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In vitro anticancer activity of methanolic extracts, non-thermal plasma and iron oxide nanoparticles on human cancer cell line [HepG2]

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Abstract

Introduction: Liver cancer is one of the most common malignancies across the globe and one of the major causes of cancer-related mortality. With limited available treatment options, there is an urgent need to look for new available options. Medicinal plants is an important and has been shown to possess tremendous pharmacological potential. The objective of the present study was therefore to evaluate the anticancer effect of the Passiflora incarnata and Arctium lappa Leaves compared with non-thermal plasma and iron oxide nanoparticles.

Methods: In this study determination of viability carried out through MTT assay, also molecular techniques were carried and proteomics assay of genes (P53 and Bcl2) compared with β-actin gene as standard.

Results: MTT assay revealed that P. incarnata and A. lappa leaves extracts reduced the cell viability of HepG2 cancer cells from 100 to 85 and 80%, respectively in a dose-dependent manner (20 0µl). The results of this study proved that, there were a significant killing effect for extracts, gliding Arc plasma exposure and nanoparticles. These results supported by analysis of genes associated with tumor which were P53 as tumor suppressor gene and Bcl2 (Bcl-xl) as antiapoptotic gene. Non-thermal plasma it had effects on increased transcription and protein product leading it increase in apoptotic effect of HEPG2 cell line at 40, 60 and 80sec, which were 74.33±1.07, 82.53±1.15 and 88.05±0.36%, respectively. Regarding the effect of methanolic extract of P. incarnata and A. lappa leaves. This study illustrated that, P. incarnata and A. lappa (group5 and group6), it has high effects on increased gene transcription P53 compared control (group1), which were 99.89±0.40 and 98.07±1.08%, while the result of iron oxide nanoparticles (group7), was 99.76±1.10%, compared with control group, which was 66.22±2.08%, respectively.

Keywords: Extracts, Non-thermal plasma, Nanoparticles, HepG2, Genes (P53 - Bcl2 - β-actin).

Introduction

Passiflora incarnata L. commonly known as passion flower belongs to Passifloraceae family and is one of the most popular and widely used medicinal plants in traditional medicine in West India, Mexico, Netherland and South America. The plant has a long history of application in herbal medicine as an anxiolytic and

sedative hypnotic which dates back to ancient time by Suvarna and Sanjay., (2012). Arctium lappa L. also known as burdock, is a plant belonging to the Compositeae (Asteraceae) family. Previous studies linked Arctium lappa to multiple therapeutic activities as antitumor on human cancer cell lines. It has a stronger antioxidant effect than other vegetables or fruits. Chemical reactions with free-radical terminations and oxygen inhibitory compounds are accounting for suppressing biochemical processes promoted by Oxygen by Tonea et al., (2016).

The nanotechnology is a multidisciplinary field, convergence of basic sciences and applied disciplines like biophysics and molecular biology. It has created powerful impact in various fields including oncology, pulmology, immunology etc., and to highly specialized areas like gene delivery, tumor targeting, and oral vaccine formulations by **Patra *et al.*, (2018).**

Physical plasmas are composed of reactive atoms, molecules, ions and radicals. NTP can produce a mixture of highly-reactive chemical species which play important roles in cell processes. At present, NTP has become as a novel tool in some biomedical areas, such as sterilization, antifungal treatments, blood coagulation, dental care, wound healing and cosmetics targeted cell and tissue removal. NTP treatment has been proven effectively in inducing apoptosis in vitro of various tumor types and inhibiting tumor growth in vivo. Moreover, NTP effects on cells were dose dependent that low doses of plasma can stop cancer cells proliferation and high doses result in cell apoptosis and necrosis. **Liu *et al.*, (2017).**

P53 protects cells against DNA damage-induced stresses, which could lead to transformation and tumor genesis. Mutations in p53 have been found in over 50% of all human tumors with loss of the gene seen in the majority of tumor types. In addition to direct loss of the p53 gene, disruption of key components in the p53 pathway have been implicated in a vast number of human tumors. Therefore, it is clear that understanding the pathway through which p53 protects the cell against oncogenic transformation, and how this pathway can be inhibited, is essential in elucidating targets for future cancer therapy. **Henry *et al.*, (2002).**

Bcl2 is a family of protein responsible for dysregulation of apoptosis and prevention of death in cancer cells**. Reed., (1999).** Antiapoptotic Bcl2 family members Bcl-Xl and proapoptotic protein, such as BAD and BAX, interplay with each other to control the pathways leading to the release of cytochrome C from the mitochondrial membrane. Then activation of Caspase cascade and finally to the execution of apoptosis. **Gross *et al.*, (1999).**

MATERIALS AND METHODS

Plant extraction:

The Sciences Academy of Experimental Researchers (SAER), located in Mansoura, Egypt, provided the *Passiflora incarnata* and *Arctium lappa* Leaves. The Botany Department of the Faculty of Agriculture at Al Azhar University graciously verified the plant sample. The samples were ground into a fine powder after being oven-dried at 55°C. The powder was separated into portions for aqueous and methanolic extract production. The plant's 500 g of powdered of dehydrated leaves were boiled in reflux at 80 to 100°C in reflux for (3h) with distilled water (5L) to produce the aqueous extract. The extract was filtered, lyophilized, and kept at 4°C until it was required for the experiments by **Kim *et al*. (2011).** 500 g of the plant's powdered leaves were soaked at room temperature six times (8h) in methanol (98% 10L) to yield a methanolic extract. The extract was stored at 4°C until it was needed in further studies. A rotating evaporator operating at 45°C was used to concentrate it by dryness while under vacuum by **Erian *et al.*, (2016).**

This work was authorized by the Sciences Academy of Experimental Research's Ethics Committee in Egypt [28 Oct 2024, No. 44713].

