Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(4): 174-188

Received: 23-05-2016
Accepted: 24-06-2016

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The use of some plant extracts as an alternative approaches for treatment of certain malignant cell lines

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Abstract
In Palestine like in other countries of the world, cancer is one of the most serious health problems that affect the duration and quality of the individuals' life. Enormous efforts are invested to cope with this problem, but unfortunately, limited success has ever been achieved with most of the therapeutic strategies. These efforts are usually complicated with the need for well experienced surgeons, lack of specificity and high cost, as well as being usually accompanied with a wide range of side effects.
As the conventional therapeutic strategies fail to fulfill the major requirements for a successful cancer therapy, the use of naturally developed anticancer agents has evolved as an alternative safe, low-cost and convenient one. Therefore, the use of plant extracts with potential anticancer therapeutic effects might be particularly significant, especially in Palestine, which is rich in thousands of plant species known for their medical uses.
The current study, investigates the effect of crude water extracts from Bottle gourd (*Lagenaria siceraria*), Fig (*Ficus carica)* and Nettle (*Urtica pilulifera*) on cell lines derived from different human tissue origins (Hep3b: Hepatocellular carcinoma; Hela: cervical epithelial cancer; and PC-3*:* prostate cancer).
The results showed a concentration-dependent reduction in the final number of cancer cells in consequence to treatment with the aforementioned crude extracts. Two kinds of anticancer effects were evaluated and found to contribute to this reduction: the anti-proliferation effect (decreased number of metabolically active cells) and cytotoxicity (decreased number of live cells). The three plants examined possess both of the effects with various degrees. *Urtica pilulifera* possess the strongest and most profound effects on the three cell lines, mainly by induction of cell death. On the other hand, *Lagenaria siceraria* probably affects the three cell lines by a combination of cytotoxicity and antiproliferation almost to a similar degree. *Ficus carica* most probably reduces the final number of metabolically active cells mainly by its antiproliferative effect.
Both *Ficus carica* and *Lagenaria siceraria* are edible plants that were chosen on the bases of being mentioned in the holy Quran. Therefore, although their effect is lower than that of *Urtica pilulifera* their amount in the diet or as a treatment can be safely scaled up when ingested in their native form. On the other hand, despite its possible toxicity *Urtica pilulifera* is frequently orally used as a medication in many conditions by traditional medicine.
Further studies are needed to assess the active ingredients of *Ficus carica*, *Lagenaria siceraria* and *Urtica pilulifera*, involved in the antiproliferative or cytotoxic effects of these plants.

Keywords: Plant extracts, cell line, hepatocellular carcinoma, cervical epithelial, prostate, cancer

1. Introduction
Cancer is one of the most serious health problems worldwide, affecting individuals from different sexes, ages, and races. It is a group of diseases, characterized by uncontrolled cellular growth with frequent cancer cells invasion to different body parts and spreading to other organs, a process referred to as Metastasis. Metastasis is the major cause of cancer related mortality (W. H. O. 2006) [1]. In 2005, cancer was the second leading cause of death among both men and women and accounted for 13% of the total 58 million deaths worldwide World Health Organization, 2006. In 2006, about 10.9 million new cancer cases are expected to be diagnosed worldwide and more than 7.8 million cancer patients may die (W. H. O. 2006) [1]. According to the latest report of cancer registry unit in Gaza strip, 5500 cases have been reported over the period from January, 1995 to December, 2003. In addition, 1026 cancer patients died in 2004 in the Palestinian territories with a mortality rate of 28.2 per 100.000. Palestine Ministry of Health, 2005.
Cancer is a heterogeneous illness, which can originate from many different organs of the human body.

However, the most frequent cancer types in the world are lung, prostate, stomach, colorectal, and esophagus in men; and breast, lung, stomach, colorectal and cervical in women (W. H. O. 2006) [1].

Prostate cancer is the most frequently diagnosed and the second leading cause of cancer death among men, with 234460 new cases estimated to occur in USA during 2006, and 27350 American men will die as a result of this disease (American Cancer Society. 2006) [3]. In Palestine, the mortality rate of prostate cancer was 1.4 per 100000 during the period from January 1995 to December 2002 (PMOH. 2005) [2]. Despite the fact there are several cell types in the prostate, nearly all of the prostate cancers are adenocarcinoma, originating in the gland cells (American Cancer Society. 2006) [3].

Liver cancer ranks as the sixth most common type of cancer worldwide (American Cancer Society. 2006) [3]. According to the Palestinian ministry of health, liver cancer mortality rate was 1.6 per 100000 over the period from January 1995 to December 2003 (PMOH. 2005) [2]. Many different liver related tumors are identified depending on the type of cells where they originate, from these types about 83% are hepatocellular carcinoma (HCC) that begin in the hepatocytes, the main type of liver cells.

Cervical cancer is the most common cause of cancer death among women in developing countries and the second most common cancer in women worldwide (W. H. O. 2006). It is caused by a change in the epithelial cells, which line the wall of the cervix, and the most common risk factor for this type of cancer is the human Papillomavirus (HPV) (W. H. O. 2006).

In the Palestinian territories, like in other countries of the world, cancer is becoming one of the most serious problems that affect the population's welfare and cause death. This problem is lately gaining dangerous dimensions and needs a convenient, efficient and safe medical intervention with minimal side effects and few expenses. In the last decades, there were great advances in the diagnosis of cancer as well as in the field of molecular oncology. However, the cure rate of most cancers remains low. Several strategies have been used to cure cancer among which the most common are surgery, chemotherapy, radiotherapy, and immunotherapy. Other modern approaches such as hormonal and gene therapy were proposed by researchers to replace conventional cancer therapy, with variable degrees of success (American Cancer Society. 2006, Ayesh *et al*. 2003) [3, 4]. All of these therapies have undesired side effects, they are usually not available all the time and they are expensive. For instance, in surgery the immune system is compromised due to the large amount of cortisol released subsequent to the surgery, which increase the probability of cancer relapse (Rebecca, 2004) [5]. Moreover, the current use of chemotherapy is accompanied with difficult side effects. It inhibits bone marrow stem cells proliferation leading to immune suppression (Rebecca, 2004) [5]. Radiotherapy, which is widely used in the world, is also accompanied by a great deal of side effects. Lymphocytes are most readily affected by radiation resulting in prolonged T-cell suppression (Rebecca, 2004) [5]. Other side effects such as, bone necrosis, lung fibrosis, skin revascularization, ulceration, nausea, vomiting, and renal damage are also associated with all types of conventional therapies.

As the conventional cancer therapies failed to completely fulfill the criteria for a successful cancer therapy, the use of naturally developed anticancer agents has evolved as an alternative safe, low-cost and convenient one. Nontoxic chemoprevention agents from natural resources were proposed by researchers for this purpose.

Historically, plants with known therapeutic potential have long been used to cure a wide range of diseases. An example for these drugs is Morphine, which is a plant product discovered in 1861 as an analgesic agent. Later, Quinine the active component of Cinchona bark was isolated in 1820 as an effective anti-malaria drug ((Rebecca, 2004) [5], Gary and Bryn, 2003) [6]. Our Arabic tradition is particularly rich in medical plants that have been used by pioneer Arabic physicians to establish the basis for modern therapies. These were also recommended by our Profit Mohammad (صلى الله عليه وسلم) and the Holly Quran. Nigella, Garlic, Onion and Fenugreek are famous examples for these plants that were recently proven to have therapeutic effects on several illnesses. The use of potentially curative plants might be particularly significant in the Palestinian territories where the plain and mountains are covered with more than 2600 plant species of which more than 700 are known for their uses as medicinal herbs or as botanical pesticides (Said *et al* 2002).

The main objective of the current research is to investigate the effect of Bottle gourd (*Lagenaria siceraria*), Fig. (*Ficus carica)* and Nettle (*Urtica pilulifera*), extracts on Hep3b from human Hepatocellular carcinoma; Hela: human cervical epithelial cancer; and PC-3- human prostate cancer cells *in vitro* based on Arabic and Islamic traditional medicine. To achieve our main objective, the research include the following specific aims which are the determination of the proliferation activities of each cell line in response to each plant extract treatment, the determination of percent viability of each cell line in response to each plant extracts treatment and the determination of any morphological changes of each cell line in each time performing viability testing assay in parallel.

2. Methodology

2.1 Plant collection

Three plants were studied in this thesis: Roman nettle *(Urtica pilulifera*), Bottle gourd (*Lagenaria siceraria*) and Edible fig (*Ficus carica*). They were collected between March to June.

All parts of the plant (roots, leaves, and fruits) were harvested by drawing the plant stem. The seeds were isolated form the fruits, then seeds and leaves were washed under tap water and dried in shadow places for seventh to ten days. The dried plant parts were grounded by hand and stored in dry and clean bottles until the time of experiments

2.2 Preparation of the crude plant extracts

Twenty grams of grounded dry parts of the upper-indicated parts of each plant were soaked in 80 ml distilled water (20% dry wt. /v). The extraction was carried out by using a reflux condenser at boiling temperature for 30 min. The condenser returns the extract vapor to the boiling flask. The extract was cooled at room temperature and filtered using a Buchner funnel with 0.4µm cellulose filter paper. Finally, the extract was sterilized-filtered using vacuum filter with 0.2µm cellulose filter paper (Sartorius, UK). About 80% of the extract volume was collected after filtration, and stored in sterilized bottles at 4 ˚C (Stock extract). The different working dilutions were prepared in the cell culture media as indicated (Dzhambazov *et al* 2002; Lee *et al* 2004; Sartippour *et al* 2001; Nair *et al* 2005 and Yao *et al* 2002) [11-15, 19].

2.3 Cell culture

The human cervical carcinoma cell line (Hela), hepatocellular carcinoma cell line (Hep3b) and Prostate cancer cell line PC-3 were obtained from the Hebrew university of Jerusalem. They were chosen based on their high proliferation rates and

availability. The cell lines were routinely maintained as a monolayer in Dulbecco's modified Eagle's medium (DMEM) Containing 10% fetal calf serum (inactivated at 55 °C for 30 min), 25mM HEPES (pH 7.4), penicillin (180 units/ml), streptomycin (100µg/ml) and amphotericin-B (0.2 µg/ml)(4,109). The cells were grown to confluence in a humidified incubator with 5% CO2 in polystyrene culture flasks. They were subcultured by removing the medium and adding 4-6 ml of 0.05% trypsin-EDTA solution. The cells were allowed to detach at (37 ˚C) for 5-10 min. About 1/6 of the trypsinized Hep3b or 1/4 of the other cells was passed twice a week to new flasks containing fresh medium.

