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Antioxidant, Anti-Proliferative, and Induction of Apoptosis by *Phlomis samia* Methanolic Extract from Algeria

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ABSTRACT

The aim of present in vivo study was to investigate the antioxidant, antiproliferative and induction of apoptosis by Phlomis Samia Methanolic Etract (PHSME) which traditional herbal medicine from Algeria. Quantitative determination of total phenolics and total flavonoids was carried out using colorimetric methods. The total phenolic content was found to be 73,14 mg of gallic acid equivalent per gram of extract, while the content of flavonoid show a value of 21,61; 46,97 mg of quercetin, ritin equivalent per gram of extract. The antioxidant activity was evaluated in vitro with the use of free radical scavenging activity method by DPPH assay, Linoleic acid peroxidation inhibition, Estimation of total reducing power, Estimation of _OH. scavenging, Lipid peroxidation inhibition assay, results indicated that dose depend% reduction againts the previous experiments mentioned. The anticancer activity was evaluated by anti-proliferation activity with MTT assay on tow kind of cells line HepG2 and MDA. The proliferation of HepG2, MDA was significantly decreased after 48 h, 72 h incubation with PHSME at 450 ug/ml. also apoptosis and celle cycle activity were evaluated by flow cytometry methods. Apoptosis observed in Annexin V-FTTCand propidium iodide (PI) staining of cells treated with PHSME for 48 h in HepG2 MDA cells. Morover PHSME arrest cell cycle progression in G1 phase, the anticancer activity of PHSME has very potent anticancer activities, which might be useful in liver and breast cancer.

Keywords: Antioxidant activity, Apoptosis, Celle cycle, MTT, *Phlomis samia*

INTRODUCTION

Plants are potential sources of natural antioxidants because they contain phenolic compounds such as phenolic acids [1], flavonoids, tannins, and phenolic diterpenes [2-4] they are used worldwide for the treatment of diseases [5,6], and novel drugs continue to be developed through research from plants [7-9]. There are more than 20,000 species of plants used in traditional medicines, and these are all potential reservoirs for new drugs [10-12]. In recent years, considerable attention has been devoted to phenolic antioxidants from different types of plant materials [4,13]. Numerous reports of antioxidant extracts from medicinal plants have appeared, strongly inspired by an increasing consumer interest in "natural" healthy diets [14,15].

The *Phlomis* genus has been instrumental in the discovery of natural medicinal products [16]. *Phlomis* is a large genus in the Lamiaceae family, with over 100 species distributed throughout Euro-Asia and North Africa continents. They have various uses that differ from one country to another. Their flowered parts are generally used as an herbal tea to treat gastrointestinal troubles and to promote good health by protecting the liver, kidney, bone and cardiovascular system. In addition, some *Phlomis* species have culinary uses. Over the last few years, there has been a rapid increase in the information available on the structures and pharmacological activities of new compounds isolated and identified from *Phlomis* species [7].

Phlomis bovei De Noé, syn. *Phlomis samia* Desfontaines (Lamiaceae) is a rare Algerian endemic plant, commonly known as *Kayat El Adjarah* [17] in the Algerian dialect or variously named Farseouan, Tarseouan, Iniji, R'ilef and Azaref throughout the North of Africa [18,19]. It is one among the nine endemic plants recorded in the 'Rapport National sur la Diversité Biologique' [17]. The present study refers to the former, which to our knowledge has never been studied of it is apoptotic activity before [18].

The chemical constituents for these plants include flavonoids, Tannins, Alkaloids are known for antioxidant properties and apoptosis activity [20,21] However, there are only a few studies of *P. samia* extracts describing their biological activities in that could confirm their traditional use in folk medicine. In this sense, the aim of the present study was to investigate the antioxidant, antiprolifative and apoptosis effects of Methanolic extracts of the aerial parts of *P. samia* (PHSME).

Chemicals

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemicals Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic., and Mumbai.

Cell line and culture medium

Tumor cell culture

The cells were cultured at 37ºC, 5% CO_2 with relatively high humidity. The culture media for HepG2 & MDA cell lines are listed in Tables 1-3.

Plant and preparation of extracts

The plant material used in present study was collected from Algeria, Oumboigui, and authenticated by taxonomist Dr. Laouar H, Department of Botany, Ferhat Abbas University Setif. Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. 500 g of the air-dried powdered plant were macerated with MeOH 100% over night at room temperature and solvent was successively evaporated via rotary-evaporation at at 40ºC. The residue was lyophilized, and the resulting dry powder was stored at 4ºC. The Methanol extract yield from *Phlomis samia* (PHSME) was 12.78% relative to the dry starting material [22,23].