Cell line and treatment:

Cell line [HepG2] obtained from the Genetic Engineering Unit were cultured in EMEM medium (ATCC) supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific) as recommended by the supplier. Cultures were kept in a humidified 5% CO2 atmosphere at 37 °C and the medium was changed once a week. Tissue sampling was approved by the Clinical and Experimental Medicine and the Alazhar University Ethics Committee. The study carried out on a cell line [HepG2] a hepatocellular carcinoma (liver cancer) model. Where the cell line [HepG2] exposed to three types of treatment, which are :-

A. Methanolic extract of *Passiflora incarnata* and *Arctium lappa* leaves.

B. Radiation, Non-thermal plasma [plasma physics] This type of plasma called Gliding Arc Discharge [GAD].

C. Nanoparticles [iron oxide].

Gliding Arc Discharge (GAD) Experiment:

The gliding arc (GA) is an intermediate system between thermal and non-thermal discharges, and is able to provide simultaneously high plasma density, power and operating pressure with high level of non-equilibrium, high electron temperature, low gas temperature and possibility of stimulation selective chemical processes without any quenching. The main peculiarities of GA are the “memory effect” and essential influence of convective heat and mass transfer on plasma properties and the space/time arc evolution. Gliding arc gas discharge (GA) is an auto-oscillating phenomenon developing between at least two electrodes that are immersed in a laminar or turbulent gas flow, which provides significantly non-equilibrium plasma region at elevated power level **Fridman *et al.*, (1999) and Richard *et al.*, (1996)**.

The reactor used for this study derives from the gliding arc or glidarc device as illustrated in Figure (1).

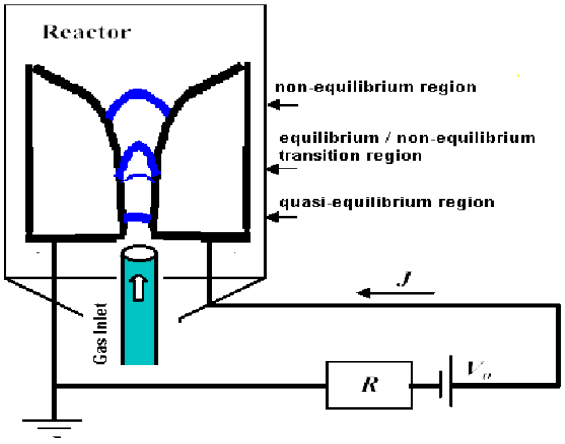


Figure (1). Simplified scheme of the gliding arc.

Fig (2), shows the photographs of gliding arc for different gas flow rate at constant discharge power. With increasing the gas flow rate, emission intensity increased in the upstream side. On the

other hand, electric discharge area increased with increasing gas flow rate in the downstream side. The positive column of the main arc discharge which can be controlled by discharge power, and downstream domain is a plasma jet plume which exists across between electrodes that depend on the gas flow.

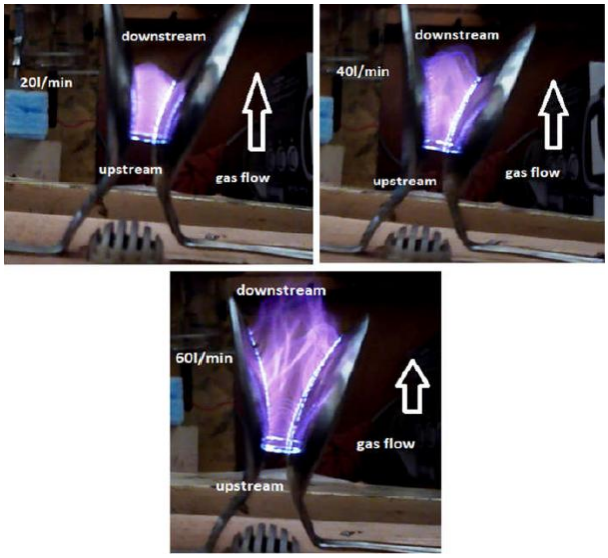


Figure (2). The photographs of gliding arc for different gas flow rate at constant discharge voltage.

The gliding arc discharge for different discharge power at 60 l/min in air gas flow rate indicate that, in the upstream side, emission intensity was strong and it increased with increasing discharge voltage. In addition, electric discharge area is small and it was increased with increasing the discharge voltage. On the other hand, emission intensity is weak and electric discharge area is large in the downstream side. Although emission intensity is almost constant independent for the discharge voltage, discharge area decreased with increasing discharge voltage in the downstream.

The species formed in air discharge plasma region were detected with spectroscopic emission, and the result has been presented in Fig (3).

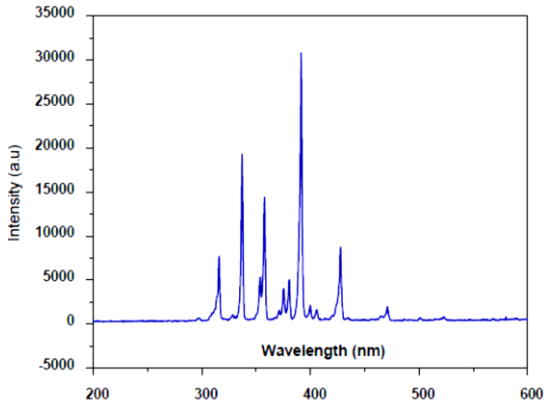


Figure (3). Spectroscopic emission in air discharge plasma region.

Preparation of iron oxide nanoparticle:

Synthesis and characterization of iron oxide nanoparticles as the following :-

Chemicals : Iron chloride ($Fecl_3$ 99%), Citrate tri sodium salt ($C_6H_5O_7 \cdot {}_2H_2O$ 95%), Ammonium thiocyanate (NH_4SCN 99%), 0.05M EDTA and methanol were purchased from fluka. Poly ethylene glycol was purchased from Aldrich, Doubled distilled water was used.

Procedures: synthesis of hematite nanoparticles according to **Arndt., (1988).** A solution of 0.1 M $Fecl_2$ (15 ml) was added drop by drop to 100 ml of vigorously stirred boiling distilled water. The color changed from yellow ($Fecl_3$) to red and upon excess addition of 0.1 M $Fecl_3$, the color turned to dark red. The resulting solution was heated to reflux and kept at the temperature for 30 min the solution was then cooled to room temperature. Such colloidal solution can exist indefinitely without any signs of precipitation. However, a reddish-brawn precipitate was obtained upon adding excess amount of concentrated solution of ammonium phosphate tribasic tri hydrate [$(NH_4)_3\ PO_4 \cdot {}_3H_2O$].

- Diameter of iron oxide nanoparticles = 6 nm = (60 angstrom).
- Volume of hydrated iron oxide nanoparticles = 1.13×10^5 A°.
- No of nanoparticles in one liter = 4.231×10^{16} A°.
- In terms of mol/l = 70.28 nm.
- The extinction coefficient ε(ʎ) of the prepared iron oxide nanoparticles. ε(ʎ)= 6.8×10^7 M^{-1} cm^{-1}.

Experimental design:

The HepG2 cell line divided to the following groups :-

Group 1 (control): cancer cell line (HEPG2) without treatment.