2.4 Experimental design

In the first (preparatory) experiments, the extracts were prepared from the desired plant parts. The working extract concentrations were then determined by testing an array of extract dilutions on one cell type.

The working extract concentrations were tested for each plant against each of the three cell lines in terms of cellular proliferation.

The effects of the same extracts working concentration were further analyzed by viability assay to determine the type of cellular effect.

Observation of the morphological changes was carried out in parallel to the viability assays. The data was statistically analyzed and comparison of the results from different methods was done and reported.

2.5 Determination of growth characteristics

Cells from Hela, Hep3b and PC-3 cell lines were seeded in 6-well plats, at a density of 100000 cells/well, as indicated earlier. Three wells from each cell line were trypsinized and harvested after 24, 48 and 72 hours and the medium was changed for the cells continuing to grow at each time point. The harvested cells were counted on hemocytometer and the average number of three wells was used for the growth curve.

2.6 Determination of plant extract working concentrations

Plant extract-DMEM preparations were prepared by incorporation of sterile stock extracts into the DMEM, media preparation (the plant extract volume was included in final volume calculation). The highest extract-DMEM plant concentration achieved this way was 16% dry wt. /v (Stock extract-DMEM), and the desired extract-DMEM concentrations were prepared by dilution with the proper volume of complete DMEM medium.

To determine the concentration with a 100% cytotoxic effect, 200000 cells were seeded onto 25cm^2 flasks containing DMEM media for 24 hours. The medium was then replaced by 8ml of 16, 8, 4 or 0.0% plant extract-DMEM. The flasks were prepared in triplicates, and the cells were incubated in extract presence for 24 hours. The viability of cells was determined as described in the following section.

2.7 Cell Proliferation Assay

A commercially purchased colorimetric kit was used to determine the proliferation activity of cells (Biological Industries, *Biet Haemic, Israel).* The method is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to orange-colored compound of formazon. The formed dye is water-soluble and the dye optical intensity can be determined at 490 nm. The intensity of the dye is proportional to the number of metabolically active cells. The test procedure includes cultivation of cells in 96-well plates, addition of the XTT reagent and incubation for 2-24 hours, during which an orange color is formed. The grater the number of metabolically active cells in the well, the grater the activity of mitochondria enzymes and the higher the concentration of the dye formed, which can then be measured and quantified (Ofek *et al* 2003) [22].

The Hela and PC-3 cells were seeded in 96-well plats at a density of 7000 cells/well, whereas the Hep3b cells were seeded at density 5000 cells/well. The cells were maintained at (37 ˚C) for 24 hours in the presence of 100 □l DMEM. The medium was replaced with 8, 4, 2, 1, 0.5 or 0.25% plant extract-DMEM concentration in triplicates. Twenty four hours later, 50 µl of the XTT reaction solution were added to each well and the plates were incubated at 37 ˚C for 3 hours. The absorbance was measured with ELISA reader at a wave length of 490 nm. The reference absorbance (nonspecific background reading) was measured at 630nm. Negative control cells were incubated with no extract in the medium.

The cells proliferative activity was estimated by calculating the ratio of remaining viable cells in each well in comparison to the control and expressed as (% of control). Each assay was repeated for two additional times and the mean and standard error was calculated for each extract concentration (Chung *et al* 2004; Frahm *et al* 2004; Ohyama *et al* 2003 and Ofek *et al* 2003) [19-22].

2.8 Trypan-blue dye exclusion Viability assay

The trypan-blue dye exclusion assay was used to determine the plant extract- mediated cell death (Konard *et al* 2000; A so K. *et al* 2004; Fimognari *et al* 2002; Fagundes *et al* 2002.) [10, 16-18]. 200000 cells/well were seeded in 6-well plates and grown as previously described. After 24 hours the medium was replaced with different plant extract-DMEM concentration (8, 4, 2, 1, 0.5 and 0.25%) in triplicates. After 48 hours incubation the medium was discarded and the cells were harvested by trypsinization and washed twice with (PBS). A volume of 0.4% trypan-blue stain that is equal to the residual PBS was then added. After 5 min incubation, the cells were counted with a haemocytometer by compound light microscope. The unstained (viable) cells and the blue-stained (dead) cells were counted separately.

Negative control cells were incubated with DMEM media without any extract and treated the same way. Positive control cells were incubated for 10 min with 0.5mM H_2O_2 before being harvested and counted as described.

The percentage cell viability was calculated using the following equation:

% cell viability = total viable cells (unstained) X 100 / total cells (stained + unstained)

Each experiment was carried out in triplicate and repeated for one more time and the average of 6 wells was considered for each extract concentration.

2.9 Determination of morphological changes of the cells in culture

The different cell lines normally grown in 6-well plates or incubated with the desired extract concentration for the purpose of viability testing were monitored by an inverted microscope in 24 hours intervals. Any morphological changes in the cells shape, level of adhesion and any other alterations were observed and documented by photography before end of experiment (Dzhambazov *et al* 2002; Frahm *et al* 2004; Wang *et al* 1999; Cao *et al* 2005) [11, 20, 23, 24].

2.10 Statistical analysis

All values expressed as mean ± standard error of the mean by Microsoft Excel. The data were statistically analyzed by SPSS by performing the correlation, regression and one-way ANOVA tests.

For each experiment, the data obtained was blotted against extract concentration and the obtained curve equation and R^2 value were calculated by the Microsoft Excel software. The extract concentration that gives 50% or 100% reduction in the number of metabolically active cells or in viability of cells (IC_{50} and IC_{100} respectively), was determined by substitution in the obtained equation (Konard *et al* 2000; Wang *et al* 1999) [10, 23].

3. Results

3.1 Determination of the cell lines growth characteristics:

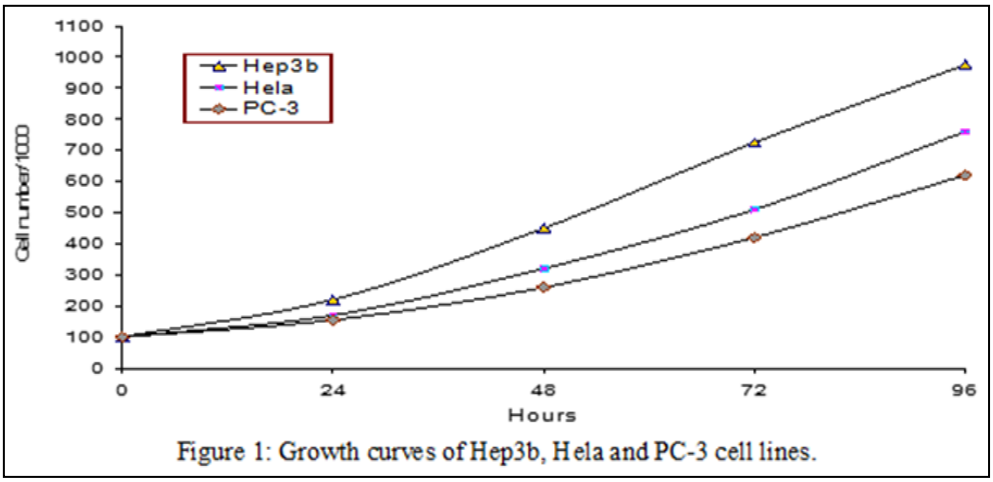

Figure 1: Growth curves of Hep3b, Hela and PC-3 cell lines.

Figure1 shows the growth curve of each of the hepatocellular carcinoma Hep3B, cervical epithelial Hela and Prostate PC-3 cell lines in normal DMEM culture media. The three cell lines maintained exponential growth characteristics until the end of experiments (96 hours). The Hep3b cells grew faster than the other two cell lines. However, no cell line growth curve reached a plateau at the end of the experiments (four days). It should be emphasized that the time of all of the following experiments did not exceeded this time.

Hepatocellular carcinoma Hep3b, Cervical epithelial Hela and Prostate PC-3 Cells were seeded at a density of 100000 cells/well. The wells were prepared in triplicates for each time point and were incubated at 37 ˚C in 5% CO2. Three wells from each cell line were harvested and counted at each time point and the rest of wells allowed continuing growing. The numbers of cells were blotted against the growth duration.

3.2 Determination of the different plant extracts working concentrations: Plant extracts-media were prepared by introducing sterile plant extracts into the DMEM culture media (the plant extract volume was included in final volume calculation). The plant extract sterilization by a 0.2µl filter was difficult to perform as the filter was blocked by the fine pieces of extract. Thus the highest plant concentration achieved (stock concentration) was 20% (wt/v). The examined extract concentrations were 0, 4, 8 and 16% for each plant type. The effect of such concentrations from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* on the viability of Hep3b cells is illustrated in figure 6. *Urtica pilulifera* concentrations less than 4% (wt. /v) showed a profound effect on the viability of cells, while 4% and higher concentrations gave maximal effect of 100% cell mortality. According to these result the gap between 0% and 4% is critical and a wider range of concentrations in this interval are necessary. *Lagenaria siceraria* gave weak reduction of Hep3b cells viability and the testing concentrations did not give more than 70% effect. *Ficus carica* had lower but considerable effect on the viability of Hep3b cells, and the highest concentrations did not reach the 50% inhibition.

From figure 2 it's noticed that concentrations lower than 4% are needed to be introduced into the experiment to assess cell death before reaching maximal values. Moreover, it seems futile to examine concentrations higher than 8% as all of the extract effects reach plateaus at those concentrations. Therefore the concentrations (0.25, 0.5, 1, 2, 4 and 8%) were used of each plant extract to be examined in the rest of the study. Any effect within this range of concentrations would be amplified theoretically if we increased the concentrations.

