International Journal of Scientific Research and Management
(IJSRM) ||Volume||5||Issue||03||Pages||5242-5252||2017||
Website: www.ijsrm.in ISSN (e): 2321-3418

Comparative Evaluation Of Anticancer Potential Of Moringaoleifera, Ganodermalucidumand Silver Nanoparticles Against Breast And Liver Cancer Cell Lines And Related Pro And Anti Apoptotic Genes Profile

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Abstract

The present work aimed to evaluate the anticancer potentials concerning the cytotoxicity and anti-proliferative activity of L-amino acid Oxidase (LAAO) ,MoringaOleifera(MO) Methanol (MLM) and water extracts (MLW), Ganodermalucidum(GL), extracts as a natural products and Silver nanoparticles (AgNP) as well. Cytotoxic effect of test extracts on both breast (MCF-7) and liver(HEPG-2) cancer cell lines varied according to test materials and could be arranged in the order of LAAO > MLM>AgNP> GL>MLW. Also, (MCF7) cell line was more sensitive to test materials compared with (HEPG2) cell line. Regarding the genotoxicity it was found that Bcl2 and survivineand caspase -3 as antiapoptotic genes were significantly down regulated and P53 as proapoptotic genes was significantly up regulated in both cell lines

Key words: Amino acid Oxidase, MoringaOleifera, Ganodermalucidum, , Silver nanoparticles, Cytotoxicity , MTT , Anticancer and apoptosis

Introduction:

Cancer is one of the greatest killers and is spreading worldwide promptly (Shastri*et al*., 1997).In medical science the methods available to treat cancer patients mainly includes surgery, chemotherapy and radiotherapy, etc. As these methods are very costly and have side effects with limitations of their use, there is need of effective and acceptable cancer therapeutic agents that should be non toxic, highly efficacious against multiple cancers, palatable, cost effective and acceptable by human population (Wealth of India-Raw material series, Vol. X, (Publication & Information directorate, CSIR, New Delhi), Reprint 1982, 581-585).Natural phytochemicals possess attribute which might protect normal cells but enhance tumor cell susceptibility followed by radiotherapy through modulating cellular molecular targets. Previous results of numerous recent studies on herbals have demonstrated significant cytotoxic activity to certain malignant tumors and protection of normal cell post exposure to ionizing radiation. Ethanolic extract of Moringa may be useful in efficient killing of tumor cells leading to establishment of improved protocol in cancer radiotherapy of patients (Amrita *et al*., 2015).In the same time Ganodermalucidum extracts in different solvents exhibited different levels of antitumor against the three cancer cell lines (including human liver carcinoma cells (HEPG-2) (ATCC® HB-8065™) that cause hepatic carcinoma, Human colonic

epithelial carcinoma (HCT-116) (ATCC® CCL-247™) that causes colorectal carcinoma, and the human cervical cancer cells (HeLa) (ATCC® CCL-2™) from an adenocarcinoma). Also, (Younis et al., 2014)reportedthatalcoholic extract ofGanodermalucidumcouldinducecellcyclearrestat G1phaseandinduce apoptosisinMCF-7cellsina time-dependentmanner.The mechanisms might be mediated through the up-regulation of the expression of p21/Waf1-a cell cycle inhibitor, down-regulation cyclin D1, and up-regulation of a pro-apoptotic Baxproteinpathway. Different biological activities of venom derivatives such as LAOs had been reported, inducing apoptotic activity on various human cell lines (Torri*et al*., 1997; Suhr*et al*., 1996& 1999).TSV-LAO possessed significant cytotoxicity on tested human leukemia T cell line – C8166 recoding an IC50 in the order of 24 nM using MTT assay. Chromatin condensation and segregation, the nuclear morphological changes of typical apoptosis phenomenons, were a common morphological changesobserved. The presence of catalase (400nM) in cell culture made an increase of the CC_{50} value to 390nM, 16-fold higher than that in the absence of the H_2O_2 scavenger (Zhang *et al*., 2003).Nanoparticles, as defined by the US National Cancer Institute, are colloidal particles in the size range of 1–100 nanometers (nm). Although there is no strict definitionof a TNP size range, it is likely that TNPs larger than 10 nm and smaller than 100 nm are most effective(Venturoli and Rippe, 2005).Park and colleagues reported cytotoxicity using silver nanoparticles prepared by dispersing them in fetal bovine serum, as a biocompatible material, on a cultured macrophage cell line, which induced cellular apoptosis (Park *et al*., 2010). Furthermore, AgNPs decreased intracellular glutathione levels, increased NO secretion, increased TNF-α protein and gene levels, and increased the gene expression of matrix metalloproteinases, such as MMP-3, MMP-11, and MMP-19. Kim and colleagues demonstrated cytotoxicity induced by AgNPs in human HepG2 cells and observed that AgNPs agglomerated in the cytoplasm and nuclei of treated cells, and induced intracellular oxidative stress, independent of the toxicity of the Ag+ ions (Kim *et al*., 2007).So, the aim of the present work was to evaluate the anticancer activity of LAAO , MLM , MLW, MSM,G. L and Ag NP against breast (MCF-7) and liver (HEPG-2) cancer cell lines concerning the anti and pro apoptotic gene profile and morphological change associated

