ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# Anti carcinogenic activity of Methanolic Extract of *Balanites aegyptiaca* against breast, colon, and liver cancer cells.

**Samir A.M. Zaahkouk[1], Ezzat I. Aboul-Ela[2], Ramadan M.A[3], Sayed Bakry[4] and *Ahmed B.M. Mhany[5]**

**1.** Depatment of Zoology, Faculty of science, Al Azhar university, Naser city, Cairo, Egypt.
**2.** Depatment of Genetics and Cytology, National Research Center, Dokki, Cairo, Egypt.
**3.** Faculty of girl for science and art , Ain Shams university, Naser city, Cairo, Egypt.
**4.** Depatment of Zoology, Faculty of science, Al Azhar university, Naser city, Cairo, Egypt.
**5.** Depatment of Zoology, Faculty of science, Al Azhar university, Naser city, Cairo, Egypt.

## *Manuscript Info*

***Manuscript History:***

Received: 04 April 2015
Final Accepted: 26 May 2015
Published Online: June 2015

***Key words:***

***Corresponding Author***

**Ahmed B.M. Mhany**

## *Abstract*

*Balanites aegyptiaca* is a widely distributed African plant of medicinal interest containing a number of cytotoxic and cytostatic compounds. The studies reported here have attempted to further characterize the anticancer activity. The current study has further indicated that its antiproliferative effects by inducing apoptotic cell death.

To study the anticarcinogenic activity of *Balanites aegyptiaca* in MCF-7 (Human breast cancer cell line), HePG-2(human hepatocellular carcinoma cell line), and HCT 116(colon carcinoma cell line). *Balanites aegyptiaca* extracts were prepared by Soxhlet extraction method using methanol as a solvent. The cytotoxicity activity of the plant extracts were determined using sulphodiamine-B assay (SRB assay), Detremenation of proteins in cancer cells line by SDS polyacylamide gel electrophoresis, DNA fragmentation induced by the plant extracts was evaluated through DNA extraction using agarose gel electrophoresis. RNA extraction to evaluate the expression of genes P53 and Bax genes using Semi-quantitave RT-PCR analysis. **Results & Discussion:** SRB assay results showed that MCF7 cells were inhibited by all the extracts with IC50 2.3±.03 while IC50 of HEPG-2 is 12.0±.01and IC50 of HCT116 is 69.3±.1µg/ml. SDS polyacylamide gel electrophoresis showed clear variation between different classes of treated and nontreated cancer cells. DNA extracted from treated cells showed fragmentation of the DNA was observed between treated and non treated (control) cancer cells suggesting the occurrence of apoptosis. RT-PCR analysis showed increase in expression of genes P53 and Bax genes.



## INTRODUCTION

*Balanites aegyptiaca* is a widely distributed african plant of medicinal interest. **Speroni *et al.*, 2005**. It is a small spinescent evergreen savanna tree with a dark brown stem which usually attains a height of 4.5-6 m. **Koko *et al.*, 2000**. In Egyptian folk medicine, the fruits are used as an oral hypoglycemic and as an anthelmintic . **Kamel *et al.*, 1998**. and an anti-diabetic. **Sarker *et al.*, 2000.** An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice **Sarker *et al.*, 2000.** The plant is used as a purge to remove intestinal parasites with the root, branches, bark, fruit and kernel extracts shown to be lethal to the miracidia and cercariae of *Shistosoma mansoni* and to *Fasciola gigantic* . **Koko *et al.*, 2005**.

*Balanites aegyptiaca* is a widely distributed african plant of medicinal interest. **Speroni *et al.*, 2005**. It is a small spinescent evergreen savanna tree with a dark brown stem which usually attains a height of 4.5-6 m. **Koko *et al.*, 2000**. In Egyptian folk medicine, the fruits are used as an oral hypoglycemic and as an anthelmintic . **Kamel *et al.*, 1998**. and an anti-diabetic. **Sarker *et al.*, 2000.** An aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice **Sarker *et al.*, 2000.** The plant is used as a purge to remove intestinal parasites with the root, branches, bark, fruit and kernel extracts shown to be lethal to the miracidia and cercariae of *Shistosoma mansoni* and to *Fasciola gigantic* . **Koko *et al.*, 2005**.