Group 2: cancer cell line (HEPG2) exposed for non-thermal plasma for 40 second.

Group 3: cancer cell line (HEPG2) exposed for non-thermal plasma for 60 second.

Group 4: cancer cell line (HEPG2) exposed for non-thermal plasma for 80 second.

Group 5: cancer cell line (HEPG2) treated with methanolic extract of *Passiflora incarnata* leaves 200µl.

Group 6: cancer cell line (HEPG2) treated with methanolic extract of *Arctium lappa* leaves 200µl.

Group 7: cancer cell line (HEPG2) treated with nano iron oxide 200µl. according to **Indrani and Devi., (2017).**

After treatment the cell line cultured for 3 days. Then viability of the cell determined by MTT assay.

MTT Cell viability Assay:

Cytotoxicity activity was evaluated on HepG2 cells via MTT (3 (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay (MTT assay kit (cell proliferation) Abcam, (ab 211391) to **Horiuchi *et al.*, (1988)** as follow:-

1- Cells (1×105/well) were plated in 0.2ml of medium / well in 96 – well plates.

2- Removing medium from the wells carefully after incubation.

3- Washing each well with MEM (WLO) FCS for 2-3 times and 200 µl of MTT (5mg) was added.

4- Incubation plates or 6-7 hrs. in 5% CO_2 for cytotoxicity.

5- Adding 1ml of DMSO (solubilizing reagent) was to each well and mixed well by micropipette and left for 45 sec.

6- Presence of viable cells was visualized by the development of purple color due to formation of formazan crystals.

7- Transferring suspension to the curette of a spectrophotometer and the OD (Optical Density) values were read at 595 nm by using DMSO as a blank.

8- Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically standard graph was plotted by taking concentration of the drug in α axis and relative cell viability in γ axis.

Cell viability (%) = Mean OD / Control OD × 100

Beside determination of viability, there were follow up of another analysis, these analysis were molecular analysis.

The molecular analysis were included proteomics analysis of P53 and Bcl2 genes compared with β-actin gene as standard.

Molecular analysis (proteomics) of genes (P53 and Bcl2) compared with β-actin gene as standard:

Blotting technique:

Blotting Solutions:

1-a- Blotting buffers: 25 mM Tris, pH 7.4, 0.15 M NaCl and 0.1% Tween 20.

1-b- Blocking solution: 2-5% nonfat dry milk in Blotting Buffer Adjust pH to 7.4.

1- c- Antibody solution: 1-5% nonfat dry milk in Blotting Buffer Adjust pH to 7.4

Blotting protocol:

Electrophoresed proteins on SDS-PAGE were transferred to a *Hybond*™ nylon membrane (GE Healthcare) via TE62 Standard Transfer Tank with Cooling Chamber (Hoefer Inc. and incubate for 1 hour at room temperature in Blocking Solution. Additionally, β-actin was applied as housekeeping protein**.**

3. Membrane was incubated the overnight at 4°C in Antibody Solution containing Anti- P53 primary antibody (abcam, ab131442). For normalizing data, Anti- β-actin primary antibody (abcam, ab228001) was used.

4. Membrane was washed at room temperature for 30-60 minutes with 5 or more changes of Blotting Buffer.

5. Membrane was incubated for 1 hour at room temperature in Antibody Solution containing appropriate dilution of HRP-conjugated secondary antibody (Antibody concentration. 0.1-0.5 microgram/mL. Adjust antibody concentration from 0.05 to 2.0 microgram/mL to obtain desired signal strength and low background.

6. Membrane was washed for 30-60 minutes with 5 or more changes of Blotting Buffer.

Western Blot Protocol References:

- Antibody Techniques, Vedpal S. Malik and Erik P. Lillehoj, 1994 Academic Press, pg 273-289.
- Immunochemical Protocols, Second Edition, John D. Pound, 1998 Humana Press, pg 207-216.
- Using Antibodies, A Laboratory Manual, Ed Harlow and David Lane, 1999 A Cold Spring Harbor Laboratory Press, pg.267-309.
- Tween is a registered trademark of ICI Americas.

Statistical analysis:

Using the statistical software package, information collected were evaluated **(CoStat, 2005)**. All comparisons were first subjected to one-way ANOVA and important distinctions between treatment means were determined using the multi range test of Duncan at $p<0.05$ as the meaning point **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Antitumor activity of non-thermal plasma, methanolic extracts and iron oxide nanoparticles on cancer cell line [HepG2]:

In this study determination of viability carried out through MTT assay, also molecular techniques were carried and proteomics assay of genes (P_{53} and Bcl_2) compared with β-actin gene as standard.

- P_{53} gene represent a tumor suppressor gene.
- Bcl_2 gene represent a proto-oncogene.

The study going through three types of treatment:-

1- Treatment through non-thermal plasma radiation (physical treatment). Cold plasma recently studied in the treatment of cancer resulting from its chemical component (Atoms, Ions radicals) and physical component of these plasma (Gliding Arc) leads to maximizing tumor cell death.

2- Treatment through methanolic extract (*Passiflora incarnata* and *Arctium lappa*) leaves.

3- Treatment through nanoparticles (iron oxide).

Cell viability in HepG2 cells using MTT assay:

The results in table (1) and figure (1), showed the percent of changes relative to normal control group. Comparing to normal control group, the levels of viability showed significant decrease in all treated group (G2 – G7), at the same time. The level of changes was high significant in (G4 treated with non-thermal plasma at 80 second). Comparison between (G2 and G3 treated with non-thermal plasma at 40 and 60 second) and (G4 treated with non-thermal plasma at 80 second) gives a significant decrease of G4 which mean that, increasing the level of plasma dosed lead to a good treatment.

The methanolic extract of *Passiflora incarnata* and *Arctium lappa* leaves showed the medium values as antitumor activity of the levels of viability, which were 85.3 and 80.2%, of (G5 and G6), respectively. Compared with iron oxide nanoparticles (G7), which

was 75.2%.

Table (1): Mean value of viability (%) of all groups.

Groups	Treatment	Cell viability (%)
Group 1	Control	100.0±0.00
Group 2	NTP 40Sec	75.0±1.09
Group 3	NTP 60Sec	62.0±0.17
Group 4	NTP 80Sec	45.1±0.22
Group 5	ME-PI 200µl	85.3±0.13
Group 6	ME-AL 200µl	80.2±1.12
Group 7	NIO 20µl	75.2±2.00

Group 1: Control cell line, Group 2,3,4,: treated cell line with non-thermal plasmas at 40, 60 and 80sec, Groups 5: treated cell line with methanolic extracts of *P. incarnata*, Groups 6: treated cell line with methanolic extracts of *A. lappa* and Groups 7: treated cell line with iron oxide nanoparticles, respectively.