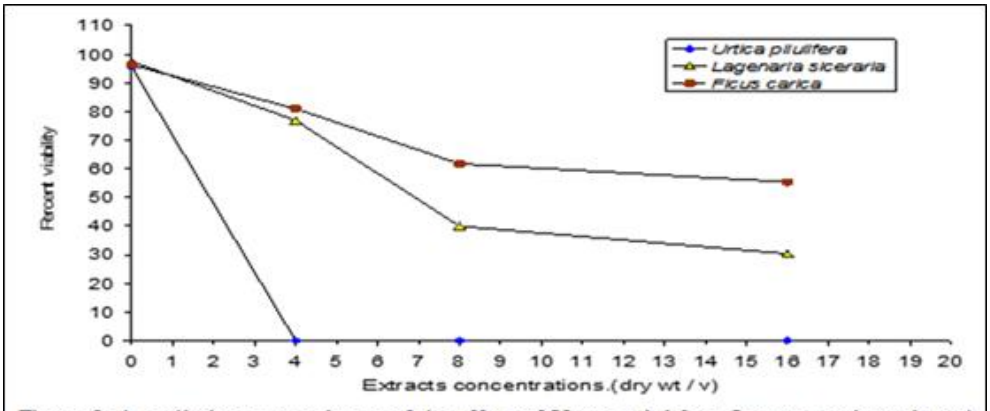

Figure 2: A preliminary experiment of the effect of *Urtica pilulifera*, *Lagenaria* siceraria and *Ficus carica* on the viability of Hepatocellular carcinoma Hep3b cells.

Hep3b cells were seeded onto 25cm^2 flasks at a density of 200000 cells/flask. The flasks were prepared in triplicates for each concentration of the different plants. After incubation at (37 ˚C) for 48h, the cells were harvested and their viability was determined by the Trypan-blue test. The percent viability of cells was calculated as stated previously and plotted against the plants concentrations.

3.3 Effect of plant extracts on cancer cells proliferation activity in culture

3.3.1. Hepatocellular carcinoma cell line Hep3b

The Human hepatocellular carcinoma cell line Hep3b was maintained in the presence of increasing concentrations of each of the three plant extracts as indicated in each experiment. The following sections describe the effect of each plant extract on this cell line in terms of proliferation activity (figure3).

3.3.1.1 Effect of *Urtica pilulifera*

Extracts from the plant *Urtica Pilulifera* were able to inhibit the proliferation of the Hep3b cells in a dose response manner. The proliferation activity of the cells was found to be significantly, inversely related to increasing the extract concentration in the medium (*P* =0.013, R =-0.86). This inhibitory effect was initiated at low extract levels and reached a 50% proliferation inhibition (IC_{50}) when the cells were grown in media with 1.9% extract concentration (R^2 =0.83). The highest proliferation inhibition level was about 85% of the control group, however was obtained at the maximum extract concentration tested (4%).

3.3.1.2 Effect of *Lagenaria siceraria*

A dose response effect was seen when the Hep3B cells were incubated with increasing concentrations of the *Lagenaria Siceraria* extracts in the culture medium. The degree of proliferation is also inversely related to the increase in medium extract content. This relationship is significant with P-values of less than or equal to 0.01and R values equal to -0.98. However, the extract inhibitory effect was initiated at higher concentrations than in the case of *Urtica Pilulifera*. Higher concentrations were thus needed to reach a 50% proliferation inhibition (IC_{50}=3.4%, R^2= 0.97). The maximum extract concentration tested (8%) was able to reach proliferation inhibition levels as high as in the case of *Urtica pilulifera* (>85% of the control group).

3.3.1.3 Effect of *Ficus carica*

The results in figure 3, indicate that the proliferation of the Hep3B cells is inversely related to the increased levels of *Ficus carica* extracts in their culture media. This behavior is significant with P-values of ≤0.005 and R =-0.908. The extract concentrations needed to elicit a considerable inhibitory effect were higher than the *Urtica pilulifera* and *Lagenaria siceraria* extracts (IC_{50}=5.7%, R2=0.87). The maximal inhibition observed (60% of the control group) was seen in media with 8% extract content.

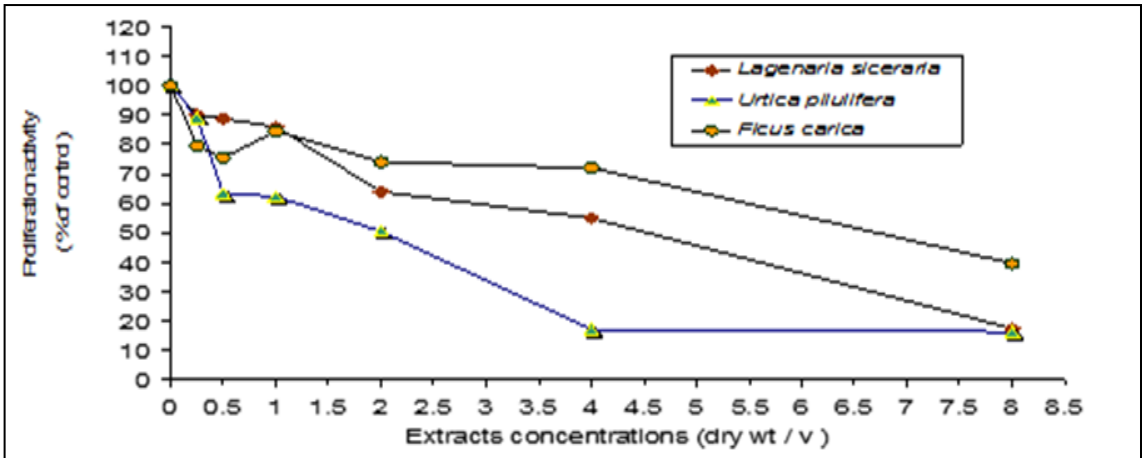

Figure 3: Reduction of proliferation activity of Hep3b cells in response to plant extracts from *Urtica pilulifera, Lagenaria* **siceraria** and *Ficus carica.*

5000 Hep3b cells/well were seeded in 96-well plates. The cells were grown in DMEM culture media containing 8, 4, 2, 1, 0.5 or 0.25% of the indicated plant extract in triplicates. The cells' proliferation activity was determined by the tetrazolium salt XTT assay as described in the material and methods. Each experiment was repeated for additional two times and the average of a total of 9 wells was calculated for each concentration. The results were calculated as percent of the control group with no plant extract and blotted against the respective extract concentration.

3.3.2 Cervical epithelial cell- line Hela

The cervical epithelial cell line Hela was grown in the presence of increasing concentrations of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*. The following sections depict the effects of each plant extract on Hela cells proliferation activity (figure 4).

3.3.2.1 Effect of *Urtica pilulifera*

Urtica pilulifera extract showed a steep inhibitory effect on the proliferation of Hela cells (figure 8). The proliferation activity of the cells was found to be inversly related to the increase in extract concentration. A dose rsponse effect was initiated at the lowest extract level used in the experiments (0.25%), and a 50% inhibition of the cells proliferation activity was achevied at extract level of (0.132%). Maximum inhibitory level of the cells proliferation was reached at relatively low concentrations 15.6% (R^2=0.6022).

3.3.2.2 Effect of *Lagenaria siceraria*

The proliferation activity of Hela cells was inhibited in a dose response manner by *Lagenaria siceraria*. The proliferation activity of cells was found to be significantly, inversely related to the increase in extract concentrations (*P*<0.01, R=-0.938). The inhibitory effect of this extract was also started at the

lowest extract concentration used (0.25%) however higher concentrations were needed (3.1%) to reach a 50% inhibition, than in the case of *urtica pliulifera*. Higher concentration also were needed to reach the maximum levels of inhibition than in the case of *urtica pilulifera*.

3.3.2.3 Effect of *Ficus carica*

The results indicate that *Ficus carica* extract is also capable of reducing the Hela cells proliferation in a concentration dependent manner. The proliferation activity is also inversely related to the increase in extract concentration. This relationship is significant with $P \leq 0.05$ and R= to -0.87. Although a considerable inhibitory effect was induced by lower concentrations, IC_{50} was reached at 3.1% extract concentration. The effect of *Ficus carica* is similar to that of *Lagenaria siceraria*, but with minor differences.

7000 Hela cells/well were seeded in 96-well plates. The experiment conditions were as in figure 3.

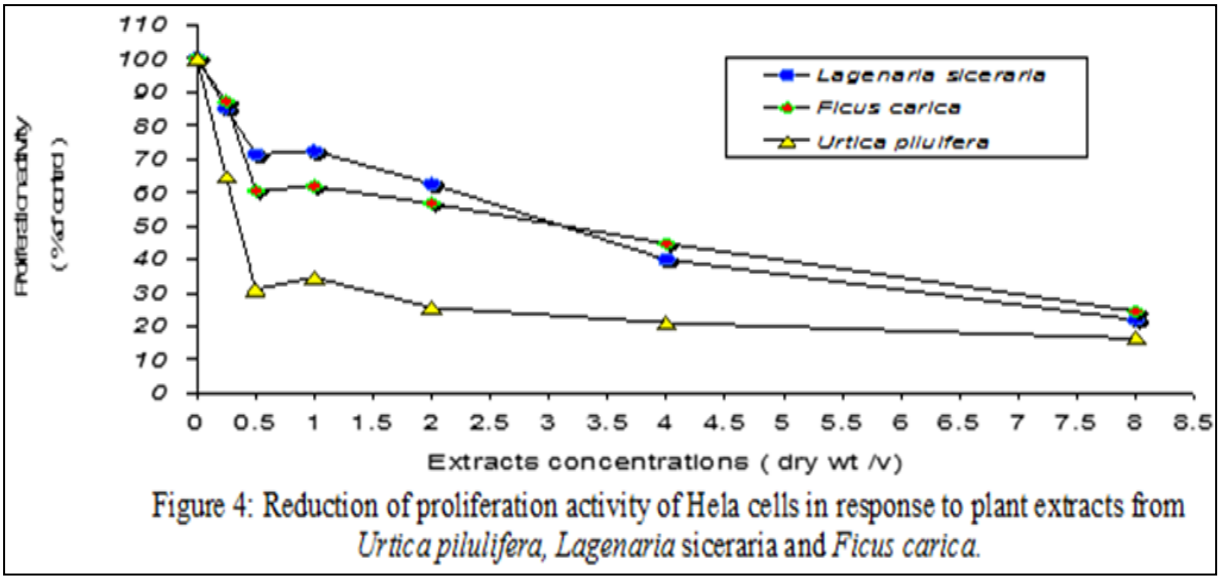

Figure 4: Reduction of proliferation activity of Hela cells in response to plant extracts from *Urtica pilulifera, Lagenaria* siceraria and *Ficus carica.*

3.3.3 Prostate cell line PC-3

The prostate cells PC-3 were incubated in the presence of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* of different concentrations. The inhibition of proliferation activity of PC-3 is illustrated in the following sections (figure 5).

