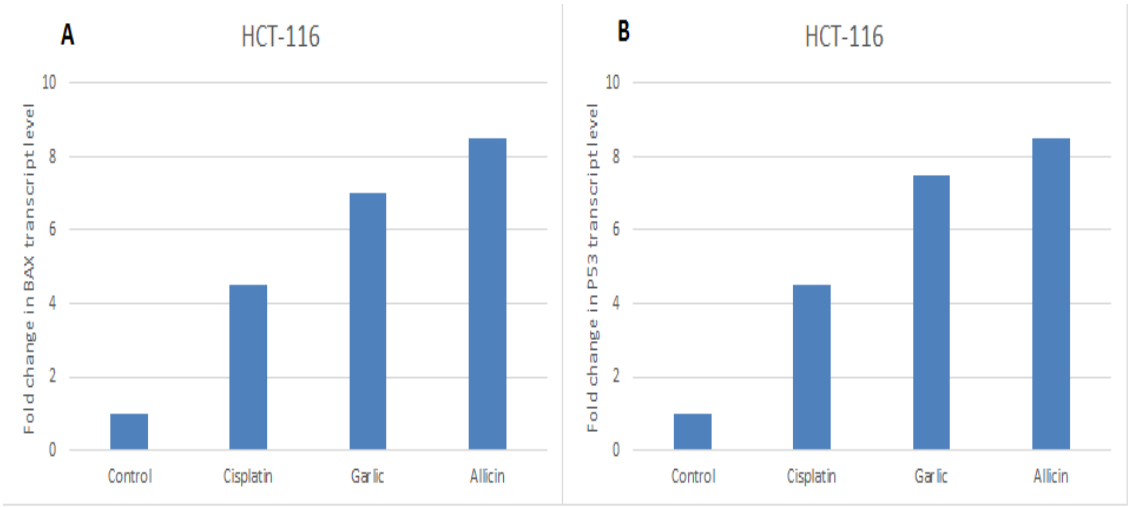


Figure 5 Effect of Allicin and Garlic extract in intinsic apoptosis-related gene and tumor suppression gene expression

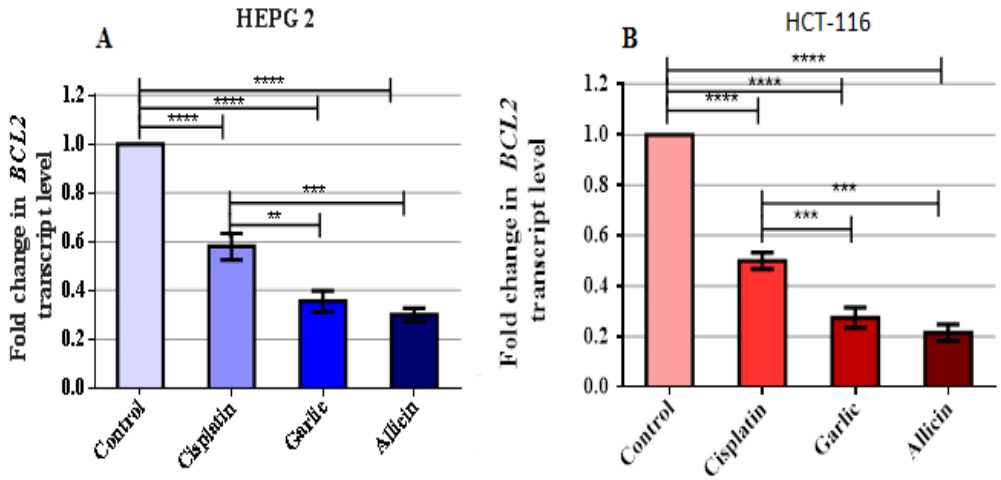


Figure 6 *Anti* Apoptosis-related gene expression *in* Hepg2 and HCT-116cells treated by Allicin and garlic extract