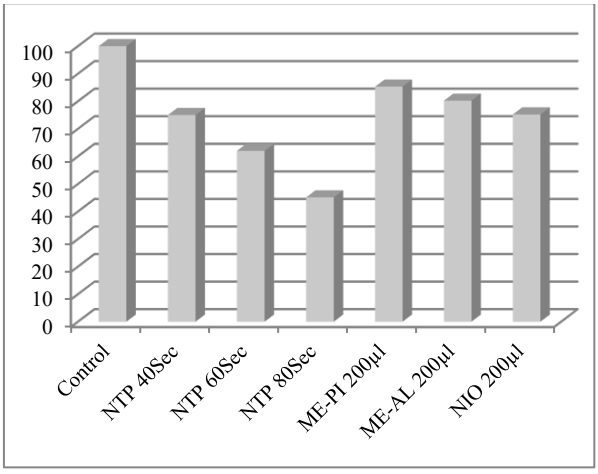


Figure (1): Mean **value** of viability (%) of all groups.

The results agree with **Sesan *et al.*, (2016),** who found that a higher values of cell viability for concentrations between 250 and 500µg/ml of *Passiflora caerulea* extract affected the normal development of culture and had a toxic effect on individual cells. It was observed a significant inhibition on the cell viability, less than 70%, at 24 and 48hrs., respectively. **Namvar *et al.*, (2014),** studied the effect of different concentrations of Fe3O4, MNPs and a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) on cancer cells. the result showed that, the inhibitory concentration (IC_{50}) value of (HepG2, was 23.83µg/ml), 72 hours after treatment.

Sadeghi., (2020). examined the cell viability by MTT assay. the result showed the cell death increased by treating with nanoparticles (Fe2O3) in a concentration dependent manner. Increased exposing time causes more significant reduction in cell viability. Viability of HepG2 decreased to 12% by giving harsh dose (100mg/ml for 24 h)and reduced to 20% by adding the nanoparticle in 100mg/ml concentration for 12 h.

Molecular analysis (proteomics) of genes (P53 and Bcl2) compared with β-actin gene as standard:

Molecular analysis (proteomics) of gene (P53):

The results of this study proved that, there were a significant killing effect for all dosses of gliding Arc plasma exposure (40, 60 and 80sec), extracts and nanoparticles as shown in table (4). These results supported by analysis of genes associated with tumor which were P53 as tumor suppressor gene and Bcl2 (Bcl-xl) as antiapoptotic gene. The results of P53 gene proved that, transcription of its gene and finally its protein product increased leading it increase in apoptotic effect of HEPG2 cell line.

Table (2) and figure (2), presented the data of molecular analysis of genes (P53) and treatment effect for all groups. Photos (1 and 2), showed the P53 gene expression level for HEPG2 cell line protein, also shows increased protein production with treatments. The figure (3 to 9), showed the dendogram of P53 protein expression level of HEPG2 cell line protein for treatments.

Non-thermal plasma it had effects on increased transcription and protein product leading it increase in apoptotic effect of HEPG2 cell line at 40, 60 and 80sec, which were 74.33±1.07, 82.53±1.15 and 88.05±0.36%, respectively. Regarding the effect of methanolic extract of *P. incarnata* and *A. lappa* leaves. This study illustrated that, *P. incarnata* and *A. lappa* (group5 and group6), it has high effects on increased gene transcription P53 compared control (group1), which were 99.89±0.40 and 98.07±1.08%, while the result of iron oxide nanoparticles (group7), was 99.76±1.10%, compared with control group, which was 66.22±2.08%, respectively.

Table (2): Data parameters of P53 protein expression level for HEPG2 cell line protein with seven treatments.

Groups	Treatment	Lane (%)
Group 1	Control	66.22±2.08
Group 2	NTP 40Sec	74.33±1.07
Group 3	NTP 60Sec	82.53±1.15
Group 4	NTP 80Sec	88.05±0.36
Group 5	ME-PI 200µl	99.89±0.40
Group 6	ME-AL 200µl	98.07±1.08
Group 7	NIO 200µl	99.76±1.10

Group 1: Control cell line, Group 2,3,4,: treated cell line with non-thermal plasmas at 40, 60 and 80sec, Groups 5: treated cell line with methanolic extracts of *P. incarnata*, Groups 6: treated cell line with methanolic extracts of *A. lappa* and Groups 7: treated cell line with iron oxide nanoparticles, respectively.

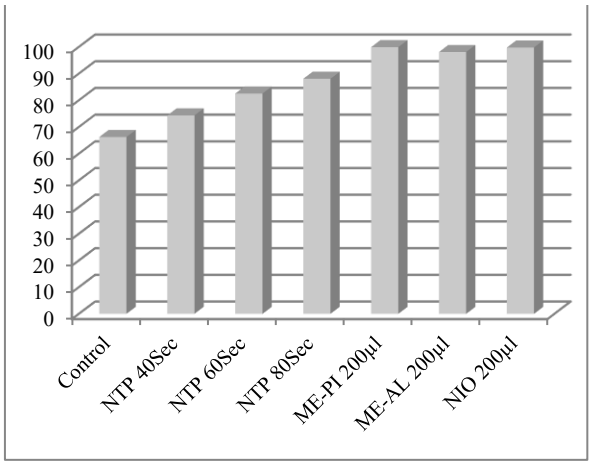


Figure (2): P53 protein expression level of HEPG2 cell line protein.

These results are in agreement with **Yan *et al.*, (2017),** who mentioned that, two types of cold atmospheric devices namely dielectric barrier discharge and plasma jet, show significantly anticancer capacity over dozens of cancer cell lines in vitro. **Li *et al.*, (2015).** studied the chrysin compound as a dietary phytochemical that is abundantly present in (*Passiflora caerulea*), which have great economic value and medicinal impact. Chrysin showed the considerably increase the apoptosis of HepG2 cancer cells. The combination of chrysin and cisplatin stabilize P53 gene expression by activating ERK1/2, which promotes P53 phosphorylation in HepG2 cells.