Materials and methods:

RPMI-1640 medium, Trypsin 0.25%, fetal calf serum, Methanol, Ethanol andL-amino acid oxidase (LAAO) were purchased from [Sigma-Aldrich-USA].Silver nanoparticles were purchased from Nanotech center- 6 October city – Egypt.*Moringaoleifera* seeds and leaves were identified by Prof. DrKhaled El Dougdoug. Department of Botany, faculty of agriculture, Ain Shams University, and *Ganodermaluciduim* fruiting bodieswere kindly supplied from Dr AhmedYounis Department of Biology, Collage ofHumanities and Science, Virginia Commonwealth University, Richmond, VA, 842012, USA. Liver (HEPG2 and breast (MCF-7) cancer cell lines were kindly supplied from R&D Sector VACSEAR –Egypt. Plastic utilities were purchased from (TPP –Swiss).

Extraction of moringa leaves and seeds

Fresh leaves of *M. oleifera* plants and dry seeds were washed, air dried and powdered using a mechanical grinder (Fisher Scientific, Inc.,Waltham, MA, USA). Methanol extract of plant leaves was obtained by macerating 40 gm of the leaves powder in 200 ml of methanol for 48 h at room temperature with intermittent agitation. The solution was filtered using Whatman No. 1 filter paper and concentrated to dryness in vacum at 40°C. The precipitate was dissolved in PBS and sterile filtrated using 0.2µm disposable syring filters (Millipore –USA). The aqueous extract of leaves was also obtained by macerating a 10 g of the plant powder in 100 ml of PBS at 37°C for 24 h. The resulting solution processed as previous

(Nworu *et. Al*. (2013). Dried moringa seeds were powdered as previous . 40g of dried ground plant material wereextracted in 200 ml methanol and concentrated to dryness under reduced pressure. Extract was redissolved in Dimethylsulphoxide (DMSO) (ASIMA SHABAN*et al*., 2012).Fruiting bodies of *G. lucidum* washed with distilled water, dried, cut into pieces (1x1 cm approximately), and added into the distilled water at 10g/100ml (w/v). The samples soaked overnight at 4° C and grounded as previous. On the second day, the samples were sonicated in using ultra-sonicator (Fisher Model 300 Sonic Dismembrator- Fisher Scientific, Inc., Waltham, MA, USA) for 30 min at 25 KHz. The mixtures were centrifuged (Beckman CS-6R Centrifuge- Beckman Coulter, Inc. Atlanta, GA, USA) at 10,000 xG for 20 minutes, and supernatants were collected. The samples were concentrated by lyophilization with a Lab-conco FreeZone Benchtop Freeze Dry System (Kansas City, MO- USA) (Youni*set al*., 2014).

Cytotoxic activity:

MTT assay was performed to determine the cytotoxic property and antiproliferetive potentials of chemical and natural test materialsagainst MCF-7 and Hep-G2 cell lines. Briefly cell lines were 24 hrs precultured in 96-well tissue culture plates. Appropriate concentrations of stock solutions were added , two fold serially diluted and incubated for 24 hours at 37° C. Non-treated cells were used as negative control. Degenerated cells were examined using inverted microscope (Hund –Germany). degenerated cells were washed out using phosphate bufere saline (ADWIA – Egypt) . MTT(3-(4,5 Dimethylthiazol-2-yl)2, 5 diphenyltetrazolium bromide, a tetrazole) was dispensed the residual live adherent cells as 0.5 mg/ ml final concentration for 4 hrs at 37^{o}C to determine cell viability. MTT is reduced in metabolically active cells to produce an insoluble purple formazan product. Optical denistyy representing the residual live cells was measured using ELx-800 Plate ELISA reader (BIOTEK-USA) at wave length of 570 nm. IC_{50} of test materials was evaluated using Masterplex -2010 software. Viability % was detrmined using the following equation

Viab %= Mean OD of test sample x100 / Mean OD of control cells

Anticancer activity

MCF-7 and HepG-2 cells precultured cell culture flasks (25 cm^2) were treated with the IC_{50} of test materials,untreated cells were considered as a negative control. Treated and untreated cells were incubated at 37°C for 24 h, microscopically examined for cellular degeneration. Detached cells were decanted using (PBS) Phosphate buffer saline (ADWIA –Egypt) and Trypsin harvested rest adherent cells were gathered centrifuged at 2000 rpm for 10 minutes (Jouan , GR-412-France) and washed using cold PBS. Pellets were collected in 100µl PBS in eppindorf tubes, labeled and stored in -70 °C.