Antioxidant assays

Total phenolic determination

Total phenolic compound amount in extracts was determined by Folin–Ciocalteu method. The procedure of Price M.P. and Butler L.G. (1977) has been used [24]. The extracts at the concentration of 100 µg/ml were added to the reaction mixture and the absorbance read at 765 nm. The results are means of three repetitions expressed in the form of Gallic Acid (GA) equivalents per g of extract [24].

Total flavonoid determination

Total flavonoid compound amount in extracts was determined by method of Bhorun et al. [25].

DPPH radical scavenging

The hydrogen atoms or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical 2,2'diphenylpicrylhydrazyl (DPPH) as a reagent [26].

This experimental procedure was adopted from Cuvelier et al. [27]. The phlomis extracts at diferent concentrations (2.5, 5.0, 12.5, 25.0, 50.0 and 250 g/ml) were mixed with the same volume of 0.2 mM methanolic solution of DPPH from Sigma. The disappearance of DPPH was read spectrophotometrically (the Shimadzu 1601 UV/VIS spectrophotometer, Shimadzu Corp., Japan) at 517 nm immediately after mixing and after 1, 5 and 30 min of incubation at room temperature [26,28].
Inhibition free radical DPPH in percent (I%) was calculated in following way.

$$I\%=(A_{blank}-A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and Asample is the absorbance of the test compound.
Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration [5,6].

Tests were carried out in triplicate. Quercetin was used as standard control.

Linoleic acid peroxidation inhibition (B-Carotene–linoleic acid assay)

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [23,29]. A stock solution of b-carotene–linoleic acid mixture was prepared as following: 0.5 mg b-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 µl linoleic acid, and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen (30 min, 100 ml/min) was added with a vigorous shaking. 2.5 ml of this reaction mixture was dispersed to test tubes and 350 µl portions of the extracts prepared at 2 g/ m1 concentrations were added and emulsion system was incubated up to 48 h at room temperature. The same procedure was repeated with synthetic antioxidant, Buthylated Hydroxytoluene (BHT) as positive control, and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank [23].

Estimation of total reducing power

The total reducing power was assessed using the Prussian blue method described by Oyaizu (1986), with little modifications. To measure the total reducing power of the tested samples, each extract (2 mL) was mixed in a test tube with 2.0 mL of 2.5 mol/L phosphate buffer (PBS, pH 6.6) containing 2.0 mL of 1% K3Fe(CN)6 solution. The mixtures were incubated at 50ºC for 20 min. After the addition of 2.0 mL 10% trichloroacetic acid, each mixture was centrifuged at 3000 g for 10 min. The supernatant (2.5 mL) was collected and mixed with 2.5 mL of deionized water containing 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer, with distilled water as the blank. Trolox was used to construct the standard curve [1].

Estimation of _OH· Scavenging

Each concentration of extract (0.5 mL) was mixed with 0.5 mL of 9.1 mmol/L salicylic acid–ethanol solution, 0.5 mL of 9 mmol/L Fe2+ solution, and 3.5 mL of distilled water. Subsequently, 5 mL of 88 mmol/L H_2O_2 was added to start the Fenton reaction. The absorbance A1 of the reaction product was measured at 510 nm. The absorbance A2 was determined with 0.5 mL distilled water instead of the 9 mmol/L Fe^{2+} solution, whereas the absorbance A3 was measured using 0.5 mL of distilled water instead of the extract. The hydroxyl radical (_OH) scavenging activity was calculated using the formula:
%scavenging activity=[1-(A_1-A_2/A_3)] ×100 [1].

Lipid peroxidation inhibition assay

One millilitre of 10% rat liver homogenate was incubated with different concentrations (1.5e1000 mg/ml) of PHSME, the lipid peroxidation was initiated by the addition of 0.1 ml of FeSO4 (25 mM), 0.1 ml of ascorbate (100 mM) and 0.1 ml of KH_2PO_4 (10 mM) and the volume was made up to 3 ml with distilled water and incubated at 37ºC for 1 h. Then, 1 ml of 5% trichloroacetic acid and 1 ml of thiobarbituric acid was added to this reaction mixture and the tubes were boiled for 30 min in a boiling water bath. This was centrifuged at 3500 rpm for 10 min. The extent of lipid peroxidation was evaluated by the estimation of thiobarbituric acid reactive substances (TBARS) level by measuring the absorbance at 532 nm [30].