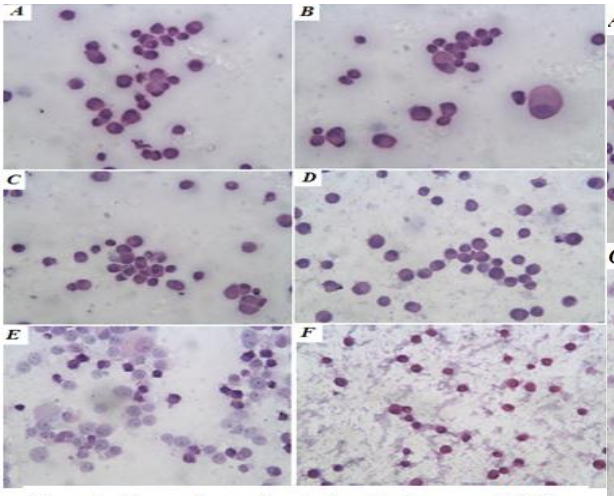


Figure 7. The nuclear and cytoplasmic changes of HCT-116 cells detected by Hematoxylin and Eosin method (x 400, magnification)HCT-116 cells control showing (A) cellular polymorphism, (B) hyperchromatism&(C) karyomegaly. HCT-116 treated cells undergoing apoptosis showing (D) Less cellular polymorphism, no hyperchromatism and less karyomegaly were found in Allicin-treated MCF-7 cells, E) Cell membrane blebbing was detected in cisplatin-treated HCT-116 cells, F) no hyperchromatism, less karyomegaly, no polymorphism and cell shrinkage (small in size, uniform size, shape) were found in. garlic-treated HCT-116cells

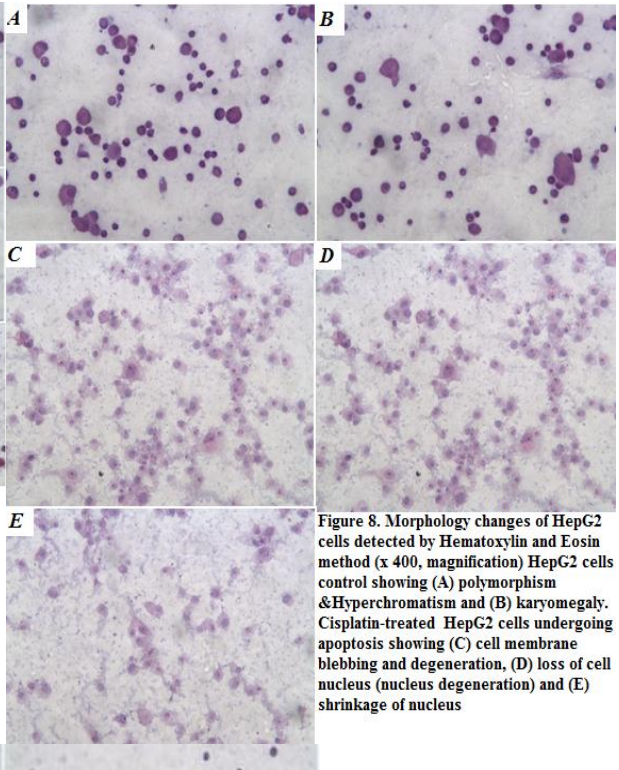


Figure 8. Morphology changes of HepG2 cells detected by Hematoxylin and Eosin method (x 400, magnification) HepG2 cells control showing (A) polymorphism &Hyperchromatism and (B) karyomegaly. Cisplatin-treated HepG2 cells undergoing apoptosis showing (C) cell membrane blebbing and degeneration, (D) loss of cell nucleus (nucleus degeneration) and (E) shrinkage of nucleus