3.3.3.1 Effect of *Urtica pilulifera*

Urtica pilulifera extract showed a strong inhibition of cell proliferation in a dose response manner. The proliferation activity is inversely related to the increase in extract concentration. This relationship is significant with *P*=0.004 and R= -0.916. The 50% proliferation inhibition (IC_{50}) was achieved at 2.3% extract content in the medium. 4% extract concentration was able to reduce the proliferation activity to reach about 10% only.

3.3.3.2 Effect of *Lagenaria siceraria*

The results indicate that the proliferation activity of PC-3 cells is inversely related to the increased levels of *Lagenaria siceraria* extracts concentration. Although this proliferation inhibition was weaker than *Urtica pilulifera* effect, a significant dose response manner was shown (*P*=0.00, R=-0.995). A relatively high extract concentration 7.9% was necessary to reach 50% proliferation inhibition.

3.3.3.3 Effect of *Ficus carica*

Ficus carica extract showed a significant inhibition of PC-3 proliferation activity *P*= 0.001, R= -0.954. The lowest concentrations of this extract were able to induce inhibitory effects more than the other extracts; despite of this noticeable effect, IC_{50} was 5.5% extract concentration.

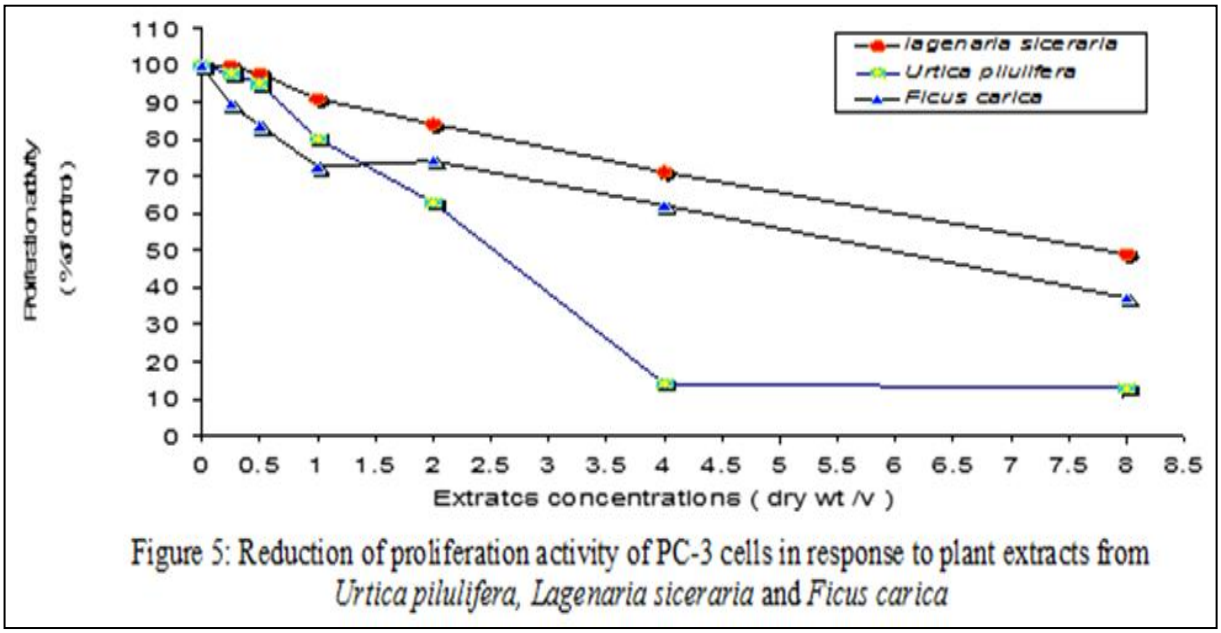

Figure 5: Reduction of proliferation activity of PC-3 cells in response to plant extracts from *Urtica pilulifera, Lagenaria siceraria* and *Ficus carica*

4. Effect of plant extracts on cancer cell lines viability in culture

4.1.1 Hepatocellular carcinoma cell line Hep3B

Hep3b cells viability was determined in the presence of increasing concentrations of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*. Figure 10, depicts the percent viability of Hep3b cells after incubation for 48 hours with different extracts at different concentrations. Hep3b cells which were maintained in DMEM without any plant extract have a percent viability ranging from 92 to 94.5% according to the three experiments.

4.1.1.1 Effect of *Urtica pilulifera*

The percent viability of Hep3b cells was found to be significantly, inversely related to the increase in *Urtica pilulifera* extract concentration (P=0.03, R=-0.787). The lowest concentration 0.25% of this extract was capable to reduce the percent viability of the cells by slightly less than 30%. The 50% viability reduction was obtained by 2.1% plant extract concentration. At 4% extract, concentration 100% reduction in cell viability was already obtained.

4.1.1.2 Effect of *Lagenaria siceraria*

The percent viability of Hep3b cells was reduced in a significant, dose response manner due to treatment with *Lagenaria siceraria* (P=0.002, R=-0.937). Although there is no noticeable inhibitory effect at the lowest concentration, the highest concentration examined (8%) was able to elicit viability reduction by 60%. In contrast with *Urtica pilulifera* behavior *Lagenaria siceraria* effect is weaker, and IC_{50} was obtained at 7.1% extract concentration.

4.1.1.3 Effect of *Ficus carica*

The results show that increasing the levels of *Ficus carica* in the DMEM media is inversely related to the percent viability of Hep3b cells. This behavior is significant with P= 0.001 and R=-0.94. Despite of that both *Ficus carica* and *Lagenaria siceraria* behave similarly at most of the concentrations examined, higher *Ficus* extract concentration (18.1%) are estimated to be needed for reaching the 50% reduction in cell viability, than *Lagenaria siceraria* extract (6.7%). The difference between both extracts arrows at high concentration (8%).

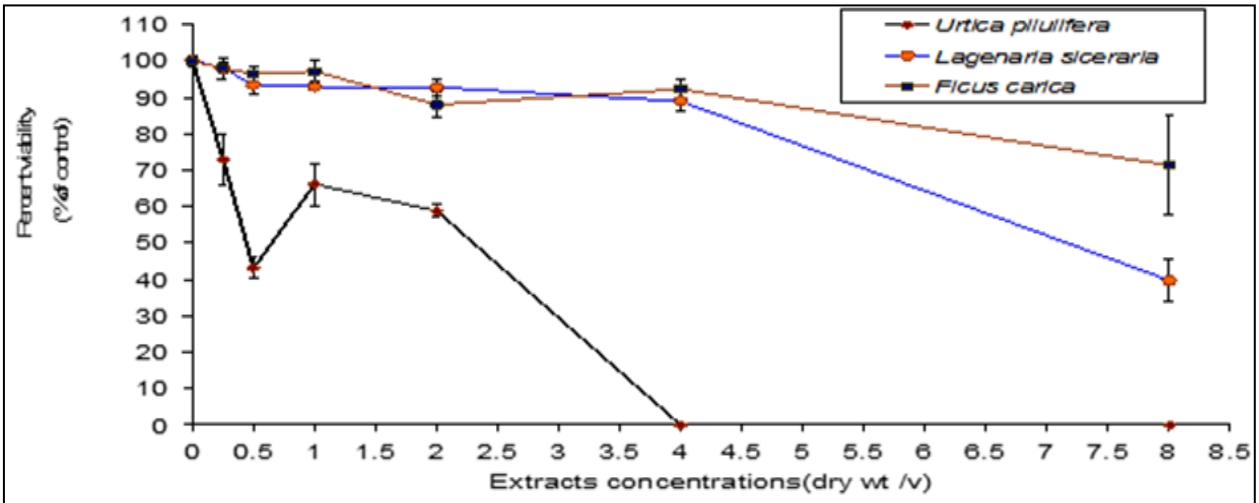

Figure 6: Reduction of Hep3b cells viability in response to extracts from *Urtica pilulifera, Lagenaria siceraria* and *Ficus carica.*

20000 Hep3b cells/well were seeded in 6-well plates. The cells were grown in DMEM culture media containing 8, 4, 2, 1, 0.5 or 0.25% of the indicated plant extract in triplicates. The percent viability was determined by the Trypan-blue test as described in the materials and methods. Each experiment was repeated for one additional time and the average ± Standard error of the mean, of a total of 6-wells was calculated for each concentration. The results were calculated as percent of the control group with no plant extract and blotted against the respective extract concentration.

4.1.2. Cervical epithelial cells Hela

The percent viability of Hela cells was determined in the presence of increasing concentrations of *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* plant extracts. Figure11 shows the effect of each plant extract on this cell line in terms of percent viability. In normal conditions without any, extract treatment, the percent viability ranges from 90 to 95% according to the results of the three experiments.\

4.1.2.1 Effect of *Urtica pilulifera*

Urtica pilulifera extract shows a steep and strong reduction of cell viability in a dose response manner. This effect is significant with P=0.004, R=-0.911. This effect was noticeable at 1% extract concentration, whereas to reach 50% viability reduction, 2.8% extract concentration was required. A complete elimination of viable cells was obtained at 4% extract concentration, and maintained at higher concentrations.

4.1.2.2 Effect of *Lagenaria siceraria*

The results illustrated in figure 7 indicate that *Lagenaria siceraria* extract causes a significant viability reduction with P=0.00 and R=-0.982. This inhibitory effect is weaker than the effect of Urtica pilulifera with IC_{50} equals to 14.1% *Lagenaria siceraria* extract concentration.

4.1.2.3 Effect of *Ficus carica*

Ficus carica behaved similar to *Lagenaria siceraria* extract in causing a considerable reduction in percent viability of Hela cells. A dose response effect was observed and significant with P= 0.003, R=-0.921. The fifty percent inhibition of cell viability was at 15.7%. No cell viability reduction was achieved as much as in the case of *Urtica pilulifera* even with the highest concentration (8%) of both *Lagenaria siceraria* and *Ficus carica*.