Antitumor activities *in vitro*

The inhibition effects of PHSME on the growths of HepG2 cells and MDA-MB-468 Human Breast Carcinoma cells were evaluated *in vitro* by MTT assay [21,31]. Cells in their exponential growth phase were seeded into flat-bottomed 96-well plates at adensity of 1 × 10^5 cells per well and incubated for 24 h at 37ºC in CO_2 incubator, then were washed with 0.10 mL of PBS. After wards, 200 uL of different concentrations of PHSME (4,5, 9, 18, 22,5, 45 mg/mL), prepared in culture medium [21,32], were added to each well. After 48,72 h of exposure, the methanoique –containing medium was removed, washed with 0.10 mL of PBS and replaced by fresh medium. The cells in each well were then incubated in culturemedium with 0.02 mL of 5 mg/mL solution of MTT for 4 h. After themedia were removed, 0.15 mL of DMSO was added toeach well to solubilize water insoluble purple formazan crystals. The absorbance was read at 570 nm using Enzyme-Linked Immuno Sor-bent Assay (ELISA) plate reader [33]. The inhibition ratio of the treatedcells was calculated based on the following formula [21]:

$$\text{Inhibition ratio (\%)} = (1 - \text{A570 of treated cells} / \text{A570 of control cells}) \times 100\%$$

Determination of apoptosis

HepG2, MDA cells were incubated with 45 mg/ml dose of PHSME for 48 h. After incubation, cells were washed twice with cold PBS and then resuspended in PBS at a concentration of 1_106 cells/ml. 5 mL of AnnexinV-FITC and PI were added to the cells. After gentle vortexing the cells were incubated for 15 min at RT in dark four hundred microliters of pbs was aded to each tube and then analysed (10.000 events) by flow cytometry within 1 h [33].

Investigation of cell cycle

Cells (1×10^5) were incubated in RPMI medium suplumented with 10% FCS with or without PHSME 45 mg/ml for 48 h after subsequent incubation cells were washed twice with PBS and centrifuged at 1200 RPM for 10 min supernatant was discarded and 500 ul of 70% ethanol was aded to the cells and incubated for 2 h after incubation cells were centrifuged at 1200 rpm for 10 min and washed with PBS twice. Supernatant was discarded and 500 ul PBS with 2 ul RNaseA were caliber added to the cells and incubated for 30 min. cells were washed with PBS by centrifuging at 1200 rpm for 10 min. supernatant was discarded and cells were suspended in 500 ul PBS with 15 ul propidium iodide (1 mg/ml) cells (10.000 events) were acquired using flow cytometer (BD, FAC caliber) [21,33].

Statistical analysis

Data are expressed as means ± S.E.M. Statistical significances were determined using a one-way ANOVA followed by the Student–Newman–Keuls post hoc test. P values ≤0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Extraction yields

The extraction yields (expressed as weight of extract relative to the weight of the initial plant material sample) is 12.78%, was influenced by several parameters, including solvent polarity and the chemical composition and physical characteristics [34,35].

Total phenols determination

Total phenolic (TP) and total flavonoid (TF) contents

In this study, the results of the TP and TF contents are presented in Table 1.

The phenolic content of the extracts was determined using the Folin–Ciocalteu method, and the values obtained were 73.14 mgGAE/g extract. Was a High concentration compared with other TP extracted from *P. umbrosa* and *P. megalantha* using different solvents varied widely, ranging from 39.43 to 55.20 mg of /g [36].

The TF content in PHSME (Table 1) were 21.61 g, 46.97 g of Quercitin & Ritin equivalents in g of extracts respectively. Which in turn is higher than that observed in the previous study was about 7.63-9.38 g from *P. umbrosa* and *P. megalantha* in same order [36].

Table 1: Total polyphenol and flavonoid contents of PHSME extract

Total phenolic (TP)	Total flavonoid (TF)	
	mg equivalent quercetin/g dry weight	mg equivalen rutun/g dry weight
73.14 ± 0.15 mg GAE/g extract.	21.61 ± 0.17 mg	21.61 ± 0.29 mg

Data were expressed as means ± SD.