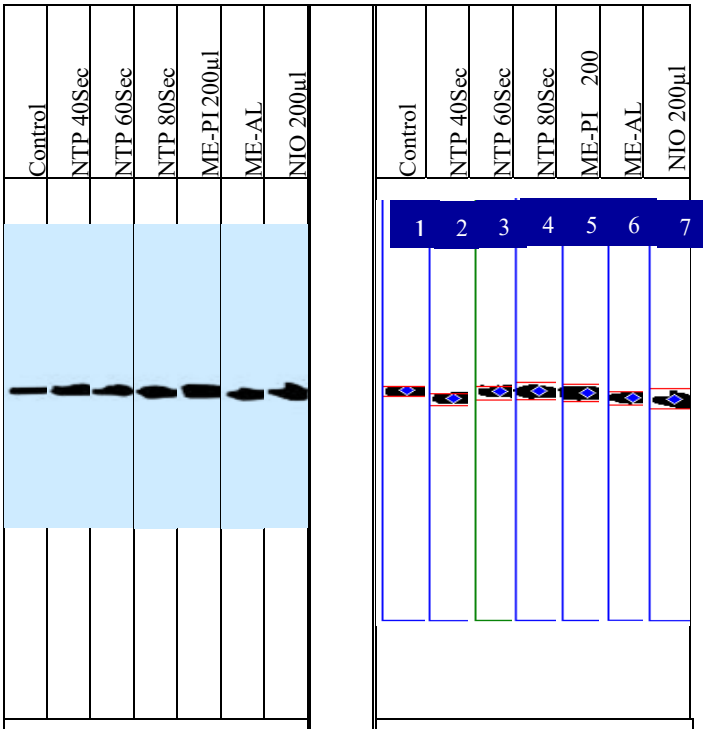


Photo (1): P53 protein expression level for HEPG2 cell line protein with seven treatments.

Photo (2): Computerized detection of P53 protein expression level for HEPG2 cell line protein with seven treatments.

Smolkova *et al.*, (2019), assessed that influence of NTP on three human liver cancer cell lines (Huh7, Alexander and HepG2) at 5, 10, 15, 25, 30, 45 and 60 sec. NTP treatment resulted in higher anti-proliferative effect against Alexander and Huh7 relative to HepG2. data showed that the NTP mediated alternation of mitochondrial membrane potential and dynamics led to ROS mediated apoptosis in Huh7 and Alexander cells. Interestingly, plasma treatment resulted in P53 down-regulation in Huh7 cells. High levels of Bcl-2 protein expression in HepG2 resulted in their resistance in response to oxidative stress- mediated by plasma.

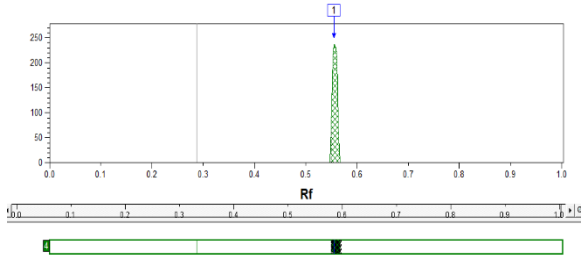


Figure (3): dendogram of P53 protein expression level of HEPG2 cell line protein for control.

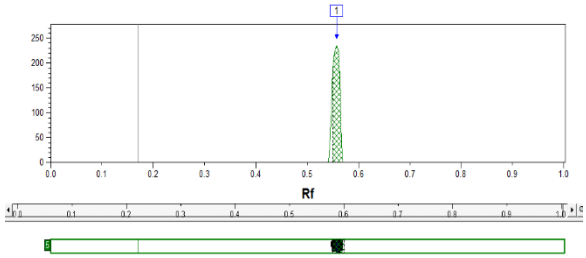


Figure (4): dendogram of P53 protein expression level of HEPG2 cell line protein for NTP 40Sec.

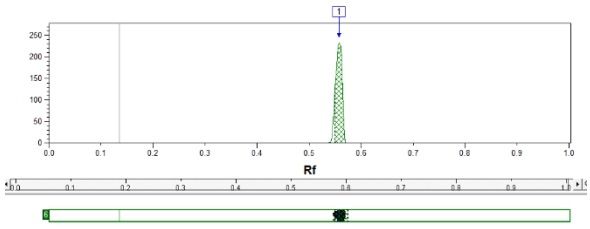


Figure (5): dendogram of P53 protein expression level of HEPG2 cell line protein for NTP 60Sec.

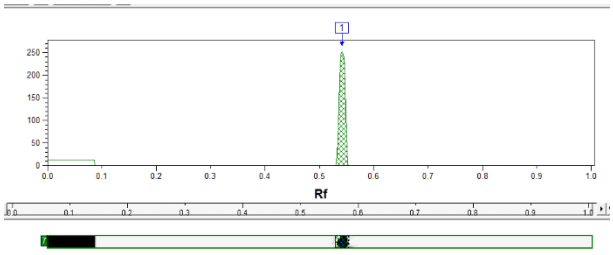


Figure (6): dendogram of P53 protein expression level of HEPG2 cell line protein for NTP 80Sec.

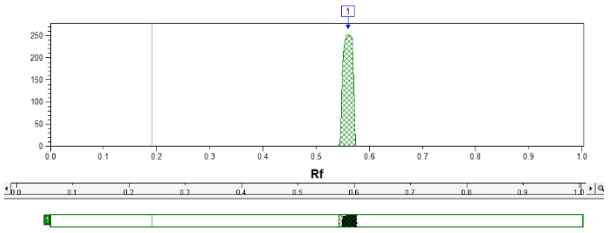


Figure (7): dendogram of P53 protein expression level of HEPG2 cell line protein for ME-PI 200µl.

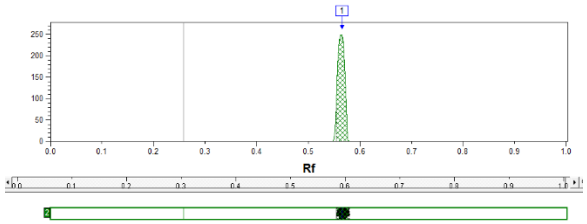


Figure (8): dendogram of P53 protein expression level of HEPG2 cell line protein for ME-AL 200µl.

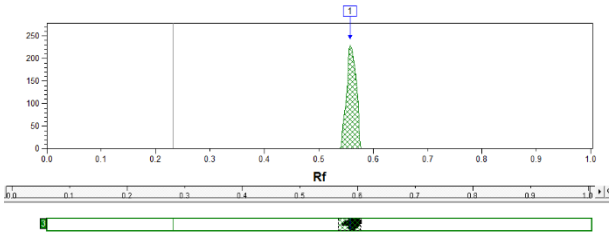


Figure (9): dendogram of P53 protein expression level of HEPG2 cell line protein for NIO 200µl.