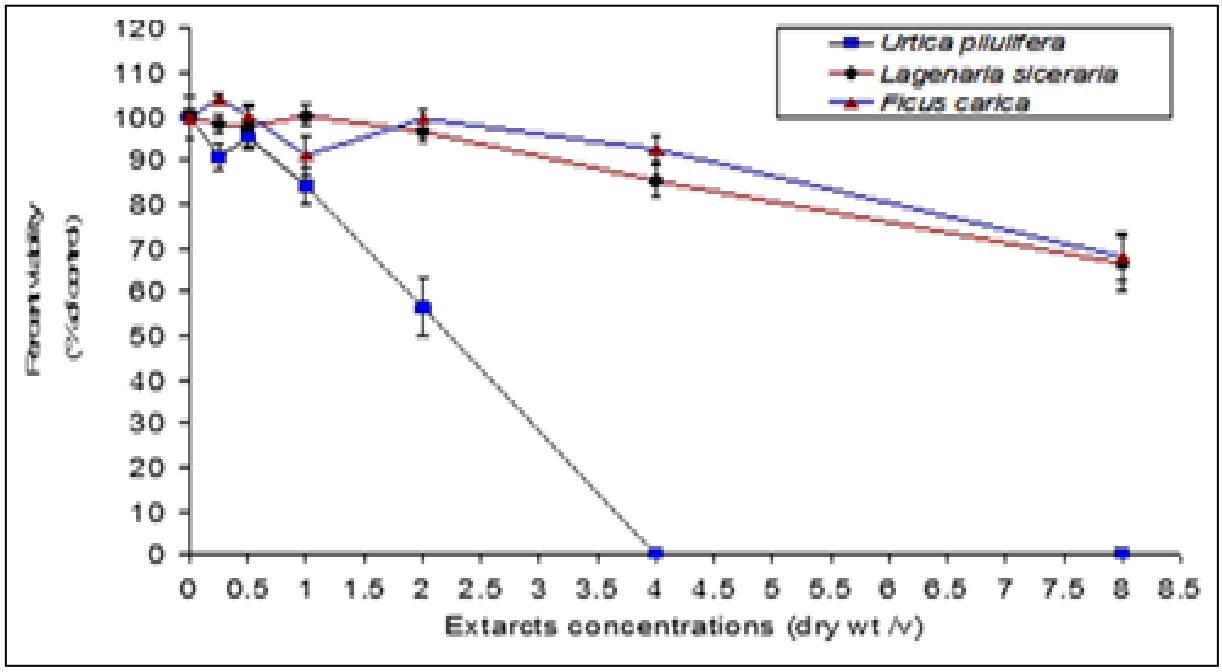

Fig 7: Reduction of Hela cells viability in response to extracts from Urttica a pilulifera, Lagenria siceraria and Ficus caria. The experiments condition as figure 6

4.1.3 Prostate cell line PC-3

Figure 8 shows the percent viability of PC-3 cells grown in the presence of increasing concentrations of plant extracts from *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*. The following sections describe the effect of each plant extract on this cell line in terms of percent viability. In normal conditions without any extract addition, PC-3 cells showed a lower percent viability than Hep3b and Hela cells. According to the three different experiments, the percent viability of PC-3 at normal DMEM was ranging between 85.5 and 90.5%.

4.1.3.1 Effect of *Urtica pilulifera*

Percent viability of PC-3 affected by *Urtica pilulifera* extract was significantly inhibited in a dose response manner (P= 0.00 and R= -0.992). A smooth and a steep relationship was initiated at the lowest extract concentration. The 50% viability was obtained by 3.6% extract concentration, while 8% extract concentration resulted in 100% elimination of any viable cells.

4.1.3.2 Effect of *Lagenaria siceraria*

The percent cell viability was found to be significantly, inversely related to the increase in extract concentrations (P= 0.001, R= -0.955). Despite of that this effect is weaker than the previous extract effect, IC_{50} of *Lagenaria siceraria* was achieved at 9.4% extract concentration.

4.1.3.3 Effect of *Ficus carica*

A dose response effect was also seen when PC-3 was grown in increasing concentrations of *Ficus carica*. Percent viability was significantly, inversely related to the increasing concentrations of this extract (P=0.00, R =-0.974). Higher concentrations were needed to reach a 50% viability inhibition (IC_{50} =13.8% extract concentration).

5. Determination of morphological changes of cell lines in response to treatment of different plant extracts

The morphological characteristics of the Hep3b, Hela and PC-3 cell lines were evaluated in parallel to each time performing viability testing assay. In each viability testing experiment the 6-well plates were examined for any morphological changes in response to treatment with the three plant extracts (*Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*) compared to the normally maintained cells (no treatment). Representative wells were pictured and shown in this study (figure 9, 10 and 11). A few cell culture flasks were frequently monitored at short time intervals.

5.1 Hepatocellular carcinoma cell line Hep3B

The normally maintained Hep3b cells (no extract) were proliferating with high rate, and formed a monolayer growth with no less than 90% confluence within 48 hours (figure 9a).

5.1.1 Effect of *Urtica pilulifera*

Urtica pilulifera showed noticeable morphological alterations with weaker adhesion level at different concentrations as evident by the cells floating. After 48 hours, the 2% extract concentration showed a potent activity. It induced Hep3b cells to round and form a grape-shaped cluster. At 24 hours after treatment of hep3b with 4 and 8%, extract most of cells were detached and aggregated in clusters (figure 12b)

5.1.2 Effect of *Lagenaria siceraria*

Hep3b cells treated with *Lagenaria siceraria* 4 and 8% concentrations for 24 hours suffered from noticeable alterations such as shrinkage, sparse, and spindle shape formation (figure12c). At the highest concentration (8%) the level of adhesion was reduced and many cells were detached.

5.1.3 Effect of *Ficus carica*

Ficus carica treated cells showed a high proliferation rat at low extract concentrations (0.25 and 0.5%). While at 4% extract concentration their growth was inhibited and slight cell shrinkage was observed (figure 12d). On the other hand 8% *Ficus carica* extract concentration induced obvious alterations to the cells morphology such as, the rounding up and detachment of many cells.

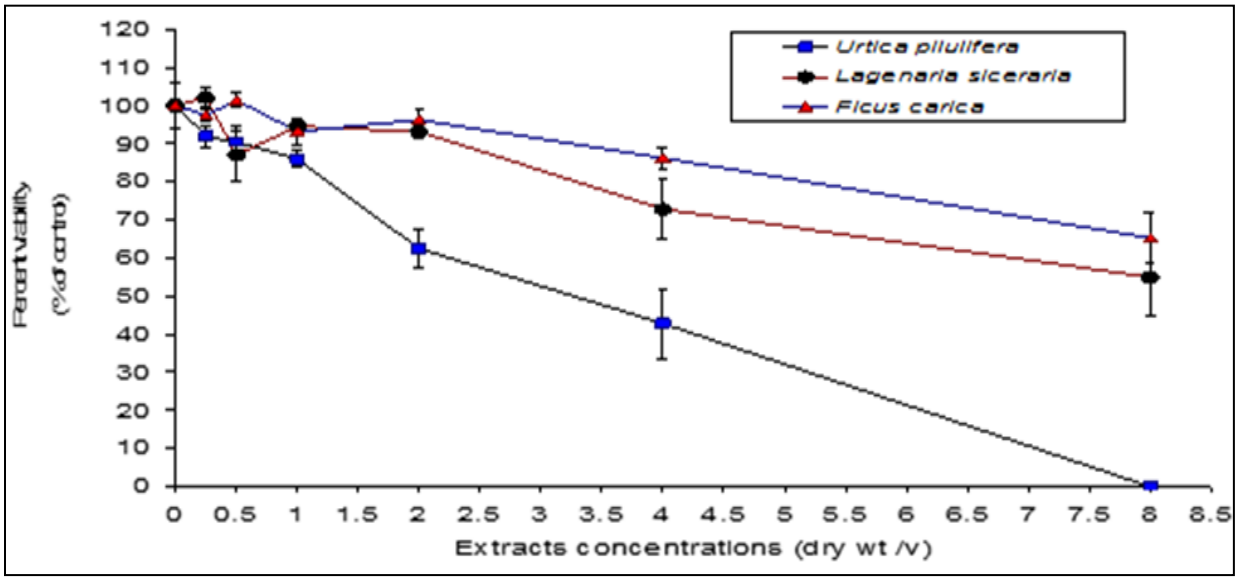

Fig 8: Reduction of PC-3 cells viability in response to extracts from *Urtica pilulifera, Lagenaria siceraria and Ficus carica*. The experiment conditions where as in Figure 6.

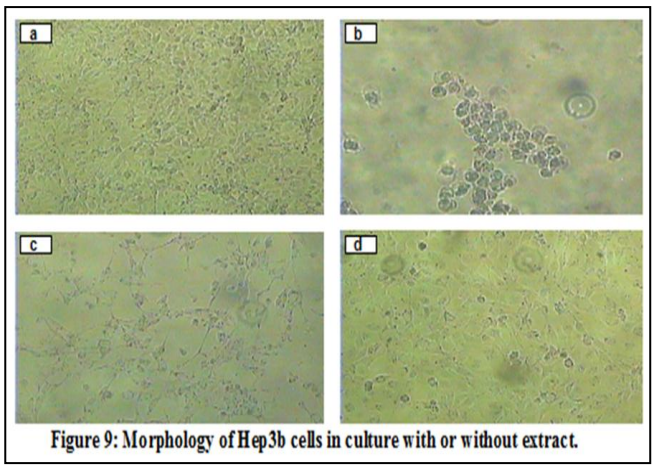

Figure 9: Morphology of Hep3b cells in culture with or without extract.

Hep3b cells were grown in DMEM culture medium without any extract (a) or with 4% *Urtica pilulifera* extract for 24 hours (b), 8% *Lagenaria siceraria* extract for 48 hours (c) and 4% *Ficus carica* for 48 hours (d). Cells in a, c and d were visualized by inverted microscope at 10X and in b at 20X.

5.2 Cervical epithelial cells Hela

The proliferation rate of Hela cells maintained in normal media (without any extract) were lower than Hep3b, as about 80% confluence was obtained after 48 hours of growth (figure 13a). Apparent alterations in cell morphology and detachment of cells from the culture surface were observed after 24 and 48 hour of treatment with different extract concentrations.

5.2.1 Effect of *Urtica pilulifera*

Five to ten hours after treatment of Hela cells with *Urtica pilulifera* (2, 4 and 8% concentrations) the cells began to round up and the level of adhesion was influenced. Many cells detached easily from the surface of the plastic flasks and moved into medium. After 24 hours of treatment with 2% *Urtica pilulifera*, most of cells were rounded and formed sparse clusters (figure 13b). Higher concentrations (4 and 8%) had more pronounced effects and nearly all cells were rounded up and detached.