RNA extraction

RNA was extracted from treated and untreated MCF-7 and HepG-2 cells 24 h post treatment according to manufacturer's protocol, where cells pellets were treated with RNA lysis buffer (175 µl) to cell pellets and thoroughly mixed by inversion followed by addition of 350 µl of RNA dilution buffer, mixed by inverting 3-4 times and heated in a water bath at 70°C for 3 minutes. Lysed cells were centrifuged at 14000 rpm for 10 minutes. The clear lysate was transferred to a clean tube, 200 µl of 95 % ethanol was added to the clear lysate and mixed well by pipetting.This mixture was transferred to spin basket assembly and centrifuged for 1 minute. Elutes were decanted, 600 µl of RNA wash solution were added and centrifuged for 1 minute. Elutes were decanted again and 50 µl of DNase incubation mix (40µl Yellow Core Buffer, 5µl 0.09M $MnCl_2$ and 5µl DNase I enzyme) were added to each spin basket and incubated at room temperature for 15 minutes. Reaction was stopped by adding 200 µl of DNase stop solution and centrifuged for 1

minute. Each spin basket was first washed with 600 µl of RNA wash solution, centrifuged for 1 min followed by second wash with 250 µl of RNA wash solution and centrifuged for 2 min to ensure removal of RNA impurities. Finally, RNA of each sample treated ell lines was eluted using 100 µl of nuclease free water. Extracted RNA was stored at - 70 °C.

Determination of RNA yield and quality

Concentration and purity of the extracted RNA were determined according to Wilfinger*et al.*, (1997), where RNA was diluted with distilled water and the optical density was measured spectrophotometrically at 260 and 280 nm and the expected absorbance range of pure extracted RNA should be within 1.7 to 2.1. RNA concentration and purity were calculated as follows:

Concentration of the extracted RNA (µg/ml) = A_{260} X dilution factor X 40

RNA purity = A_{260} /A_{280}

Reverse transcription

For each sample, extracted RNA (1 µg), random hexamer primer (1 µl) and DEPC-treated water (to 12 µl) were mixed, centrifuged briefly and incubated at 65°C for 5 minutes. Samples were placed on ice and the following components were added to each sample in the indicated order (Table 2).

Table (2): Reverse transcription reaction mixture.

Reagent	volume
Reaction buffer 5X	14 µl
RibolockRNase inhibitor (20 u/µl)	2 µl
dNTP Mix (10 mM)	2 µl
RvertAid H Minus Reverse transcriptase (200 u/µl)	2 µl
Total volume	20 µl

Samples were mixed gently, centrifuged and incubated at 25°C for 5 minutes followed by 42°C for 60 minutes. Reaction was terminated by heating at 70°C for 5 minutes. Reaction products (cDNA) were stored at - 70°C.

Verification of cDNA synthesis

GAPDH specific control primers (designed to be complementary to human GAPDH genes) were used to verify the synthesis of cDNA from the extracted RNA, where the following were mixed as follows:

Reagent	volume
cDNA	4 µl
Dream *Taq* green master mix	25 µl
Forward GAPDH primer	2 µl
Reverse GAPDH primer	2 µl
Nuclease free water	17 µl
Total volume	50 µl

PCR was performed according to the following program (Table 3).

Table (3): Amplification conditions of PCR.

Step	Temperature	Time/ sec	Number of cycles
Pre denaturation	94°C	180	1
Denaturation	94°C	30	35
Annealing	58°C	30	
Extension	72°C	45	
Final extension	72°C	300	1

RT-PCR product (10 µl) was loaded on 1.5 % agarose gel. Band detection was achieved at 100 volts for 20-30 minutes. PCR product should be visible at 496 bp

Polymerase Chain Reaction (PCR)

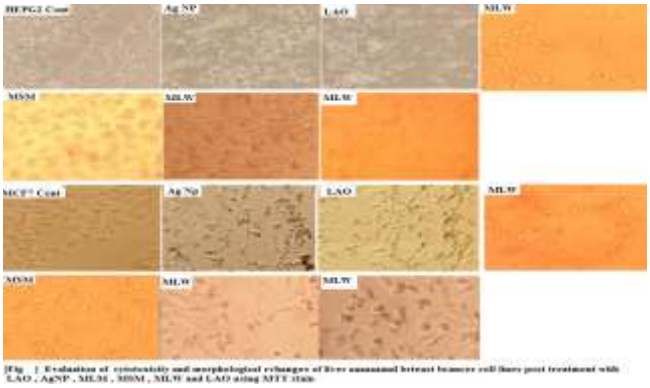
Evaluating the expression of pro-apoptotic gene (p53), and anti-apoptotic genes ;caspase- 3, Bcl-2 and survivin was carried out using the newly synthesized cDNA as templates for PCR. Twenty five µl dream *Taq* green master mix, 4 µl cDNA, 2 µl forward primer (10 picomole / µl), 2 µl reverse primer (10 picomole / µl) and 17 µl nuclease free water were pre-denaturated at 94°C for 3 minutes. Amplification was performed (35 cycles) with each cycle consisting of denaturation at 94°C for 30 sec, annealing at 57°C (p53), 55-60°C (caspase 3), 58°C (survivin) and 55°C (Bcl-

2) for 30 sec followed by extension at 72°C for 45 sec. Reactions were terminated by heating at 72°C for 5 minutes. Non-reverse transcribed RNAs were included to confirm the absence of genomic DNA. Negative control without adding template was also included to assess for reagent contaminationHuang *et al*., (2006).