Additionally extracts of the tree display abortive and antiseptic properties . **Koko *et al.*, 2000**. *Balanites aegyptiaca* contains a number of alkaloids such as N-trans-feruloyltyramine and N-cisferuloyltyramine and common phenolic compounds such as vanillic acid, syringic acid and 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone. **Sarker *et al.*, 2000**. The roots and bark also contain numerous steroidal saponins and yamogenin or diosgenin glycosides. **Speroni *et al.*, 2005**. *Balanites aegyptiaca* is a medicinal interest containing a mixture of steroidal saponins: balanitin-6 (28%) and balanitin-7 (72%) isolated from *Balanites aegyptiaca* have anticancer activity **Morsy, 2008**. Also, the importance of *Balanites aegyptiaca* is back to presence of a steroidal sapogenin compound named diosgenin which is useful in pharmaceutical industries as a natural source of steroidal hormones. **Speroni *et al.*, 2005.** Recently saponins proved to have wide range of uses, including protection of crops from mealy bugs **Patil *et al.*, 2010**, antitumor effects **Gnoula *et al.*, 2008**, Studies have also revealed that diosgenin produces changes in the lipoxygenase activity of human erythroleukemia cells and is responsible for morphological and biochemical changes in megakaryocyte cells **Beneytout *et al.*, 1995**, **Nappez *et al.*, 1995**. *Balanites aegyptiaca* protected the livers of treated mice against paracetamol. It hepatotoxicity as evidenced by a significant improvement of liver function tests. Also,

*Balanites aegyptiaca* had a relatively modest hepatoprotective activity. **Ali *et al.*, 2001.** *Balanites aegyptiaca* was known to be an all purpose tree with various uses and values. However, the most important valued part is its fruits. **National Research Council, 2008**. Fruit from *Balanites aegyptiaca* known as desert date (common) and lalobe (Arabic) is widely used for food, fodder and traditional medicine. **Elfeel and Warrag, 2011**. The fruit is a drupe, pubescent when green, becoming yellow and glabrous, after ripening. **Arbonnier, 2004**. It contains four layers (the outer skin called epicarp, the fleshy pulp called mesocarp, the woody shell called endocarp and the inner seed called kernel). The fleshy pulp of the fruit contains a large amount of carbohydrates. The kernel produces a high quality oil that can be used in human food **Obidah *et al.*, 2009**, medicine **Hanan *et al.*, 2010** or biodiesel production **Gutti *et al.*, 2012**, **Chapagain *et al*, 2009.**

The present study evaluated the efficacy of *Balanites aegyptiaca* methanolic extract as an agent with an antitumor activity in cancer cells.

## 2. Materials and methods:

### 2.1. Preparation of methanolic *Balanites aegyptiaca*.

Extracts were prepared by soxhlet apparatus for 10 hours according to the Association of Offecial Analytical Chemists **(AOAC, 1970)** procedure using methanol as a solvent fifty grams of dried sample were poured into a 200 ml of methanol . The heat given to the solvent was just to evaporate it slowly. Then crude extract was left at room temperature to dry.

### 2.2. Cell lines and culture maintenance.

Human breast cancer cell line (MCF-7), human hepatocellular carcinoma cell line (HePG-2), and colon carcinoma cell line (HCT116) were obtained from VACSERA - Cell Culture Unit, Cairo, Egypt. This cell lines originally obtained from the American Type Culture Collection (ATCC). The cell count was done and the cell viability was tested by trypan blue using haemocytometer. Cells were cultured in RPMI medium (Gibco, Glasgow, UK) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, 1% non-essential amino acids in tissue culture flasks and incubated in a CO2 incubator in a 5% CO2 and 95% humidity atmosphere. Once the cells attained confluent growth, the cells were trypsinized using Trypsin-EDTA for carrying out various assays. **Freshney, R.I. (2000).**

### 2.3. Measurements of cytotoxicity by sulphodiamine-B assay(SRB)

The cytotoxic assay was performed at the Center of Genetic Engineering at Al-Azhar university, using the sulforhodamine B assay. **Vichai, et al., 2006.** The stock cells maintained in 75 cm2 polystyrene flasks (Falcon) with minimal essential medium containing 10% fetal bovine serum, penicillin 100 IU/mL and streptomycin 100 IU/mL. in a humidified atmosphere of 5% CO2 at 37 $^{O}$C. The cells were dissociated with 0.2% trypsin (Sigma) in phosphate-buffered saline (PBS) solution (Sigma). Cell lines in exponential growth phase were washed with PBS solution and trypsinized and resuspended in complete culture media. Cells were equal to 20.000 cells /well and were plated in 96-well plates for 24 hours before treatment to allow attachment of the cells to the wall of the plate. After incubation, the cells were exposed to various concentrations. The plant extracts were added to the cells at serial concentrations of 100 , 10 , 1 , 0.1 and 0.01 µg /ml. The control well received only maintenance of medium