5.2.2 Effect of *Lagenaria siceraria*

When Hela cells were treated with 2% *Lagenaria siceraria* extract for 24 hours a number of cells were dead as evident by cellular rounding up, floating and fragmentations. The time and concentration dependent extract effect was observed in the degree of confluence. (Figure 13c) illustrates the effect of 8% extract concentration after 48 hours of incubation.

5.2.3 Effect of *Ficus carica*

Treatment of Hela cells with *Ficus carica* 4 and 8% for 48 hours resulted in reduced density of the monolayer, and some of the cells were round and detached (figure 13d).

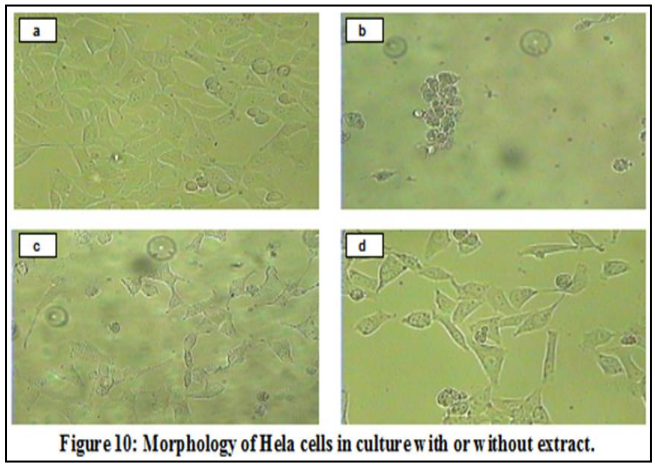

Figure 10: Morphology of Hela cells in culture with or without extract.

Hela cells were grown in DMEM culture medium without any extract (a) or with 2% *Urtica pilulifera* extract for 24 hours (b), 8% *Lagenaria siceraria* extract for 48 hours (c) and 8% *Ficus carica* for 48 hours (d). Cells in all were visualized by inverted microscope at 20X.

5.3 Prostate cell line PC-3

Figure (14a) shows PC-3 cells which are characterized by a considerably lower proliferation rate than both Hela and Hep3b cell lines in normal conditions (without any extract).

5.3.1 Effect of Urtica pilulifera

After 24 hours of PC-3 treatment with 4% *Urtica pilulifera* the cells were rounded up, and detached from the monolayer (figure 14b). There were relatively large amounts of cellular fragments and cytoplasm condensation that appeared at 4% *Urtica pilulifera* concentration.

5.3.2.1 Effect of *Lagenaria siceraria*

PC-3 cells treated with 4 and 8% *Lagenaria siceraria* showed a noticeable decrease in confluency after 48 hours of treatment, but no evident morphological changes (figure 14c).

5.3.2.2 Effect of *Ficus carica*

The reduction of confluence degree was visible in PC-3 cells when incubated for 48 hours in the highest extract concentration used, but also with no noticeable morphological changes (figure 14d).

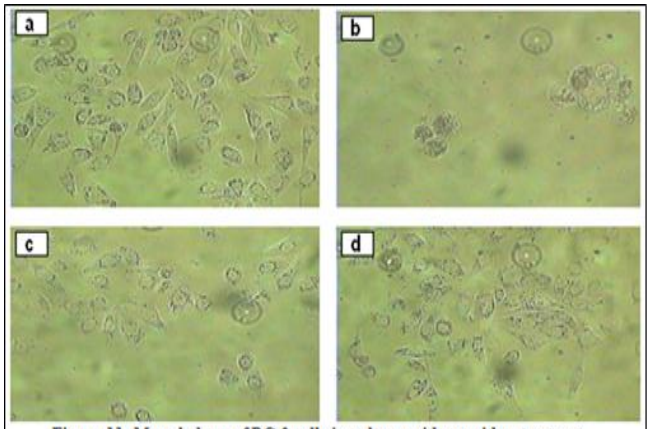

Figure 11: Morphology of PC-3 cells in culture with or without extract.

PC-3 cells were grown in DMEM culture medium without any extract (a) or with 4% *Urtica pilulifera* extract for 24 hours (b), 8% *Lagenaria siceraria* extract for 48 hours (c) and 8% *Ficus carica* for 48 hours (d). Cells in all were visualized by inverted microscope at 20X.

6. Discussion

Cancer is a term describing conditions characterized by unscheduled and uncontrolled cellular proliferation (Ponder. 2001). It is a very common disease, and its incidence is increasing at an average annual rate of 1.2% (Ponder. 2001). Lately, there has been improvement in the treatment strategies of cancer, which has resulted in prolonged survival of patients with chronic cancer disease. However, there is a growing need for additional means of cancer therapy, in the form of both palliative and curative treatments. The strategies available today, are sophisticated, and are only able to affect 50 to 60% of cancer patients, while the others will eventually die from their disease (Verweij *et al* 2003; Talback *et al* 2003 and Socialstyrlesn 2005) [27-29].

Chemotherapy has been used for cancer treatment for more than 50 years; sometimes in combination with or parallel to surgery and radiotherapy. After surgical ablation of progressive cancer, metastasized tumor cells continue to progress and this is one of the faultiest associated with surgery. One the other hand, radioactive rays and most anticancer chemotherapeutic agents damage DNA or suppress DNA duplication to kill the rapidly growing tumor cells. At the same time, they also affect normal cells causing serious adverse effects, such as bone marrow function inhibition, bone necrosis, lung fibrosis, skin devascularization, ulceration, nausea, vomiting, renal damage and alopecia (Cassady *et al* 1981) [30]. Thus it is evident that a wide array of selective and potent components is needed to match the growing problems associated with cancer.

Plants and natural products play an important role in medicine and provide important prototypes for the development of novel drugs (Cragg 1998). They offer a valuable source of compounds with a wide variety of biological activities and chemical structures. Many anti-cancer agents have been derived from natural sources; directly as pure native compounds, or as semi-synthetic analogues (Pezzuto 1997; Schwartsmann 2000 and Lee 1999) [32-34].

Our Arabic and Islamic tradition is particularly rich in medical plants that have been used by pioneer Arabic physicians to establish the basis for modern therapies. But a few of these plants have been examined scientifically. In this extent we studied the potential effect of three Arabic and Islamic traditional plants as anticancer agents. These were Fig (*Ficus carica*) and Bottle gourd (*Lagenaria siceraria*) that were mentioned in the holy Quran in more than one occasion and Roman nettle (*Urtica pilulifera*) which is traditionally used as a popular medication (Shtayeh *et al*, 2000) [8]. To fulfill this aim, the proliferation, viability and morphological characteristics of three cell lines (Hep3b, Hela and PC-3) were studied in response to treatment with extracts from the aforementioned plants.

The cellular proliferation activity was tested by a colorimetric method which is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to orange-colored compounds of formazon. The intensity of the formed water soluble dye is proportional to the number of metabolically active cells. The proliferation activity of the treated cells was normalized to that of normally growing cells from the same type with no treatment. This normalized value was expressed as percent of the control group. Theoretically, any reduction in the number of metabolically active proliferating cells might mean that the proliferation pathway itself was halted (cell cycle arrest), or that a fraction of the cells went through a death pathway. Therefore, a viability test was necessary to determine which of these two options may play a role in this study. Therefore, the trypan-blue dye exclusion test was used to determine the plant extract-mediated cell death. The unstained cells (viable) and the blue stained (dead) were counted separately, and the percent cell viability was calculated as previously described. The viability assay was performed with the same cell-extract combinations as in the proliferation assay. The percent viability of normally grown cells from the same types was determined for comparison with extract-treated cells. If we consider the results of the proliferation and viability assays in parallel, then we would be able to say whether reduction of the number of the metabolically active cell has resulted from death of part of them or due to any other reason. Any conclusion drawn from such comparison may be confirmed by morphologically following up the treated cells in comparison with normally maintained control cells. In this study a combination of the three different assays was performed in parallel for each plant extract and each cell line.

In order to assess the degree of proliferation inhibition and viability reduction obtained by each extract in comparison to the others, the IC_{50} values were summarized in tables 3 and 4. The findings of this study indicated that the proliferation activity of Hep3b cells was inhibited by all of the three plant extracts (*Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica*). A dose response effect was obtained with all of these extracts, but the degree of this effect varied from one extract to the other. The results indicated that *Urtica pilulifera* is the most potent extract, even at low concentrations with IC_{50} of (1.9%) followed by *Lagenaria siceraria* with IC_{50} of (3.4%) and *Ficus carica* with of IC_{50} of (5.7%). At the highest, extract concentration used in the study (8%) both *Urtica pilulifera* and *Lagenaria siceraria* reached a similar proliferation inhibition level of about (90%). However, this degree of inhibition was

reached at lower concentration of *Urtica pilulifera* extract than of *Lagenaria siceraria* extract (figure7). Ficus carica on the other hand did not reach the same degrees of proliferation inhibition obtained by both *Urtica pilulifera* and *Lagenaria siceraria* even at the highest concentration. When considering the results of Heb3b cells viability assay, the three plant extracts showed various degrees of Heb3b cells viability reduction. *Urtica pilulifera* showed a steep reduction of the cells viability with IC_{50} of 2.1% extract concentration, which equals the IC_{50} of the same extract in cell proliferation assay. These compatible results (Figure 16) suggest that reduction in the metabolically active Hep3b cells in consequence to *Urtica pilulifera* extract treatment is due to cell death. However such compatibility was not evident in the case of *Lagenaria siceraria* (Figure 17) and *Ficus carica* (Figure 18). *Lagenaria siceraria* has a less potent cytotoxic effect against Hep3b cells (IC_{50} = 7.1%). This might indicate that this extract counteracts the proliferation activity of Hep3b cells mostly by mechanisms other than induction of their death. This deviation between the cytotoxic effect and proliferation effect was more profound in the case of *Ficus carica* with IC_{50} of 18.1% in case of cytotoxicity compared to 5.7% in the case of proliferation.