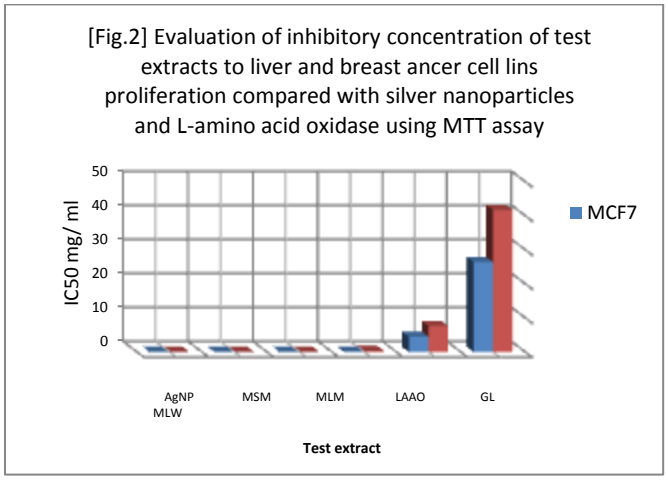
Results:

The toxicity of test extractswas assessed quantitatively using MTT assay. It was noticed that cytotoxicity induced by test materials was a dose dependent, cell viability significantly inhibited. Regarding the untreated control it showed normal cell behaviors and morphology , (Figure 1 – A). Interestingly, a clear difference between the toxic effect of MCF7 and HEPG2 cell lines was detected even at the highest concentrations tested, allowed to determine the cytotoxic concentration of test materials that reduced cell viability by 50%, CC_{50}. Cell morphology showed alterations subsequent to the treatment, as cells exhibited a thin and elongated shape, (Figure 2 – A, B and C). However, after incubation with serially dilutedtest extracts, those produced detectable changessuch as various morphological abnormalities was recorded. Also,the alterations, induced, included reduction of nuclear sizes, which often displayed diverse degrees of chromatin condensation,cell rounding and some areas devoid of cells were also noticed in the same culture (Figure 2 – D). Furthermore, cell shrinkage and the formation of blebs on cell surface finally resulted in the generation of apoptotic bodies (Figure 2 – E and F).

Data recorded revealed that cytotoxicity of test materials evaluated using MTT assayshowed a variable IC_{50}concentrations relative to cell lineswhere toxicity varied and it was clear that test plant extracts , *G. L* , LAAO and Ag NP were toxic in a significant way ($P<0.05$) toMCF-7 than HEPG-2 cells Fig [1]. Also, it was found that LAAO , and Ag NP were significantly toxic than G.L and MLW ($P<0.05$).

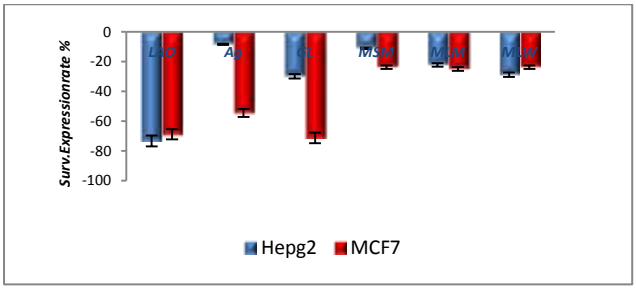


[Fig.1] Cytotoxic effect of test extracts on liver and breast cancer cell lines using



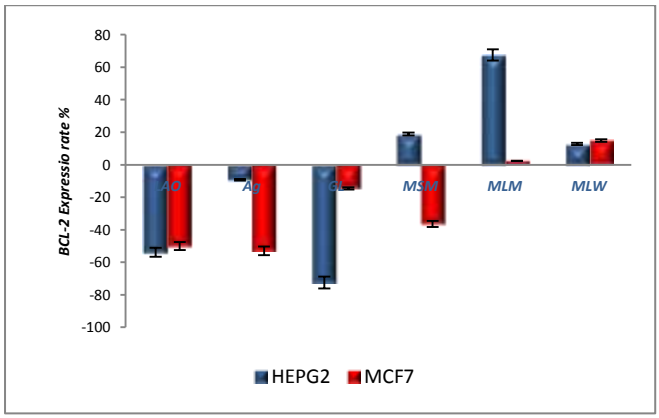
Regarding the cytotoxic effect of test extracts compared with the effect of AgNP and LAAO as a natural and synthetic products. Data recorded revealed that the IC50 of test extracts and materials was arranged in the order of AgNP> MLM> MLM> LAAO> GL> MLW in both HEPG2 and MCF7 cell lines.Regarding the genotoxicity of extracts , it was noticed that the antiapoptotic genes up and down regulation was monitored, and the Survivin gene inHEPG2 cell linetreated with test extracts was down regulated compared with that innon treated control cells as the gene down regulation pattern was arranged in the AAO> GL> MLW>MLM> MSM>AgNP> Control [Fig.]. In the same way the survivinegen in MCF7 cell line showed a better down regulation rate than in HEPG2 cells and the variation in the expression rate was arranged in the order of LAAO>AgNP>MLM,MLW&MSM> GL> control [Fig 3]. Data indicated a significant down regulation ($P<0.05$) of the gene under the