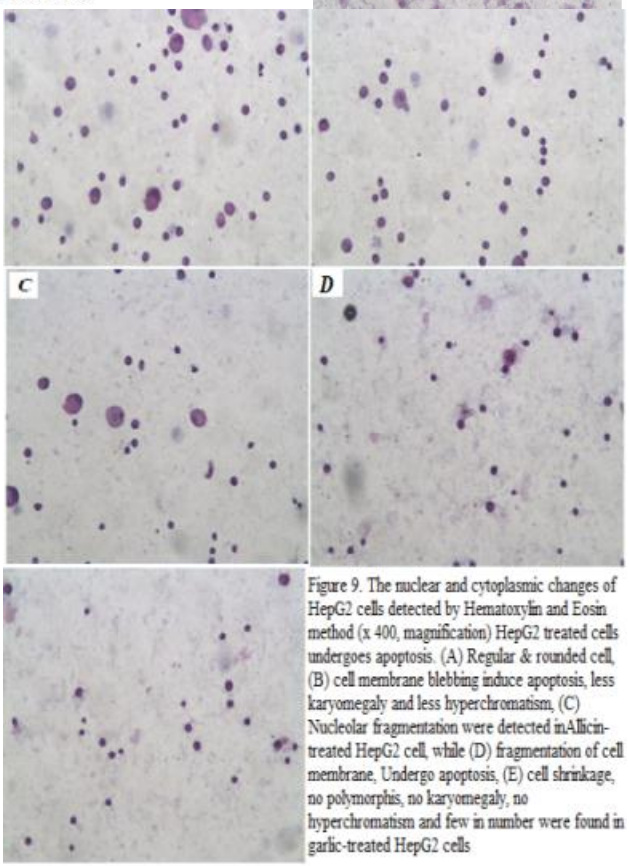


Figure 9. The nuclear and cytoplasmic changes of HepG2 cells detected by Hematoxylin and Eosin method (x 400, magnification) HepG2 treated cells undergoes apoptosis. (A) Regular & rounded cell, (B) cell membrane blebbing induce apoptosis, less karyomegaly and less hyperchromatism, (C) Nucleolar fragmentation were detected inAllicin-treated HepG2 cell, while (D) fragmentation of cell membrane, Undergo apoptosis, (E) cell shrinkage, no polymorphis, no karyomegaly, no hyperchromatism and few in number were found in garlic-treated HepG2 cells

DISCUSSION

Garlic has been used worldwide as a folk medicine. It contains alliin [(1)-S-allyl-L-cysteine sulfoxide] as a major sulfur-containing compound. When the raw garlic clove is damaged, alliin is hydrolyzed to sulfenate, pyruvate, and ammonia by alliinase. Condensation of 2 mol of sulfenate gives allicin (diallyl thiosulfinate), Allicin, however, is rapidly converted to diallyl disulfide (DADS) and other sulfides because of its instability.[19] Garlic also contains S-propylcysteinesulfoxide (PCSO) and S-methylcysteine-sulfoxide (MCSO). PCSO can generate over 50 compounds depending on the temperature as well as the water content.[20] DADS are more effective than water-soluble compounds in