Table 1: Summary of calculated concentrations of the three plant extracts *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* that give 50% (IC_{50}) reduction in proliferation activity of Hep3b, Hela and PC-3 cancer cell lines. The R^2 value for each curve equation is illustrated

Plant		Hep3b cells	Hela cells	PC-3 cells
Urtica pilulifera	IC_{50}	1.98	0.132	2.31
	R^2	0.837	0.6022	0.8563
Lagenaria siceraria	IC_{50}	3.4	3.1	7.9
	R^2	0.97	0.9761	99.82
Ficus carica	IC_{50}	5.7	3.1	5.5
	R^2	0.8675	0.9059	0.9583

Table 2: Summary of calculated concentrations of the three plant extracts *Urtica pilulifera*, *Lagenaria siceraria* and *Ficus carica* that give 50% (IC_{50}) reduction in the % viability of Hep3b, Hela and PC-3 cancer cell lines. The R^2 value for each curve equation is illustrated.

Plant		Hep3b cells	Hela cells	PC-3 cells
Urtica pilulifera	IC_{50}	2.1	2.8	3.6
	R^2	0.7056	0.8305	0.9843
Lagenaria siceraria	IC_{50}	7.16	14.18	9.4
	R^2	0.8496	0.9585	0.9367
Ficus carica	IC_{50}	18.12	15.7	13.8
	R^2	0.8886	0.852	0.9442

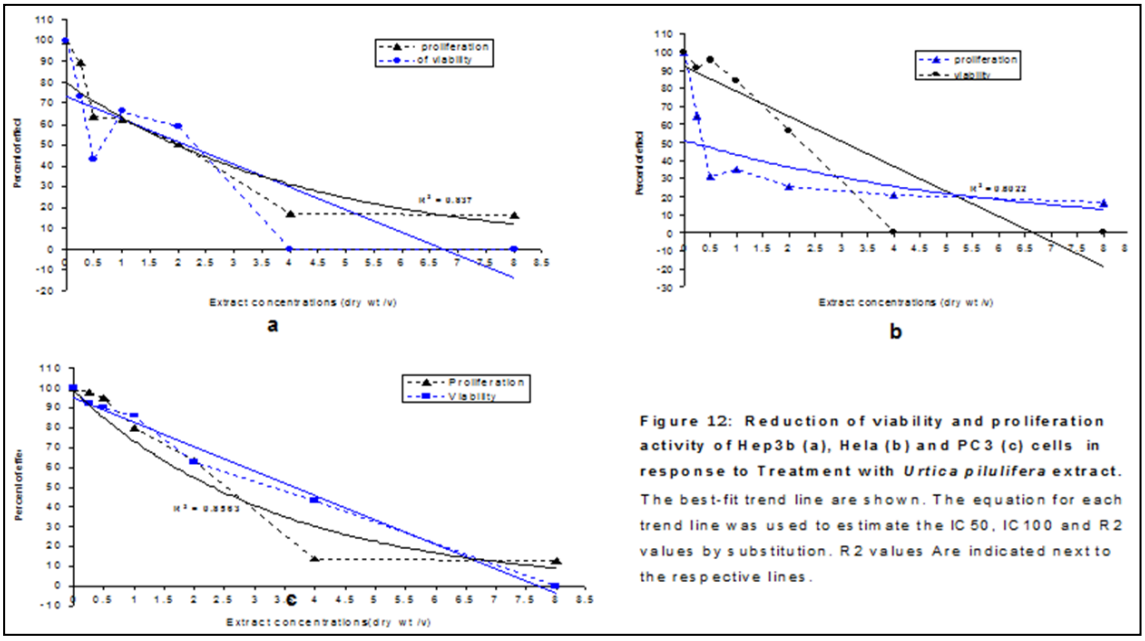

Figure 12: Reduction of viability and proliferation activity of Hep3b (a), Hela (b) and PC3 (c) cells in response to Treatment with *Urtica pilulifera* extract. The best-fit trend line are shown. The equation for each trend line was used to estimate the IC 50, IC 100 and R2 values by substitution. R2 values Are indicated next to the respective lines.

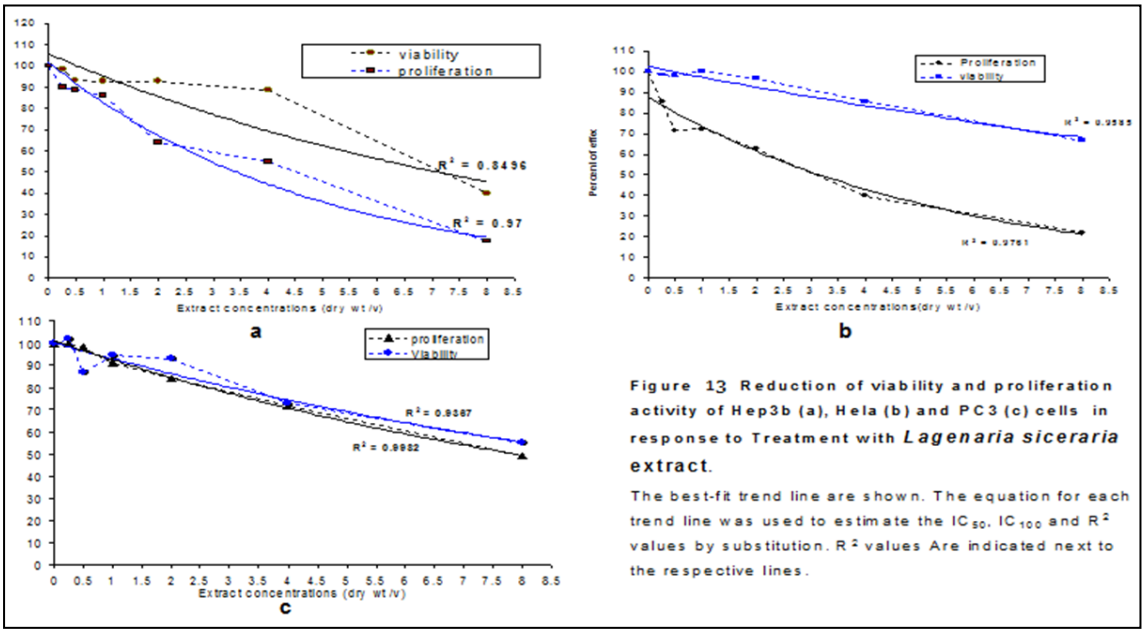

Figure 13 Reduction of viability and proliferation activity of Hep3b (a), Hela (b) and PC3 (c) cells in response to Treatment with *Lagenaria siceraria* extract.

The best-fit trend line are shown. The equation for each trend line was used to estimate the IC_{50}, IC_{100} and R^2 values by substitution. R^2 values Are indicated next to the respective lines.

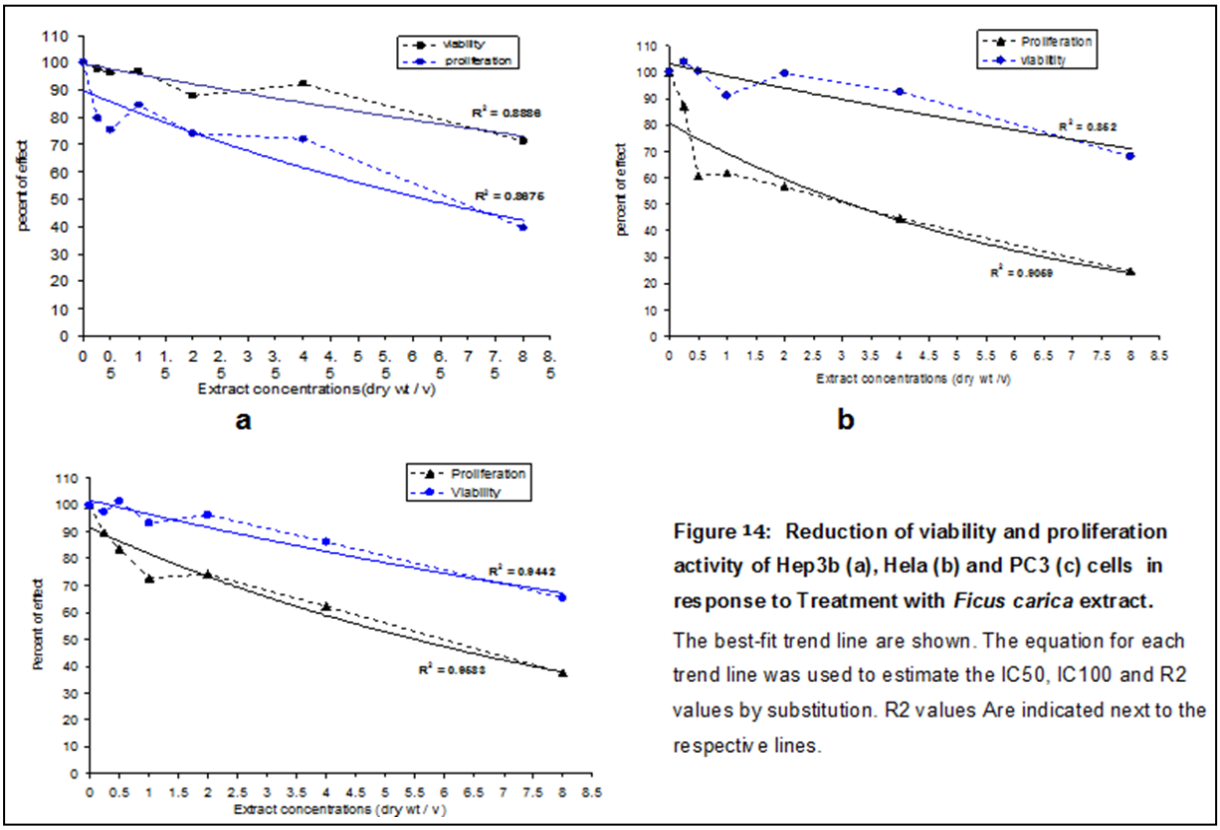

Figure 14: Reduction of viability and proliferation activity of Hep3b (a), Hela (b) and PC3 (c) cells in response to Treatment with *Ficus carica* extract.

The best-fit trend line are shown. The equation for each trend line was used to estimate the IC50, IC100 and R2 values by substitution. R2 values Are indicated next to the respective lines.

These data were confirmed by the inspection of morphological alterations of Hep3b cells due to treatment with the three plant extracts (figure13). As shown in figure 13b *Urtica pilulifera* had the most potent effect compared to the control (figure 13a). Most of cells were rounded up and formed a grape shaped cluster, which is one of the prominent morphological features of cellular death in culture. Such features were also noticeable to lesser extent when the cells were incubated with *Lagenaria siceraria*, and could hardly be detected in the case of *Ficus carica*. Moreover, the confluence of Heb3b cells was reduced in response to treatment with the three plant extracts and in accordance to the previously discussed results of proliferation inhibition.