DPPH radical scavenging activity

The free radical scavenging activity of the methanolic extracts was determined by the DPPH method and the results are shown in Table 2. The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods [37,38]. Percent DPPH radical scavenging activities of all the extracts concentrations were dose dependent. Similar to the reducing power, the amount of DPPH scavenging activity appeared to depend on the phenolic concentration of the plants methanolic extracts, The highest DPPH scavenging activities were shown in this extracts 32.4 µg/50% DPPH scavenging activity under the experimental conditions) and this value was comparable to each of BHT (Figure 1) This radical scavenging activity of extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. Antioxidants intercept the free radical chain oxidation by donating hydrogen from the phenolic hydroxyl groups, thereby forming stable end products, which does not initiate or propagate further oxidation [39,40]. Nitrogen based radicals such as DPPH react with phenols by two mechanisms: (i) Direct abstraction of phenol H-atom and (ii) Electron transfer from ArOH or its phenoxide anion (ArO_) to DPPH. The contribution of one pathway or another depends on the nature of the solvent and/or the redox potentials of the species involved [41]. Radical scavenging activity of phenolic acids and their esters generally depends on numbers of phenolic hydroxyl groups [42-44].There is a relatively high contents of phenolic compounds plant extract (Figure 1).

Table 2: Radical scavenging activity DPPH of PHSMEand BHT

Concentration of PHSME (mg/ml)	% of DPPH inhibition by PHSME	% of DPPH inhibition by BHT
0.069	93.65 ± 2.88	86.99 ± 0.46
0.057	84.35 ± 1.68	85.79 ± 3.91
0.053	77.81 ± 1.01	82.96 ± 1.21
0.046	66.76 ± 0.80	78.83 ± 0.21
0.034	57.03 ± 2.37	77.46 ± 1.23
0.026	45.18 ± 2.76	69.68 ± 1.23
0.023	33.09 ± 1.35	38.27 ± 2.23
0.015	27.08 ± 1.30	34.004 ± 2.18
0.011	15.17 ± 0.75	20.24 ± 2.01
0.001	6.51 ± 1.00	12.15 ± 0.92

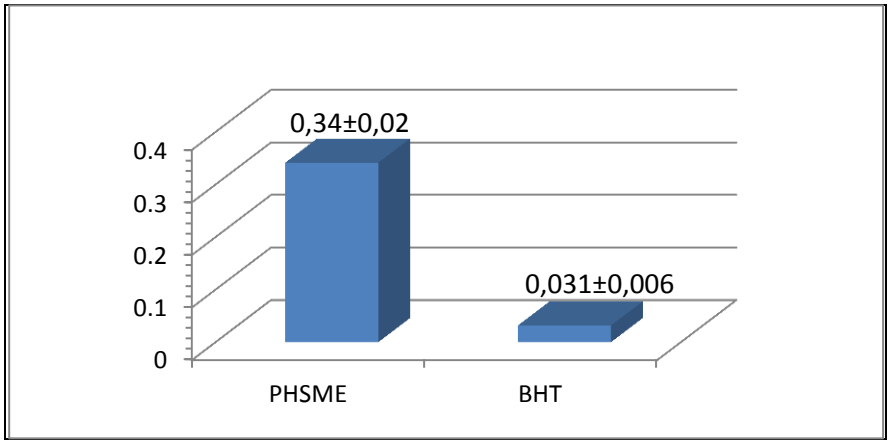

Figure 1: Extract concentration providing 50% inhibition (IC50) of DPPH*

Linoleic acid peroxidation inhibition (b-Carotene–linoleic acid assay)

The antioxidant activity in terms of peroxidation inhibition of PHSME was investigated and the results are presented in Figure 2. At a concentration of 250 µg in the final reaction mixture, the extracts inhibited 93% peroxidation of linoleic acid after incubation for 48 h. the absorbance of the control H_2O at 470 nm decreased to a minimal value after 20 h, while the methanolic extract was remains constant in the same period and decreased to 93% after 48 h. Further, this value was comparable to that of the natural antioxidant, BHT (100%). the results indicated that the equivalent antioxidant activity of extract needs exploitation of cost effective natural antioxidants.