effect of test samples compared with its values in cell control.Also, LAAO and Ag NP showed the highest down regulation potential rate (P<0.05) in case of HEPG-2 and MCF-7 cell lines treatment compared with the rest of test extracts [Fig. 3]



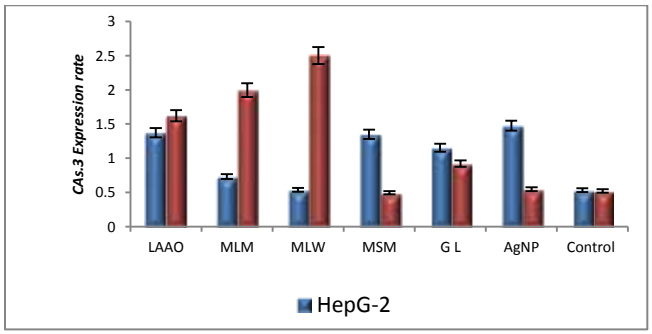
[Fig.3] Detection of Survivin gene pattern in MCF7 and HEPG2 treated cells using Real time PCR

In the same way regarding the antiapoptoticgene ; BCL-2 data revealed thatMCF-7 cell line was mostly affected under the effect of test extracts than HEPG-2 cells and as previously ,mentioned LAAO and AgNP affected gene down regulation in both cell lines compared with the down regulation rate in cell control and expression rate was arranged in the order of GL>LAAO>MLM>AgNP> MLW>MSM > control . While , In case of MCF-7 cells the BC L-2 gene expression was arranged in the order of AgNP>LAAO> MSM>GL > control in the mean time the BCL-2 gene was not affected compared with its expression rate in cell control in case of cell treatment with MLM and MLW(P>0.05), [Fig.4]



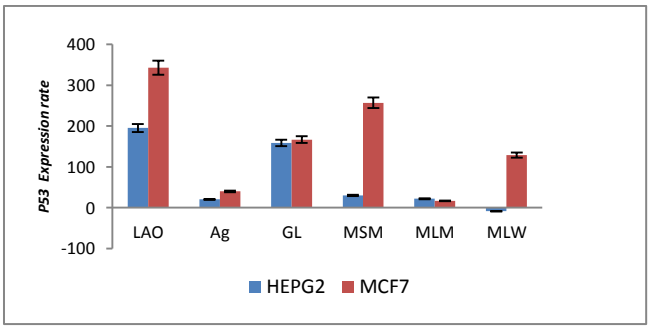
[Fig.4] Detection of BCL-2 gene pattern in MCF7 and HEPG2 treated cells using Real time PCR

caspase 3 showed up regulatedpattern by the effect of MLW extract (P>0.05) and there was a significant upregulation of the gene in case of treatment with rest of test extracts (P<0.05) compared with caspase-3 values in HEPG-2 cell control group and the effect was arranged in the order of Control > MLW> MLM>GL>MSM> LAAO> AgNP. Also, I case of MCF-7 treated group expression rate was arranged in the order of MSM>AgNP>Control>GL>LAAO>MLM>MLW respectively [Fig 5] .



[Fig.5]Detection of caspase -3 gene pattern in MCF7 and HEPG2 treated cells using Real time PCR

Finally regarding the pro-apoptotic gene (P53) it was noticed that it was significantly upregulated in MCF7 more than in HEPG2 (P<0.05) and the expression rate was arranged in the order of LAAO> MSM >GL > MLW>Ag NP > Control and the pattern of expression rate in MCF-7 was arranged in the order of LAO>GL>MSM>MLM>AgNP>control [Fig.6].



[Fig.6]Detection of Survivin gene pattern in MCF7 and HEPG2 treated cells using Real time PCR