suppressing breast cancer. Mechanisms of action include the activation of metabolizing enzymes that detoxify carcinogens, the suppression of DNA adduct formation, the inhibition of the production of reactive oxygen species, the regulation of cell-cycle arrest and the induction of apoptosis. DADS synergizes the effect of eicosapentaenoic acid, a breast cancer suppressor, and antagonizes the effect of linoleic acid, a breast cancer enhancer. Moreover, garlic extract reduces the side effects caused by anti-cancer agents. Thus, garlic and garlic-derived compounds are promising candidates for breast cancer control.[21] The results recorded assured that Allicin and garlic extract can suppress the growth of cancer cells in vitro. The immortal cell line obtained from human Hepatocellular carcinoma (HepG2 cells) was largely utilized in the present hepatic and colon centration of Allicin and ethanolic cancer research. HepG2 and HCT-116 cells were initially treated with different conaqueous extract of garlic using MTT assay; the rate of cell viability were inhibited by Allicin and garlic extract in a dose-dependent manner, thus suggesting that tumor growth was significantly inhibited. The present study focused on apoptosis molecular pattern using gene expression quantification of pro-apoptotic and anti-apoptotic genes in HepG2 and HCT-116 cell lines. The records of pro-apoptotic and anti-apoptotic genes was in accordance with.[22] reporting that coherent analysis done on the administration of Alliums and stomach tumor on the studies from 1966 to2010.the results revealed that the reduction in the risk of cancer in stomach recognized to be correlated with elevated administration of garlic ; odds ratio 0.53 and 95% confidence intervals, 0.40-0.65. In the same times,[32] recorded that in another meta-analysis in European prospective investigation into cancer and nutrition (EPIC) analysis, there was a reciprocal relation between total intake of Alliums including onion and garlic, and probability of occurrence of tumor in intestine and stomach (24) also reviewed the researches, which studied the influence of garlic on colorectal cancer. The results of one of the reviewed researches revealed that the extent and quantity of colorectal adenoma were significantly down regulated (29%) with the consumption of aged garlic extract. Moreover,[25] examined the impact of SAMC on the cell proliferation and apoptotic cell death on human stomach cancer cells line (SGC 7901). Various doses of SAMC were treated on the SGC 7901 cell line. The cytotoxicity was measured by AO/EB staining. JNK(c-Jun N-terminal kinases) and p38 signaling were examined using PCR. The result showed that SAMC could repress growth and induce apoptotic cell death of SGC 7901 cells through JNK and p38 signaling. Concurrently,[26] studied the impact of Allicin on the proliferation of HepG2 cells and the study based mainly on the stimulation of autophgic cell death by Allicin on HepG2 cells. The results showed that Allicin stimulated p53-mediated autophagy and suppressed the proliferation of HepG2. Recently.[27] examined the putative impact of Allicin on apoptosis of the human colon cancer cell line HT29, in vitro. HYT29 was treatreed with various doses of Allicin (0-40µg/ml) to determine its impact on cell viability. Results revealed that doses > 5µg/ml significantly elevated the apoptotic characteristics at 6-24h using the tunnel assay. Regarding the viability assessment techniques MTT assays, cell cycle analysis, cologenic invasion, and apoptosis assays were carried to investigate the impact of SAC on the proliferation and metastasis of hepatocellular carcinoma- metastatic cell line MHCC97L. The beneficial effects of SAC alone and in combination with cisplatin were investigated using orthotopic xeno graft model, in vivo. The result indicated that SAC repressed the viability and cologenic survival of MHCC97L cells correlated with repression of growth indicators including proliferating cell nuclear antigen (PCNA) and Ki-67.SAC also significantly down regulated anti-apoptotic BCl-2 and BCL-XL and up-regulated caspase-9 expression. In addition, SAC prevented the motility and penetration of MHCC97L cells associated with elevation of E-cadherin and decrease of expression of VEGF. Moreover, SAC significantly stimulated apoptotic cell death in concentration –dependent manner and raised the number of cells in S-phase leading to cell cycle arrest and decrease entry of cell to G2/M phase. SAC also suppressed the cell cycle inducer protein including cyclin B1, cdc25c (cycle division cycle 25c) and cdc2. Finally, the result of xenograft model showed that SAC alone or in combination with cisplatin constrained the development and spreading of hepatic cancer to the lung.[28] In addition,[29] recorded that Allicin triggered apoptotic cell death of SGC-7901 cells via induction of BCL2 regulated and death receptor (FAS/FASL) signaling and suppressed cell viability.[30] Also, recorded that different concentration of DADS reduced the proliferation and stimulated apoptotic cell death of stomach cancer AGS cell lines in a concentration – dependent manner. DADS also elevated levels of BAX, CASAPASE-3 and FAS, and regulated BCL-2 level. Regarding the impact of SAMC on the growth of human gastric cancer SNU-1 cells, there was a clear suppression of viability of SNU-1 cells in dose-dependent with the appearance of many apoptotic characteristics such as DNA degradation and arise in the sub-G1 cell content . The results indicated that the induction of apoptosis by SAMC treatment might be correlated to the activation of caspase-3 via the induction of Bax and P53.[31-32] Also reported that Allicin (6.2-310µM) induced the cytotoxicity of colorectal carcinoma cell lines such as HT-29, HCT-116,Caco-2 and LS174T in concentration and time dependent manner. Allicin at concentration of 26µm induced cytotoxicity of HCT-116 and apoptotic cell death through a process correlated with trans-activation of the transcription factor Nrf2 (nuclear factor – erythroid 2- related factor 2). Regarding the role of antioxidation that related to anticancer activity, it was reported by,[33-34] that DADS at concentration of 200µM suppressed the viability of HCT-116 cells by stimulation of reactive oxygen species causing cell cycle arrest in G2/M phase.[35] and also showed that DATS presented the highest effect including cytotoxicity and stimulation