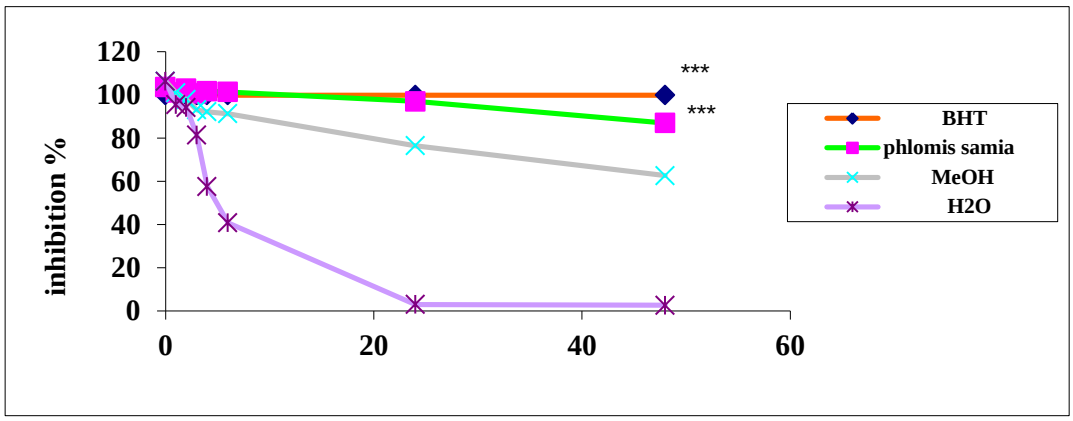

Figure 2 :% inhibition of linoleic acid peroxidation by *PH.samia* extract

The antioxidant activity of *PHSME* in the present study may be attributed to its phenolic contents. Antioxidant property is widely used as a parameter for medicinal bioactive components. The antioxidant property of *PHSME*, revealed in the present investigation may be important in future research works to study the underlying mechanism of its various medicinal properties. Further research can also explore the particular antioxidant principle(s) from the *PH, samia* extracts which can be one of the potent lead molecule(s) from the arsenal of natural products.

Reducing ability

Figure 3 shows the reductive capabilities of PHSME and Vitamin c (Vc). The reducing power of extract increased concentration dependently and it showed reducing power approaching to those of Vc. The reducing power of PHSME and Vc was dose dependent. The reducing power assay measures the electron donating ability, of antioxidants using potassium ferricyanide reduction method, antioxidant reduce form, the perl's prussian blue complex, this ability may serve as a significant indicator of it's potential antioxidant activity [44].

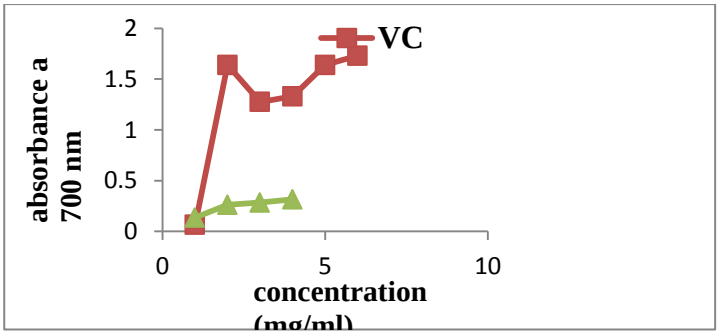

Figure 3: Reducing power activity of varying concentrations of PHSME extract with comparision to ascorbic acid

Estimation of _OH· scavenging

As shown in Figure 4, both Vc and Methanolic extract exhibited obvious scavenging activity on hydroxyl radicals in a concentration-dependent manner. The scavenging rates were improved with the increasing of concentration. This increased dramatically from 28.67% to 64.20% at the concentration range from 0.001 to 0.025 g/L for PHSME as well as the VC reached 74.97% at 0.01 g/L. When the concentration of PHSME and Vc respectively were over 0.15 and 0.05 g/L, the scavenging effect on _OH of two experience were both very significant difference. The EC50 values for PHSME and Vc were 0.22 and 0.08 g/L respectively. So Vc had a higher level of hydroxyl radical scavenging effect than PHSME. In the present study, PHSME which contained flavonoids exhibited similar activity compared with Vc in a High concentration. The result demonstrated that PHSME content was important factor which can reduce the generation of hydroxyl radicals by chelating $Fe^{2+}$and promote their reducing power.