Molecular analysis (proteomics) of gene (Bcl2):

Table (3) and figure (10), showed the data of molecular analysis of genes (Bcl2) and treatment effect for all groups. Photos (3 and 4), showed the Bcl2 gene expression level for HEPG2 cell line protein, also shows decreased protein production with treatments. The figure (11 to 17), showed the dendogram of Bcl_2 protein expression level of HEPG2 cell line protein for treatments.

Non-thermal plasma it had effects on reduced transcription and protein and increases the apoptotic effect of the HEPG2 cell line at 40, 60 and 80sec, which were 72.71±1.04, 76.54±0.20 and 67.02±1.06%, respectively. Regarding the effect of methanolic extract of *P. incarnata* and *A. lappa* leaves. Group (5) and (6), high effects on reduced gene transcription Bcl2 compared control (group1), which were 66.47±0.18 and 70.46±0.15%, while the result of iron oxide nanoparticles (group7), was 72.17±1.09%, compared with control group, which was 86.82±0.35%, respectively.

Table (3): Data parameters of Bcl_2 protein expression level for HEPG2 cell line protein with seven treatments.

Groups	Treatment	Lane (%)
Group 1	Control	86.82±0.35
Group 2	NTP 40Sec	72.71±1.04
Group 3	NTP 60Sec	76.54±0.20
Group 4	NTP 80Sec	67.02±1.06
Group 5	ME-PI 200µl	66.47±0.18
Group 6	ME-AL 200µl	70.46±0.15
Group 7	NIO 20µl	72.17±1.09

Group 1: Control cell line, Group 2,3,4,: treated cell line with non-thermal plasmas at 40, 60 and 80sec, Groups 5: treated cell line with methanolic extracts of *P. incarnata*, Groups 6: treated cell line with methanolic extracts of *A. lappa* and Groups 7: treated cell line with iron oxide nanoparticles, respectively.

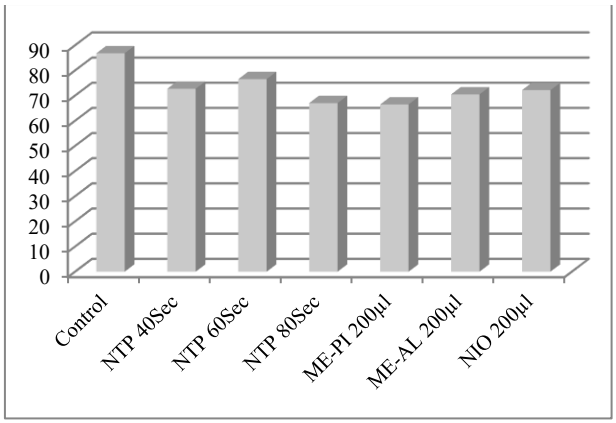


Figure (10): Bcl_2 protein expression level of HEPG2 cell line protein.

The obtained results correspondent with **Aguillon *et al.*, (2018).** who found that, the *P. edulis* leaf extract contains a higher polyphenolic content, whereas in the juice extract more polysaccharide content was observed. On the other hand, a significant decrease in viability was observed with juice extract at 400µg/ml and a significant increase in cytotoxicity by leaf extract at 25µg/ml; finally, both extracts significantly increased proapoptotic activity. **Brulle *et al.*, (2012).** showed that, the treatments non-thermal plasma (NTP) and gemcitabine present a significant antiproliferative effect on pancreatic cells and the IC_{50}, as determined using Hill slope method, was 9 sec and 10nM for NTP and gemcitabine, respectively. Combined treatment NTP with gemcitabine, was (5nM) lead to an IC_{50} of 4 sec.

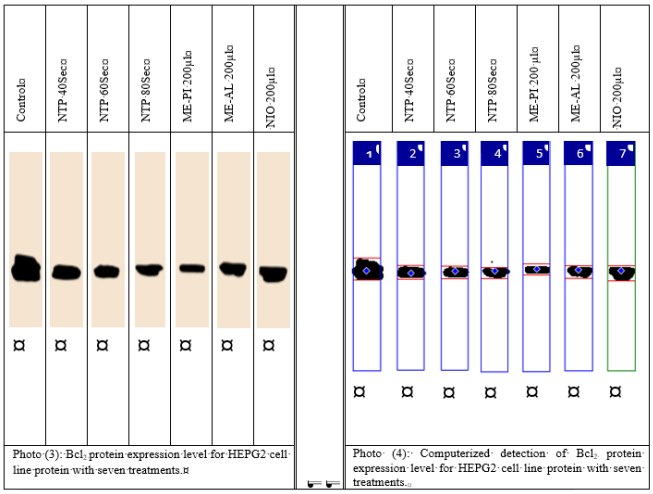


Photo (3): Bcl_2 protein expression level for HEPG2 cell line protein with seven treatments.

Photo (4): Computerized detection of Bcl_2 protein expression level for HEPG2 cell line protein with seven treatments.

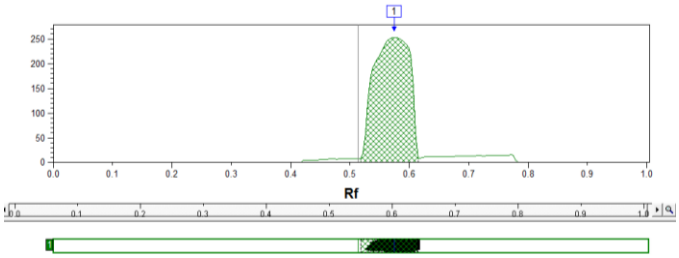


Figure (11): dendogram of Bcl_2 protein expression level of HEPG2 cell line protein for control.

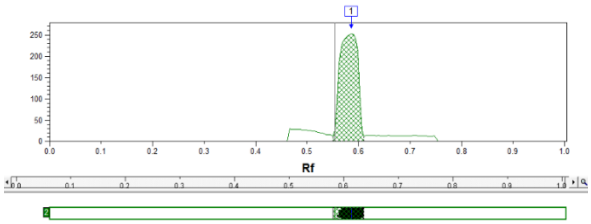


Figure (12): dendogram of Bcl_2 protein expression level ofHEPG2 cell line protein for NTP 40Sec.