of apoptosis through caspase-3 activation on HepG2 cell compared to DAS and DADS. DADS are more effective than water-soluble compounds in suppressing breast cancer. Mechanisms of action include the activation of metabolizing enzymes that detoxify carcinogens, the suppression of DNA adduct formation, the inhibition of the production of reactive oxygen species, the regulation of cell-cycle arrest and the induction of apoptosis. DADS synergizes the effect of eicosapentaenoic acid, a breast cancer suppressor, and antagonizes the effect of linoleic acid, a breast cancer enhancer. Moreover, garlic extract reduces the side effects caused by anti-cancer agents. Thus, garlic and garlic-derived compounds are promising candidates for breast cancer control.[18] DADS also affects cell proliferation activity and viability and induces apoptosis. Temporary activation of MAPKs in HepG2 cells and phospho-p38 and phospho-p42/44 regulated cell apoptosis were reported.[32-33] Despite garlic extract wa used in iuts crowed form and proved a potential against different cancer cell lines it was reported in china by Ban et al 2007. that thiacremonone, another component found naturally in garlic as organ-sulfur c ompound was able to induce apoptosis in HCT-116 ,cancer cell line as well, in addition to another line SW60. Apoptosis was induced in cancer cells by suppressing the anti-apoptotic gene; Bcl-2 gene, while inducing the pro-apoptotic gene; Bax gene.[34] Also, it was proved by.[35] that diallylsulphide, a component found in garlic, has the ability to arrest the cell cycle at G2 phase, alongside inducing apoptosis in colon cancer cell. While another study carried out by.[36] showed that aged garlic extract (AGE) inhibited the invasive action of colon cancer in SW620 and SW480 cancer cell lines. Matsuura who is this later stated that the action of AGE depended on the type of the cancer cell line. Due to their study they were able to observe that garlic extract improve endothelial cells, adhesion while inhibiting motility and invasion, resulting in AgE's ability to inhibit tumor formation by preventing angiogenesis. This led to the conclusion that garlic extract could be used as an anti-cancer agent for colorectal carcinoma.[37] A randomized clinical trial carried out in 2006 exhibited a significant reduction in both the size and the number of adenomas present in 51 patients diagnosed with colorectal carcinoma. Tanaka and Colleagues, the people responsible for this trial, ensured that the subjects in the trial were treated with garlic extract for a period of 12 months, using different concentration to determine the most effective dose. They reached the conclusion that the a high-dosage treatment with aged garlic extract decreased the adenomas' size profoundly, aiding in the presumption that garlic extract has components that by a number of pathways are able to suppress cancer growth and proliferation thus decreasing the said cancer's incidence rate.[37] An additional approach that was performed in 2003 by Sengupta, et al, as to the study effects of garlic in vivo in Sprague –Dawley rates. The study showed that even oral intake of garlic in addition to treatment with azoxymethane resulted in a decrease in colon cancer cells. Azoxymethane has been a known carcinogen that has significant effect on colon carcinoma.[38] Later in 2005 Sengupta et al carried out another test that led to the postulation that garlic extract has the ability to modulate and adjust azoxymethane induced carcinogenesis. This test was carried out on Swiss albino mice, and it was observed that the mice oxide synthase expression was elevated, leading to the apoptosis of colon cancer cells.[39] Another study by Nagini in 2008 presented remarkable beneficial effect of garlic and its derivatives had on numerous carcinogenesis mechanisms, which include eradicating free radicals, angiogenesis, mutagenesis, cell differentiation and cell proliferation.[40] Different allium derivatives were found by Howard et al in 2007 to have an 90% inhibition rate of metastasis of prostate cancers. The study was performed in vivo on mice infected with prostate cancer, and S-allyll mercaptocysteine was injected into them resulting in the prevention of metastasis of the cancer to the adrenal glands and lungs.[40] Other studies were conducted to use garlic as preventative, rather than a treatment. In a study carried out by,[41] a comparative analysis was carried out on the effect of garlic in preventing multiple forms of tumors malignancies. Primitive cell lines were cultured, and compared to control cell lines. The effectiveness was compared using multi-dimensional techniques such as two dimensional or three dimensional conformal modules. It was concluded that, the components of garlic was able to inhibit the proliferation of the cells, hence proving the capabilities of its preventative nature. Allicin exerted a time-and dose-dependent cytostatic effect on colon cancer cell lines HCT-116, LS174T, HT-29, and Caco-2 at concentrations ranging from 6.2 to 310 µM .While the Allicin (62 µM) exerts cytotoxic effects on HCT-116 and induces apoptosis via a mechanism associated with transactivation of the transcription factor Nrf2.[28] When compared to the oil-soluble garlic extract DADS had pronounced effect in HepG2 cells.[42] Water-soluble extracts induced p53/p21-dependent cell cycle arrest in G2/M phase and apoptosis by the activation of c-Jun-NH(2) terminal kinase (JNK)/c-Jun phosphorylative cascade. Also DADS/DATS were effective in GSTP expression mediated through JNK-AP-1 and ERK-AP-1 signaling inducing phase II detoxification system.[43] antigenotoxic potential of purified garlic compounds like Allicin, DAS, DADS, S-allyl cysteine (SAC) and allylmercaptan (AM) in the human hepatoma cell line HepG2and found to protect human hepatic cells against the genotoxicity induced by indirect-and direct-acting genotoxic agents primarily by the inhibition of CYP enzymes and induction of phase II enzymes.[43]