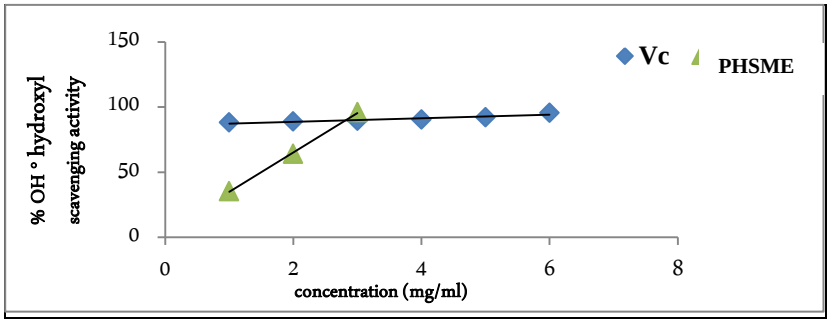

Figure 4: Inhibition of hudroxyl scavenging activity of varying concentrations of PHSME extract with comparision to ascorbic acid

Among the reactive oxygen species, the hydroxyl radical is the most reactive and could induce severe damage to adjacent biomolecules functioning in living cells, which can be prevented and/or inhibited by antioxidants. Therefore, removing the hydroxyl radical is important for antioxidant defense in cell or food systems [45].

The inhibition on liver lipid peroxidation induced by $FeSO_4$ and H_2O_2

The lipid peroxidation inhibition effects of PHSME increased with the increase of sample concentrations as shown in Figure 5.

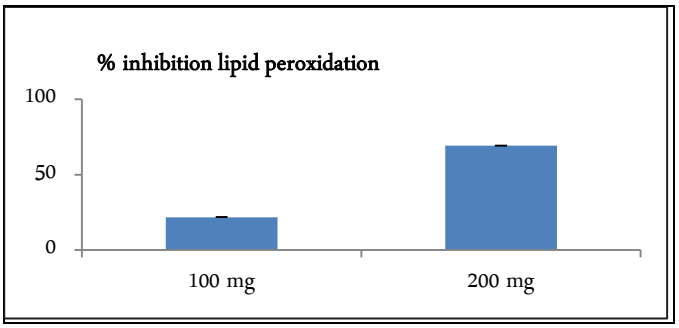

Figure 5: Inhibition of liver lipid peroxidation with PHSME

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products [46]. MDA is one of the products of lipid peroxidation, which can react with TBA, yielding a pinkish red chromogen with absorption maximum at 532 nm [47]. In the present study, $FeSO_4$–H_2O_2 system was used to induce lipid peroxidation in rats liver microsome. The lipid peroxidation inhibition effects of PHSME increased with the increase of sample concentrations as shown in Figure 5. At the concentration of 0.1 g/L, the inhibition effects was 50% and 72%, at the concentration of 0.2 g/L, According to previous studies, most polyphenols derived from plants are relatively effective natural antioxidant [30,48].

Table 3: Cell lines and media used for their growth

Cell line	Source	Cultur media
HepG2 cells	ATCC (American Type Culture Collection)	DMEM supplemented with 10% FBS, 100 units/ml of penicillin, 100 ug/ml of streptomycin, 1 mM sodium pyruvate, 2 mM glutamine.
MDA-MB-468 Human Breast Carcinoma	ATCC (American Type Culture Collection)	RPMI 1640 medium with L-glutamine, vitamins, non-essential amino acids, sodium pyruvate, 10% fetal bovine serum

***In vitro* Antiproliferative Activity**

Cell prolifer-ation was evaluated using MTT assay, After incubated with PHSME for 48 h, 72 h at the concentrations from 4,5 to 45 mg/mL. The inhibition effects of MDA, HepG2 cells were observed and compared with the control group. Our *in vitro* assay showed that the herbal extract displays strong anti-proliferative effects on the two cancer cells. These effects were found to be dose-and time-dependent (Figures 6 and 7). There was a 18% decrease of viability cells after 72 h treatment compared to those of 48 hour. The IC50 (the concentration at which the herb exhibits a 50% inhibition of tumor cell growth) for MDA, HepG2 cell lines was in the range of 2.72-5.47 mg/ml after 72-hour treatment (Table 4). Were HepG2 the most sensitive to the herbs with an IC50 of 2.7 mg/mL.

It is good to note that the herbal extract exerted a High Significantly ($P<0.05$) inhibition of viability cells, between 56-71% at the highest concentration (45 mg/mL) (Figure 1).

This study it is the first in terms of influence of PHSME on cancer cells, which is encouraging to see any other effects, and the mechanisms which are involved.