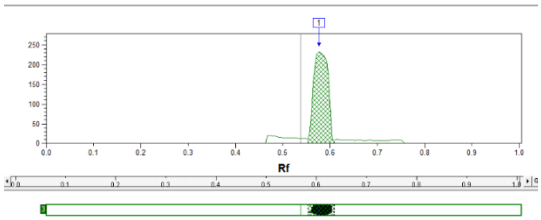


Figure (13): dendogram of Bcl_2 protein expression level ofHEPG2 cell line protein for NTP 60Sec.

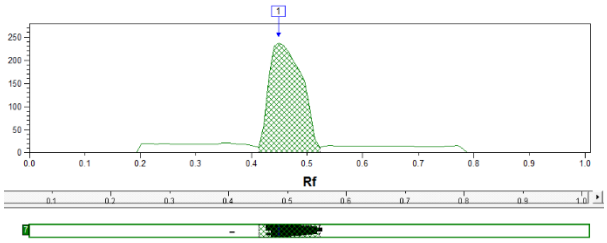


Figure (14): dendogram of Bcl_2 protein expression level ofHEPG2 cell line protein for NTP 80Sec.

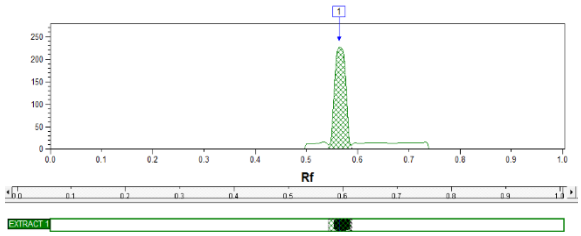


Figure (15): dendogram of Bcl_2 protein expression level ofHEPG2 line protein for ME-PI 200µl.

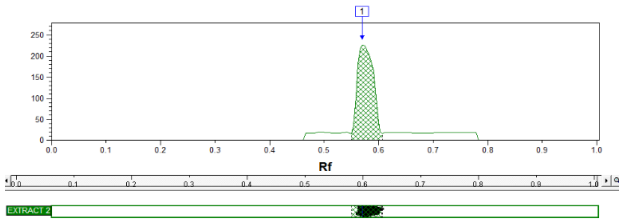


Figure (16): dendogram of Bcl_2 protein expression level of HEPG2 cell line protein for ME-AL 200µl

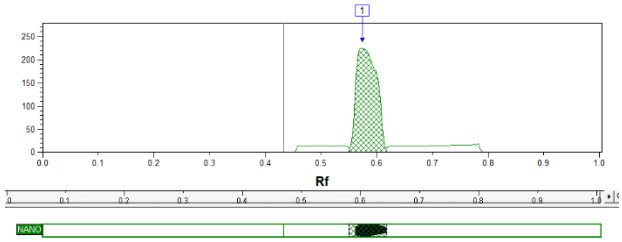


Figure (17): dendogram of Bcl_2 protein expression level ofHEPG2 cell line protein NIO 200µl.

Molecular analysis (proteomics) of gene (β-actin):

Actin participates in countless cellular functions ranging from organelle trafficking and pathogen motility to cell migration and regulation of gene transcription. Actin's cellular activities depend on the dynamic transition between its monomeric and filamentous forms, a process exquisitely regulated in cells by a large number of actin binding and signaling proteins. **Drazic *et al.*, (2018).**

The results of table (4) and figure (18), showed that, the data of molecular analysis of genes (β-actin) and treatment effect for all groups. Photos (5 and 6), showed the β-actin gene expression level for HEPG2 cell line protein, also shows convergent protein

production with treatments. The figure (19 to 25), showed the dendogram of β-actin protein expression level of HEPG2 cell line protein for treatments.

Table (10), β-actin results are shown when treated with Non-thermal plasma at 40, 60 and 80sec, methanolic extract of *P. incarnata*, methanolic extract of *A. lappa* and iron oxide nanoparticles as follows 54.21±0.08, 51.52±0.02, 51.77±0.06, 51.05±0.11, 51.04±0.09 and 50.06±1.09%, compared with control group, which was 52.97±0.01%, respectively.

Table (4): Data parameters of β-actin protein expression level for HEPG2 cell line protein with seven treatments.

Groups	Treatment	Lane (%)
Group 1	Control	52.97±0.01
Group 2	NTP 40Sec	54.21±0.08
Group 3	NTP 60Sec	51.52±0. 02
Group 4	NTP 80Sec	51.77±0.06
Group 5	ME-PI 200µl	51.05±0.11
Group 6	ME-AL 200µl	51.04±0.09
Group 7	NIO 200µl	50.06±1.09

Group 1: Control cell line, Group 2,3,4,: treated cell line with non-thermal plasmas at 40, 60 and 80sec, Groups 5: treated cell line with methanolic extracts of *P. incarnata*, Groups 6: treated cell line with methanolic extracts of *A. lappa* and Groups 7: treated cell line with iron oxide nanoparticles, respectively.

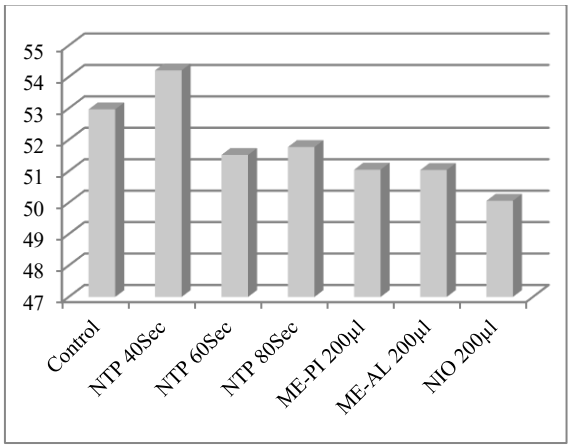
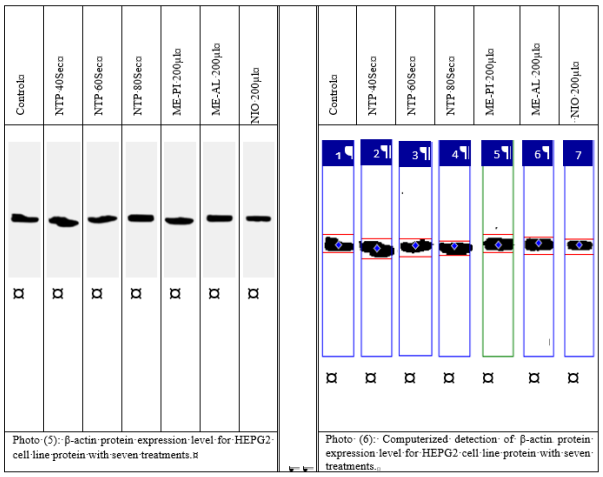


Figure (18):β-actin protein expression level of HEPG2 cell line protein.