It has been reported that treatment of pancreatic cancer cells by H2S has been shown to induce apoptosis, resulting from the activation of caspase 3, decreased protein level of Bcl-2 and activation of Bax expression.[44] As Bax is known to induce mitochondria-driven apoptosis. it found that activation of cystathione-γ-lyase CSE and increased H2S levels by S-propargyl-

cysteine SPRC treatment were coupled with elevated p53 and Bax expressions in gastric cells and tumors. These results suggested a novel mechanism for the anticancer effects of SPRC via CSE/H2S-induced cell growth inhibition and apoptosis through the p53/Bax pathway.[45] As a conclusion, this study shows that the garlic extract and Allicin induces inflammatory responses, inducing apoptosis in HCT-116 and HEPG2 cancer cell lines through the up-regulation of pro-apoptotic gene, Bax and p53 all and down-regulation of anti-apoptosis gene, Bcl-2. As seen in the studies stated above, a large number of garlic components are believed to play a role in inhibiting cancer proliferation, of a number of cancer cell lines. Therefore, a better understanding of the pathways of the organo-sulfurs found as components of garlic are crucial in the advancement of garlic usage as drugs.

CONCLUSION

So, it can be concluded the active potential of garlic crowd and purified Allicin derivative to be active agents against cancer. Toxicity of both Allicin and garlic extract can induce cell arrest during the G2/M phase. Also, garlic extract and Allicin can suppress carcinogenesis via up-regulation of antiapoptotic genes and down regulation of antiapoptotic genes, in addition it may enhance the angiogenesis as well. Apoptotic effect of test derivatives could be proved via the detected histopathological alteration detected

RECOMMENDATIONS

It is recommended that a higher rate of fractionation of garlic extracts for more active derivatives detection followed by evaluation as anticancer and antimicrobial. A preclinical studies must be started to massive investigation of applicability of these extracts in human cases

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