Table 4: IC50 values of the cell lines

Cel line	IC50 at 48 h	IC50 at 7 h
HepG2 cells	4,129 ± 0,063	2,729 ± 0,014
MDA-MB-468 Human Breast Carcinoma	5,06 ± 0,017	5,47 ± 0,023

The Figure represents the percent viability of MDA, HepG2 cells. PHSME decreased cell viability in a dose-dependent Data are means ± SD (n=3).
*Significantly different from control ($P<0.05$).
**High significantly different from control cells ($P<0.05$).

The Figure represents the percent viability of MDA, HepG2 cells. MEPHE decreased cell viability in a dose-dependent Data are means ± SD (n=3).
*Significantly different from control ($P<0.05$).
**High significantly different from control cells ($P<0.05$).

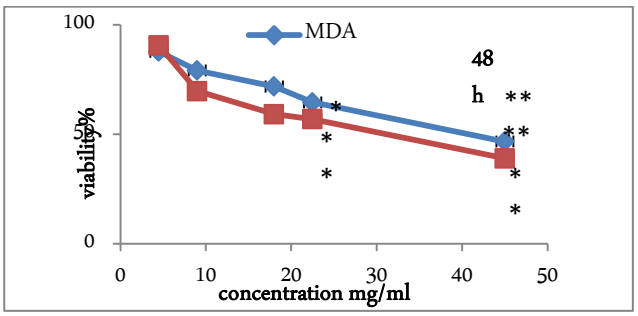

Figure 6: Effect of MEPHS treatment on the MDA, HepG2 cell viability after 48 H

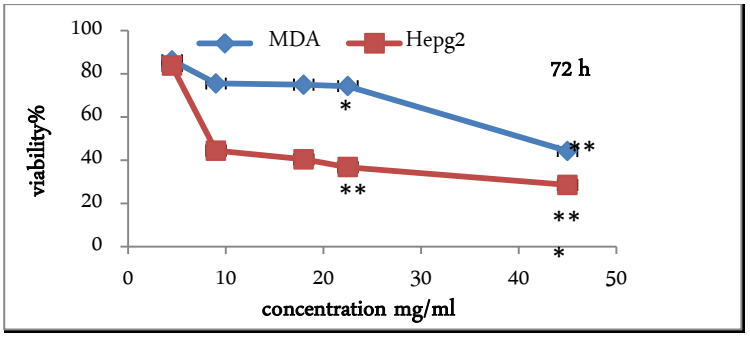

Figure 7: Effect of PHSME treatment on the MDA, HepG2 cell viability after 72 H

Effect of PHSME on apoptosis

HepG2, MDA cells were treated with PHSME (45 mg/ml) for 48 h to study the apoptosis by staining with Annexin V-FTTC(FL1) and propidium iodide. About 20% to 36% of cells were positive for Annexin V-FTTC(FL1) and PI in untreated control while around 1,5-1,7%. Cells were positive for staining in PHSME treated cells showing a marked increase in apoptosis (Figure 8).

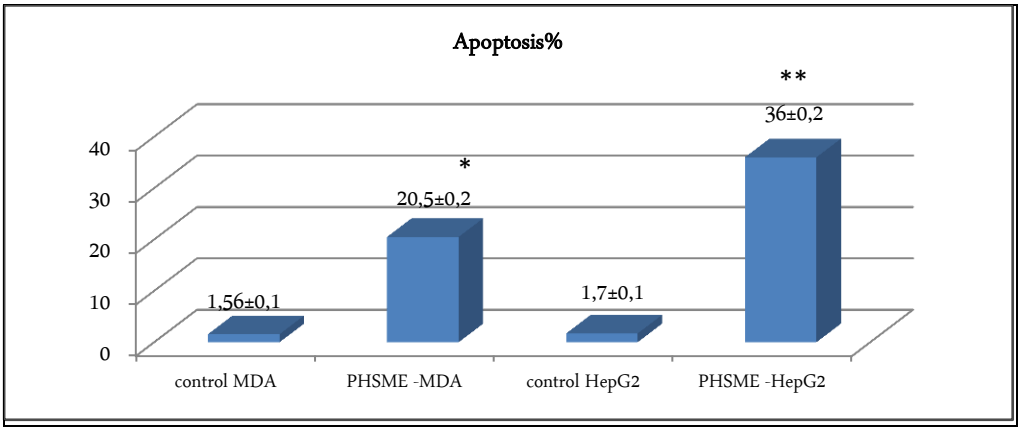

Figure 8: Effect of PHSME on apoptosis of MDA & HepG2 cells were incubated with 45 mg/ml dose for 48 h cells were stained with annexin V-FTTC(FL1)and propidium iodide FL2. Results are mean values ± SD of three indep