Discussion

Moringaoleifera (Moringaceae) and *Ganodermalucidum* [3-5] are highly valued plant, they have an impressive range of medicinal uses with high nutritional values. Different parts of M. Olifera and *G. lucidium*contain a profile of important minerals, and are a good source of protein, vitamins, β-carotene, amino acids and various phenolicslectin ,laccaseisozym eslanostanoidtriterpenes polysaccharides inhibitor for peptidylprolylcis-trans isomerase (PPIase) Cyclophilin inhibitor amino acids triterpenoids. Also, they are enriched with rare combination of zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferolGanoderic acid T (Tang *et al.*, 2006). Biological derivatives from various parts of Moringa and G. lucidumhave immature products used as cardiac and circulatory stimulants, possess antitumor, antiepileptic, anti-inflammatory, antiulcer, antihypertensive, antioxidant, antidiabetic, hepatoprotective, anti microbial activities. Also, *LAAO* derived from snake venom and Silver nanoparticles were of the interesting bioactive materials drew attention for its medicinal importance. So, the present work aimed to *In Vitro* evaluation of moringa seed and leaf water and methanolic extracts, G. lucidum fruits ,LAAO and Ag-NP anticancer potentials against two type of cancers namely Liver and breast cell lines. Data recorded regarded the anti proliferative effect of test material showed a variable IC_{50} values to test cell lines , that was approved in different works where Hui *et al*., 2010, investigated on the molecular level that the apoptotic potentials of *G. lucidum*peptides (GLP) to human hepatoma (HepG2) cells and the result of western blotting showed that the expression levels of Bcl-2 and survivin ; apoptosis inhibiting genes, were down-regulated and p53, as a pro apoptosis gene, was up-regulated in a dose dependent manner. The activity of caspase-3, was the key proteinase of cell apoptosis, was activated by GLP in a dose-dependent manner. It was suggested that GLP could induce HepG2 cells apoptosis, and the mechanism of GLP was probably associated with the down-regulation of Bcl-2 and surviving expression, up-regulation of p53 expression and activation of caspase-3 activity. These results were very close to our results were with HepG2 and MCF7 cell lines treated with GL extracts that enhanced the down regulation of Survivin and Bcl2 strongly with Bcl2 >survivin, up regulation of p53 and activation of caspase-3 gene. Results showed that novel polysaccharide SeGLP-2B-1 isolated from Se-enriched *Ganodermalucidum*, showed anti-proliferative activity towards several cancer cell lines in vitro. SeGLP-2B-1 inhibited the growth of MCF-7 cells in a time- and dose-dependent manner were it increased the activities of caspase-9 & 3 and poly (ADP-ribose) polymerase in a time-dependent manner. This result is closed to our result where *Ganodermalucidum*extract induced up regulation in MCF7 cells with 76.6%. Also, Tiloke et al., 2013 showed that *Moringaoleifera*aqueous leafs extract pro-apoptotic action on cancerous A549 lung cells was confirmed by the significant increase in p53 protein expression ;1.02-fold, ($p < 0.05$), p53 mRNA expression (1.59-fold), caspase-9 (1.28-fold, $p < 0.05$), caspase-3/7 (1.52-fold) activities and an enhanced expression of Smac/ DIABLO. Our results regarding *Moringaoleifera*aqueous leaf extract showed up regulation of p53 gene with HEPG-2 cell line than that of MCF-7 cells. While with caspase3 it showed max up regulation with MCF7 cells treated with MLW extract with minimal effect on hepG2 cells. Also, (Michelle et al., 2011) recorded that Ganodermalucidum (Reishi) contains biological compounds that are cytotoxic against cancer cells. Results show that Reishi selectively inhibits cancer cell viability although it does not affect the viability of noncancerous mammary epithelial cells. In the mean time *Gamilaetal* (2004), reported that the cytotoxicity and antimicrobial activity of different *moringa oleifera* seeds extracts were tested