Similarly Hela cells proliferative activity was inhibited with various levels due to their treatment with the three plant extracts. As shown in figure 8, *Urtica pilulifera* has a strong inhibitory effect on the proliferation activity in extract concentration dependent manner with IC_{50} of 0.13% extract concentration. Both Ficus *carica* and Lagenaria *siceraria* had a similar but weaker effect on proliferation of Hela cells, and the IC_{50} for both extracts was 3.1%. The percent viability of Hela cells was also reduced in a dose response manner by all of these plant extracts. *Urtica pilulifera* extract was the most effective with IC_{50} of 2.8%, while the *Lagenaria siceraria* and *Ficus carica* effect on cells viability were similarly less potent with IC_{50} of 15.7% for *Ficus carica* and 14.1% for *Lagenaria siceraria*. Being the most potent plant, *Urtica pilulifera* mostly exerts its effects by inducing cell death although other effect can be present (IC50 for proliferation=0.13% and for cytotoxicity =2.8%). The difference between the IC_{50} of both *Ficus carica* and Lagenaria *siceraria* in proliferation inhibition and the IC_{50} of cell viability reduction indicates that these extracts exert their effect by cell cycle arrest with low cytotoxicity. The morphological observations of Hela cells maintained in increasing concentrations of the three plant extracts show that *Urtica pilulifera* had the most rapid and potent effect of these extracts. Figure 14b shows that most of the cells were rounded up and formed sparse clusters due to treatment with 2% *Urtica pilulifera*. These results suggest that most of Hela cells were dead in the case of *Urtica pilulifera*. A reduction of Hela cells confluence was the most prominent observation when these cells were incubated with *Ficus carica* or *Lagenaria siceraria*, but cell death indications were almost not seen. These observations indicate an antiproliferative effect of both Ficus *carica* and Lagenaria *siceraria* but less likely cytotoxicity.

The proliferation activity of the prostate cell line PC-3 was inversely related to increasing the levels of the three plant extracts. The degree of proliferation reduction varied from one plant extract to the other. As shown in figure 9, *Urtica pilulifera* was again the most potent extract with IC_{50} of 2.3%, whereas *Ficus carica* comes second with IC_{50} of 5.5%, and *Lagenaria siceraria* comes last with IC_{50} of 7.9%. When comparing these results with results from PC-3 cells viability assay, we find that *Urtica pilulifera* also has the strongest effect with IC_{50} of 3.6%. These results are supported by the morphological changes shown in figure15b including cells rounding up and detachment from the monolayer indicating that cell death might has occurred.

Lagenaria siceraria induced a reduction of PC-3 cell viability with IC_{50} of 9.4% extract concentration similar to IC_{50} from the same extract in the proliferation assay. This fact suggests that this extract also caused PC-3 cell death. The reduction of the cells confluence due to *Lagenaria siceraria* treatment observed in figure 14c gives evidence of the effect of this extract. A Higher concentration of *Ficus carica* was needed to reach a 50% viability inhibition (IC_{50} =13.8%) than that needed to reach 50% inhibition in cell proliferation (IC_{50}=5.5%). This again suggests that the effect of this extract did not occur only through cell death.

Based on the previous results, *Urtica pilulifera* have a potent antiproliferative effect on the three tested cancer cell lines. While there is no previous studies about the role of *Urtica pilulifera* extract as anticancer agent, many studies investigated the role of its genus member *Urtica dioica*. *Urtica pilulifera* and *Urtica dioica* are similar in many chemical and

morphological aspects (Shtayeh *et al* 2000 and Maitai *et al* 1980) [8, 9]. Our results on *Urtica pilulifera* are in agreement with previous studies on *Urtica dioica.* For example an aqueous extract of *Urtica dioica* roots was shown to directly inhibit the proliferation of Hela cells and block its binding by epidermal growth factor (EGF) (Wagener 1994) [35]. In the same study, a polysaccharide mixture from an aqueous root extract was shown to exert an anti-inflammatory activity in a rat Paw oedema test. Investigation of the effect of a 20% methanol extract of *Urtica dioica* roots on prostate cell line (LNCap) resulted in a selective and significant concentration-dependent proliferation reducing effect on prostate cells (89). In the same study, the cell proliferation activity was determined by a colorimetric assay and the cytotoxicity was examined by Trypan-blue test. Our results are also comparable with data obtained from an in vitro study that aimed to investigate the effect of *Urtica dioica* leaves aqueous extract on the enzyme activity of prostate cancer tissue (Durak *et al* 2004) [36]. The results of the study indicated that the extract caused a significant inhibition of adenosine deaminase activity (ADA) of these tissues in a dose dependent manner. These data might be of importance because ADA is a key enzyme in the nucleotide metabolism and DNA turn over. Extracts *of Urtica dioica* were also used in the treatment of adult mouse with bingeing prostate hyperplasia (Lichius and Muth 1997) [37]. Five differently prepared root extracts were tested on these rats. The 20% methanol extract was the most effective with 51.4% inhibition of induced growth.

Despite all of these data, the mechanism(s) of action of *Urtica dioica* extract as an antiproliferative agent has yet to be established. Different modes of actions are proposed in this regard. For example, it has been observed that some sterols and hydroxyl-fatty acids, given their low concentrations in *Urtica dioica*, can inhibit aromatase, which is a key enzyme in steroid hormone metabolism mediating the conversion of androgens into estrogens (Gansser and Spiteller 1995) [38]. Another mechanism involves a dose dependent inhibition of the binding of sex hormone binding gluobin (SHBG) to its receptor in response to *Urtica dioica* extract (Hryb *et al* 1995). Some lignans present in *Urtica dioica* were shown to interfere with the binding of androgens to SHBG, thereby reducing the transport capacity of androgens (Gansser and Spiteller 1995) [38]. Based on the similarity between *Urtica pilulifera* and *Urtica dioica* we would suggest that *Urtica pilulifera* might exerts its antiproliferative effect via a similar fashion.

The results of this study strongly indicate a possible cytotoxic effect of *Urtica pilulifera* against cancer cell lines. This was evident from the results of Trypan-blue viability assay as well as from the inspection of cells morphology in culture. Only one study was found to deal with this issue in the scientific debate. In this study, the cytotoxicity of fixed and volatile oils extracted from *Urtica pilulifera* leaves and seeds were tested on Swiss albino mice (Qzbek *et al* 2004) [40]. The results indicated that both oils of *Urtica pilulifera* are completely nontoxic even at doses reaching 12.8 ml/kg. However, this study involved only the oil fractions of *Urtica pilulifera* extract. Other components of the extract may play a role in the observed toxicity in our study. Moreover, the aforementioned study was performed in vivo on mice and the only cytotoxicity parameter considered was the mice death. No other mice toxicity indicators were analyzed such as, histopathological alterations of the different mice tissues and organs.

According to results discussed earlier the inhibitory role of *Ficus carica* on the proliferation of the three cell lines was more profound than its cytotoxic effect on the same cell lines. This conclusion is in accordance with the results of previous studies. For instance, the proliferation of different cell lines was inhibited by components of *Ficus carica* (Rubnov *et al* 2001) [41]. Such proliferation inhibition was also obtained when cow teat papillomatosis skin surface benign tumors, were treated by *Ficus carica* latex (Hemmatzadeh; Fatemi and Amini 2003) [42]. Furthermore, *Ficus carica* was found to have an in vivo antioxidant effect after being consumed by human (Vinson *et al* 2005) [43]. Accordingly, the dried fruits of this plant should be eaten more frequently as they are rich in phenol antioxidants and fibers. Compared with vitamins C and E, the well-known antioxidants, the dried fruits of *Ficus carica* were found to be a superior one.

Lagenaria siceraria was found to possess both antiproliferative effects as well as a cytotoxic effect to a considerable degree. Unfortunately no literature was found regarding the anticancer activities of this plant. However, members of its family (*Cucurbitaceae*), were shown to have a class of biologically active compounds (Cucurbitacins) with well-established anticancer cytotoxic activity (Rehm *et al* 1960; Guha and Sen 1975 And Miro1995) [44-46]. For example, Cucurbitacins were shown to have strong cytotoxic effect on KB cell line, derived from human nasopharyngeal carcinoma and on Hela cells by different proposed mechanisms (Miro1995 and Witkowski; Woynarowska and Konopa 1984) [46, 47]. Moreover, (Cucurbitacins) were shown to have a proliferative inhibitory activity in vivo against several carcinoma, sarcoma, and leukemia models (Gitter *et al* 1961; Gallily *et al* 1962 and Reddy *et al* 1988) [48-50].

The level of these compounds and/or any of their derivatives in Lagenaria siceraria is unknown. However the results of this thesis indicate both antiproliferative and cytotoxic activity of *Lagenaria siceraria*. Therefore we expect *Lagenaria siceraria* extracts to behave in a similar manner like other members of its family via (Cucurbitacins) involvement.

7. Conclusion

In conclusion, all of the three plants examined in this study possess varying levels of anticancer activity in vitro. This is evident by the concentration dependent manner reduction in the final number of cancer cells as a consequence to treatment. Two kinds of anticancer effects were examined and found to take part in this study. The first is anti-cell proliferation effect (decreased number of metabolically active cells) and the second is cytotoxicity (decreased number of live cells). The three plants examined possess both of the effects with various degrees. *Urtica pilulifera* possess the strongest and most profound effects on the three cell lines, probably by cytotoxicity mainly. On the other hand *Lagenaria siceraria* probably affects the three cell lines by combination of cytotoxicity and anti-proliferation almost to a similar degree. *Ficus carica* most probably reduces the final number of metabolically active cells mainly by its antiproliferative effect, although cytotoxicity likely contributes to viability reduction at high concentrations.

Both *Ficus carica* and *Lagenaria siceraria* are edible plants that were chosen on the bases of being mentioned in the holy Quran. Therefore, although their effect is lower than that of Urtica *pilulifera* their amount in the diet or as a treatment can be safely scaled up when ingested in their native form. On the other hand, despite its possible toxicity *Urtica pilulifera* is frequently orally used as a medication in many conditions by traditional medicine.

8. References

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