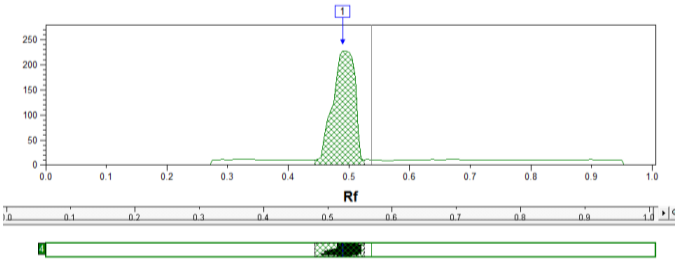


Figure (19): dendogram of β-actin protein expression level of HEPG2 cell line protein for control.

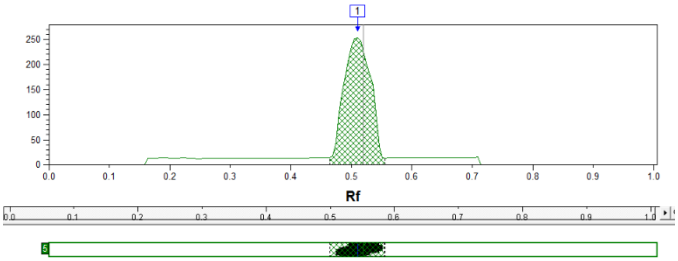


Figure (20): dendogram of β-actin protein expression level of HEPG2 cell line protein for NTP 40Sec.

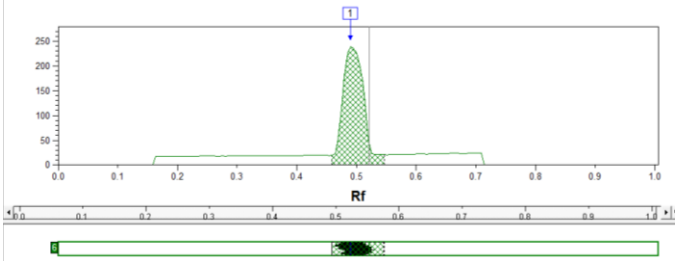


Figure (21): dendogram of β-actin protein expression level of HEPG2 cell line protein for NTP 60Sec.

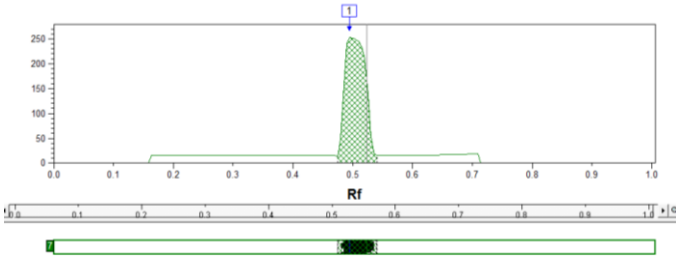


Figure (22): dendogram of β-actin protein expression level of HEPG2 cell line protein for NTP 80Sec.

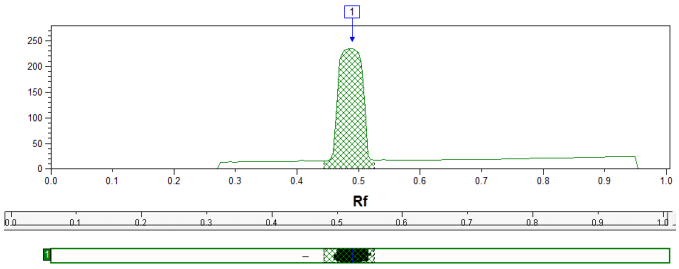


Figure (23): dendogram of β-actin protein expression level of HEPG2 cell line protein for ME-PI 200µl.

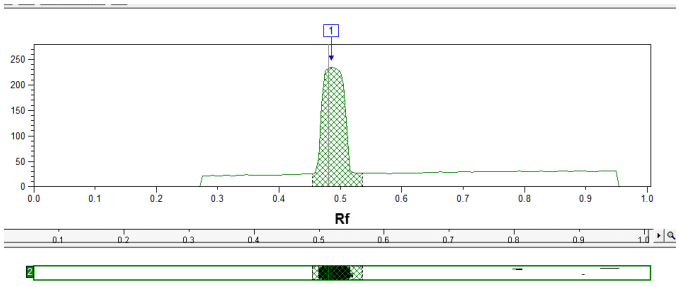


Figure (24): dendogram of β-actin protein expression level of HEPG2 cell line protein for ME-AL 200µl.

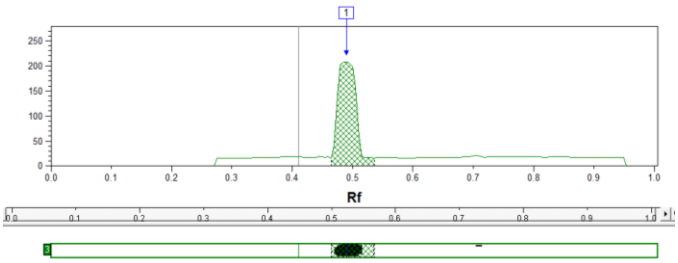


Figure (25): dendogram of β-actin protein expression level of HEPG2 cell line protein for NIO 200µl.

The results of present investigation exhibited that non-thermal plasma and iron oxide nanoparticles treatment is higher in its effect on cell death compared to biological extracts, while on the contrary, in molecular analyzes on genes, we found that the effect of extracts is the highest impact and best treatment. This is due to the ability of non-thermal plasma and iron oxide nanoparticles to penetrate cells, while extracts may neutralize negative effects and secondary compounds interact to produce a synergistic effect.

CONCLUSION

This study showed that both leaf extracts of *Passifloraceae* and *Arctium lappa* have antitumor activity in the in vitro model of liver cancer cell line (HEPG2. The results of the study showed that non-thermal plasma, biological extracts and nanoparticles have effects on increased transcription and protein products (P53), which led to an increased apoptotic effect of the HEPG2 cell line. While the same treatments showed an effect in reducing transcription and protein output (Bcl2), which proves the efficacy of these treatments in vitro cancer cells inhibition and genetically treated

Availability of data and materials: The data sets used in this study are available with the corresponding author and will be provided on a reasonable request.

Competing interest: The authors have no conflict of interest to declare.

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