Effect of PHSME on cell cycle

Analysis of the cell cycle after treatement of HepG2, MDA cells with PHSME showed that, the percentage of SG1 phase was increased while the percentage of cells at G1, S M were decreased. PHSME caused a significant arrests cell cycle progression in SG1 phase (Figure 9).

The induction of apoptosis in cancer cells has been demonstrated to be an efficient strategy for cancer therapy [49,50]. A variety of stimuli are known to be capable of triggering apoptosis in cancer cells, which includes irradiation [51,52], heat shock [53,54], nitric oxide [55] and some other chemicals [49,56]. However, most of these stimuli are often facing the problem of poor specificity, since normal cells are also damaged. Therefore, it rendered such treatment less appropriate for clinical use. To find a potential apoptosis-inducing drug with high specificity for cancer cells becomes the focus of related cancer research [57].

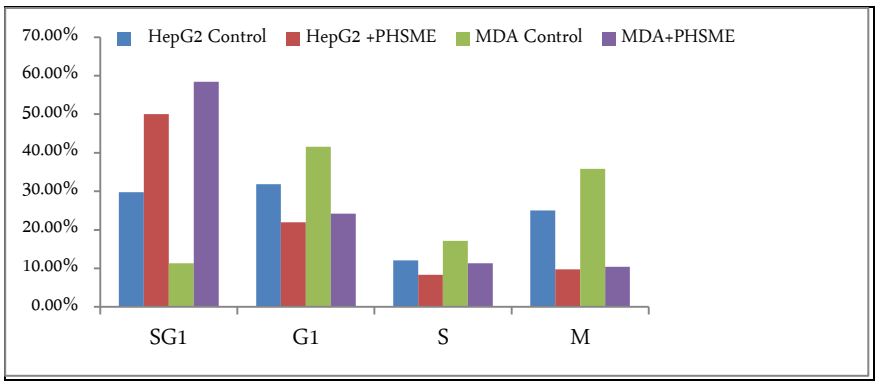

Figure 9: Cell cycle analysis by flow cytometry: the percentage of untreated cells in SG1, G1, S, M phases of the cell cycle after treatement 48 h with (45 mg/ml)PHSME in MDA & HepG2 cells. Results are mean values ± SD of three independent experiments

Chemotherapeutic drugs are known to induce cytotoxicity in tumor cells through diverse mecchanisms, in which signaling events play an important role depending upon the cell type and stumulus [33,58] there is a need to find new anticancer drugs thats can kill cancerous cells with minimal toxicity, the cytostatic effect of whole plant extracts on cancer cells is often much better than the effect of their particular biologically active compounds [59].

It is well known that plant-derived polyphenols induce thier cytotoxicity to tumor cells by ROS mediated mechanisms aour result shows that *P. samia* extract is rish with polyphenol and flavonoids witch may be can increased intracellular ROS that participate in a set of response that lead to apoptosis in HepG2, MDA cell lines [60].

PHSME may exert its cytotoxic effect on tumor cell lines through its major component polyphenols [60] which have been demonstrated to generate large amounts of intraccellular peroxides in tumor cell lines [61,62], studies have also shown that gallic acid and certain plant polyphenols are capable to indicing cytotixicity in tumor and normal cells [59,20].

Many anticancer agents arrest the cellcycle at one phase and then induce apoptosis cell death [20,21,33,63] in the present study we found that PHSME arrest cell cycle in G1 phase and induce apoptosis may be this attribute to the plant derived polyphenols including tanins and gallic acid ase well ase, tanins are well known inducers of apoptosis [31,56,64] which explain apoptosis cells death.

CONCLUSION

We concluded that *Ph. samia* extract could induce apoptosis so quickly in HepG2, MDA cells within a few hours, by arrest cell cycle in sub G1 phase and this extract is rich with compounds thats have antioxidants activity. This may again suggest that the anticancer effect of *Ph. samia* extract is the combined result of various plant constituents rather than any single component. However, further investigation is needed for more characterization on these potential active components.

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