against Scenedesmusobliquus (greenalgae),Escher

ichia coli ATCC 13706, Pseudomonas aeruginosa ATCC 10145, Staphylococcus aureus namru325923,Bacillus sterothermophilus . Also Herpes simplex virus type 1 (HSV1) and polio virus type 1 (Sabin vaccine) are affected. Fixed oil extracted from the seeds was found to activate the growth of Scenedesmusobliquus with high algal biomass production. aqueousmethanolic extracts (IC_{50} 207.5 mg/l) and water extract (lC_{50} 287.5 mg/l) has a cytotoxicity effect on Scenedesmus growth. on the other hand, the antibacterial effects of fixed oil, aqueous methanolic and water extracts concentrations showed a fluctuation on its effects. Although, P. aeruginosa was more resistant to all M. oleifera extracts, B. sterothermophilus was more sensitive than other organisms to all extracts. the effect of aqueous methanolic extract and fixed oil on HSV-1 was non significantly different, recording 52.22% and 45.2% respectively . very low activity was observed for fixed oil on PV-1. Regarding the anticancer potential of LAAO derived from snake venom our data was in accordance with *(Bregge-Silva et al., 2012*) despite his lAAO source was differ from another snake species and different cell line used ,where they evaluated the cytotoxic effect of LAAO (denominated Lm LAAO) isolated from *Lachesismuta* snake venom on AGS (gastric adenocarcinoma) and MCF-7 (breast tumor) cells, with IC_{50} of 22.7 µg/mL and 1.41 µg/mL, respectively.The catalase (0.1 mg/mL) completely abolished the cytotoxic effects of Lm LAAO on MCF-7 tumor cells. This result was nearly to ours despite we concerned the proapoptotic and antiapoptotic genes and the cell cycle pattern post cell treatment with the IC_{50} of test extract, LAAO and Ag NPs These results may be due to the release of H_2O_2, in addition to the inhibition of cycline kinase dependent enzymes (CDKs). Also, the phenolics content and elagic acid may interfere in cellular arresting and changes the genetic profile. Recently*(Fathia et al., 2015)* reported that the cytotoxic effects of L-Amino acid oxidase (svLAAO), was tested against HepG2 and MCF-7 cell lines and IC_{50} was calculated. All the tested compounds had anti proliferative effects on the tumor cell lines. The cytotoxicity of svLAAO was much higher than SV with IC50 = 3.65 and 0.48 µg /ml. SvLAAO was the most effective and safe compound (than its crude venom) to be used as antitumor agent. And svLAAO induced proapoptotic genes up regulation and down regulation of anti apoptotic genes in a significant way compared with nontreated cells in addition it induced different cellular arrest at G0/G1 phase. In the mean times evaluation of anticancer activity of Ag NPs and its antimicrobial activity , data recorded was in agreement with *(Kulandaivelu et al., 2016)* demonstrated thatthe cytotoxic effects of the silver nanoparticles could significantly inhibited MCF7 cancer cell line proliferation in a time and concentration-dependent manner by MTT assay. This result is much closed to our result. Western blot analysis has revealed that nanoparticles were able to induce cytochrome C release from the mitochondria, which was initiated by the inhibition of Bcl_2 and activation of Bax genes. Our data was confirmed as anticancer by (Selim and Hendi, *2012*) recording that the induction of apoptosis by chemically synthesized Ag NP in MCF-7 cell line through down regulation of both survivin and BCL2 genes and up regulation of p53 gene but with very small percent down regulation of caspase3 gene.Also, (Ismail *et al*., 2015) reported that Ag-NPs induced DNA fragmentation and apoptosis in HepG2 and MCF-7 via suppressing of Bcl-2 gene expression, up-regulation of BAX, down-regulation of Bcl2, simulation of caspases, P53 and cytochrome C gene expression and concluded that Pleurotusostreatus edible mushroom extracts showed antitumor cytotoxicity against HepG2 and MCF-7 via caspase-dependent apoptosis, associated with the activation of p53 and the down-regulation of Bcl-2. Finally it can be concluded that test extracts whatever its extraction regimen showed a variable anticancer potentials and despite its variable potential; either are

promising agents must be considered also its antiviral potential are promising as well

References

1. Ahmed Younis, Jennifer Stewart, Fang-Sheng Wu, Hussien El Shikh, Fathy Hassan, Mahmoud Elaasser. Effectiveness of different solvents extracts from edible mushrooms in inhibiting the growth of tumor cells. Cancer Biology 2014;4(4):1-15]. (ISSN: 2150-1041).
2. Hongbo HU et al., Ganodermalucidum extract induces cell cycle arrest and apoptosis in Mcf-7 human breast cancer cell. Int. J. Cancer: 102, 250–253 (2002).
3. K. D. Mishra et al., Review Article; Promising Role of MoringaOleifera (Lam.) In Improving Radiotherapy: An Overview, JIPBS, Vol 2 (2), 182-192, 2015.
4. Shastri A D, SushurtSamhita, NidanSthan, Part-I, 11th edn, (Choukhamba Sanskrit Sansthan, Varanasi), 1997, 272-273.
5. (Wealth of India-Raw material series, Vol. X, (Publication & Information directorate, CSIR, New Delhi), Reprint 1982, 581-585).
6. Venturoli, D. and Rippe, B. (2005) Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability. Am. J. Physiol. Renal. Physiol. 288, F605–613
7. Torii S, Naito M, Tsuruo T: Apoxin I, a novel apoptosis-inducing factor with L-amino acid oxidase activity purified from Western diamondback rattlesnake venom. *J Biol Chem*1997,272(14):9539-9542.
8. Suhr SM, Kim DS:Identification of the snake venom substance that induces apoptosis. *Biochem Biophys Res Commun* 1996,224(1):134-139.
9. S.M. Suhr, D.S. Kim, Comparison of the apoptotic pathways induced by LL-amino-acid oxidase and hydrogen peroxidase, J. Biochem. 125 (1999) 305–309.
10. Zhang YI, Wang JH, Lee WH, Wang Q, Liu H, Zheng YT, et al. Molecular characterization of Trimeresurusstejnegeri venom L-amino acid oxidase with potential anti-HIV activity. BiochemBiophys Res Commun 2003;309:598–604.
11. Venturoli D, Rippe B. Ficoll and dextran vs. globular proteins as probes for testing glomerular permselectivity: effects of molecular size, shape, charge, and deformability.Am. J. Physiol. Renal. Physiol. 2005; 288:F605–F613.
12. Park EJ, Yi J, Kim Y, Choi K, Park K. Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. ToxicolIn Vitro. 2010;24:872–878. doi: 10.1016/j.tiv.2009.12.001.
13. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3:95–101.
14. Nworu, C. S.1,3*, Okoye, E. L.2, Ezeifeka, G. O.2 and Esimone, C. O. Extracts of Moringaoleifera Lam. showing inhibitory activity against early steps in the infectivity of HIV-1 lentiviral particles in a viral vector-based screening. 2013;12:30: 4866-4873.
15. AsimaShaban, Ghanshyam Mani Mishra, Rajesh Nautiyal, SaumyaSrivastava, KiranTripathi , PoonamChaudhary and SatishK.Verma. In vitro cytotoxicity of MoringaOleifera against different human cancer cell lines. Asian J Pharm Clin Res, Vol 5, Suppl 4, 2012, 271-272.
16. Ahmed Younis, Jennifer Stewart, Fang-Sheng Wu, Hussien El Shikh, Fathy Hassan and Mahmoud Elaasser. Cytotoxic activity of edible mushrooms extracts against tumor cell lines. 2014, Volume 3 No. 11.
17. Cory AH , Owen TC , Barltrop JA and Cory JG. Use of an aqueous soluble

tetrazolium/formazan assay for cell growth assays in culture. 1991, 3(7):207-212.

18. Michael V. Berridge, Patries M. Herst and An S. Tan. Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. 2005, Volume 11, Pages 127–152.
19. W.W. Wilfinger, K. Mackey, P. Chomczynski. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity.(1997), BioTechniques, 22 pp. 474–481.
20. Lulu Huang, Lou Massa and Jerome Karle. The Kernel Energy Method: Application to a tRNA. 2006. vol. 103 no. p 1233–1237.
21. Charlette Tiloke, Alisa Phulukdaree and Anil A Chuturgoon.The antiproliferative effect of Moringaoleifera crude aqueous leaf extract on cancerous human alveolar epithelial cells. 2013. 13:226.
22. Michelle M. Martínez-Montemayor, Raysa Rosario Acevedo, Elisa Otero-Franqui, Luis. A. Cubano, and Suranganie F. Dharmawardhane.Ganodermalucidum (Reishi) Inhibits Cancer Cell Growth and Expression of Key Molecules in Inflammatory Breast Cancer. 2011; 63(7): 1085–1094.
23. ‘’Bregge-Silva C, Nonato MC, de Albuquerque S, Ho PL, de Azevedo IL J, VasconcelosDiniz MR, Lomonte B, Rucavado A, Díaz C, Gutiérrez JM, Arantes EC: Isolation and biochemical, functional and structural characterization of a novel L-amino acid oxidase from Lachesismuta snake venom. Toxicon 2012, 60(7):1263–1276.
24. FathiaZaky El SharkawiShaimaa Saber Saleh and AlyFahmy Mohamed El Sayed.Potential anti cancer activity of snake venom, bee venom and their components in liver and breast carcinoma.2015; Vol. 6(8): 3224-3235.
25. Kulandaivelu, Balaji1; Gothandam, K M1.Cytotoxic Effect on Cancerous Cell Lines by Biologically Synthesized Silver Nanoparticles. 2016. Vol.59: e16150529.
26. Amel F. M. Ismail, Mahgoub M. Ahmed and Asmaa A. M. Salem. Biosynthesis of silver nanoparticles using mushroom extracts: Induction of apoptosis in HepG2 and MCF-7 cells via caspases stimulation and regulation of BAX and Bcl-2 gene expressions. 2015. 5(1)1-9.
27. Selim ME1 and Hendi AA.Gold nanoparticles induce apoptosis in MCF-7 human breast cancer cells. 2012. 13, 1617-1620.
28. Gamila H. Ali , Gamila E. El-Taweel & M. A. Ali (2004). The cytotoxicity and antimicrobial efficiency of *Moringaoleifera* seeds extracts.
29. Wen Tang, TingyueGu, Jian-Jiang Zhong (2006):*Ganodermalucidum* by reversed phase liquid Separation of targeted ganoderic acids from chromatography with ultraviolet and mass spectrometry detections. Biochemical Engineering Journal Volume 32, Issue 3, 1 December 2006, Pages 205–210.
30. Amrita Singh1, K. P. Mishra, Renu Dayal1 and Rudra P. Ojha1.Promising Role of MoringaOleifera (Lam.) In Improving Radiotherapy: An Overview.Vol 2 (2), 182-192, 2015.
31. Asimashaban, Ghanshyam Mani Mishra, Rajesh Nautiyal, SaumyaSrivastava, KiranTripathi ,PoonamChaudhary and SatishK.Verma. In vitro cytotoxicity of *Moringaoleifera* against different human cancer cell lines. 2012. Vol 5, Suppl 4, 2012, 271-272.
32. HE Hui, SHI Yan-ling, XU Shu-fen, ZHANG Sheng and GUO Hui.Hepatoprotective effect of *Ganodermalucidum* peptides against alcohol-induced liver injury in mice.Food Scienc. 2010-03.
33. https://books.google.com.qa/books?id=urhwlkqvs2qc&printsec=frontcover.

Ganodermalucidum extract induces cellcyclearrest and apoptosis in MCF-7 human breastcancercell