(negative control). Triplicate wells were prepared for each individual concentration. The monolayer cells in the plates were incubated at 37$^{o}$C in a humidified incubator with 5% CO2 for 48hours, the cells were fixed with 10% trichloroaceticacide for one hours, washed, and stained with sulforhodamine B stain. Excess stain was washed off with acetic acid, and the attached stain was recovered with 50 µl/well of 10 mM tris base (PH 7.4) for 5 min. on a shaker at 1600 rpm. The optical density (OD) of each well will be measured spectrophotometrically at 570 nm with an ELISA microplate reader. The relationship between the surviving fraction and the drug concentration was plotted to determine inhibitory concentrations (IC50) for each tumor cell line. The IC50 values will be calculated using sigmoidal concentration response curve fitting models (Sigmaplot software).

**2.4. SDS polyacylamide Gel Electrophoresis. Laemmli, 1970; Stegemann et al., 1988.**

The cells were precipitated and lysed directly utilizing 150 µl of lysis buffer and heated for 10 min at 100$^{o}$C. Each sample was separated in 12% SDS polyacylamide gel. The separating gel was inserting between the glass plates and allows polymerizing for 15- 30 min at room temperature. The stacking gel was inserting on the top of separating gel and comb was inserted to form wells. After polymerization, the comb was removed wisely to avoid tearing the edges of the wells before polymerization. The slab gel apparatus chamber was filled with running buffer.15µl of the prepared samples were loaded into each well. The power supply was connected to the gel electrophoresis tank at a constant volt (100-volt) till the tracking dye reach the bottom of the gel and the current was switched off. The gel was removed by prying the glass plated apart gently, and then the separated protein gel was stained by using comassie blue stain over night. After gel staining, the gel was transferred to destaining solution which changed several times the destaining solution removed the background dye from the gel leaving stained proteins as visible blue bands. The protein pattern in terms of molecular weight, band matching and band density was analyzed using gel documentation system.

**2.5. Determination of DNA fragmentation according kit of promega (England).**

The characteristic of DNA breakage was analyzed by 1% agarose gel. Cancer cells were placed in aflask at a concentration of 5 × 105cell/mL. The cells were treated with IC50 concentration and were further incubated for 48 h. The DNA was isolated and electrophoretically analyzed on 1% agarose gel containing 10 µL ethidium bromide. **Lerman and Frisch, 1982; Lumpkin and Zimm, 1982; Stellwagen, 1983.**

**2.6. RNA Extraction from HePG-2, HCT 116 and MCF7 Cell line**

Cancer cells were placed in a flask at a concentration of 5 × 10$^{5}$cell/mL. The cells were treated with IC50 concentration and were incubated for 48 h. 1ml trizol was added to the cells pellet. The cells pellet was layside with trizol by vortex. The cells were incubated for 5min. at R.T. and 200µl of chloroform was added .Vortex was performed for 15 sec. and cells were incubated at R.T. for 3min. The cells were spin at 13000 rpm. For 15 min. at 4$^{o}$C. Upper aqueous phase -colorless- containing RNA was transferred in new tube and equal volume of ice cold Isopropanol was added. The tube was vortexed briefly 3 sec. The tube was incubated at R.T. for 10 min. & Spin 13000 rpm at 10 min. at 4$^{o}$C.The pellet was washed with 1ml cold 75% Ethanol & spin7500 rpm. 10 min. The pellet was dissolved in 20µl DEPC water. Store at -80$^{o}$C. The RNA was isolated and electrophoretically analyzed on 1% agarose gel containing 10 µL ethidium bromide. . **Lerman, 1982; and Lumpkin 1982.**

**2.7. RT-PCR (semiquantitative reverse transcriptase polymerase chain reaction)**

RT-PCR reaction was done using two steps RT-PCR Kit RT-PCR program started while the PCR tubes still in ice bath. Annealing temperature is 55°C for all genes. The numbers of cycles were 35 cycles. After PCR amplification.10 µl of samples was loaded in the gel to detect the precise size of PCR products. The gel was running at 100 volts for 20-30 min. using submarine unit.

## Results

The present study aimed to evaluate of anticancer effect of *Balanites aegyptiaca*. The evaluation parameters include cell viability, protein analysis, DNA damage and analysis of expression of some relevant genes by RT – PCR.

### 1.1. Morphological changes

Morphological changes were obvious where treated cells started showing gradual cell shrinkage, cell rounding and detaching from the surface of tissue culture flasks, finally followed by cell swalling and rupture (the morphological features give an indicator of cell apoptosis).

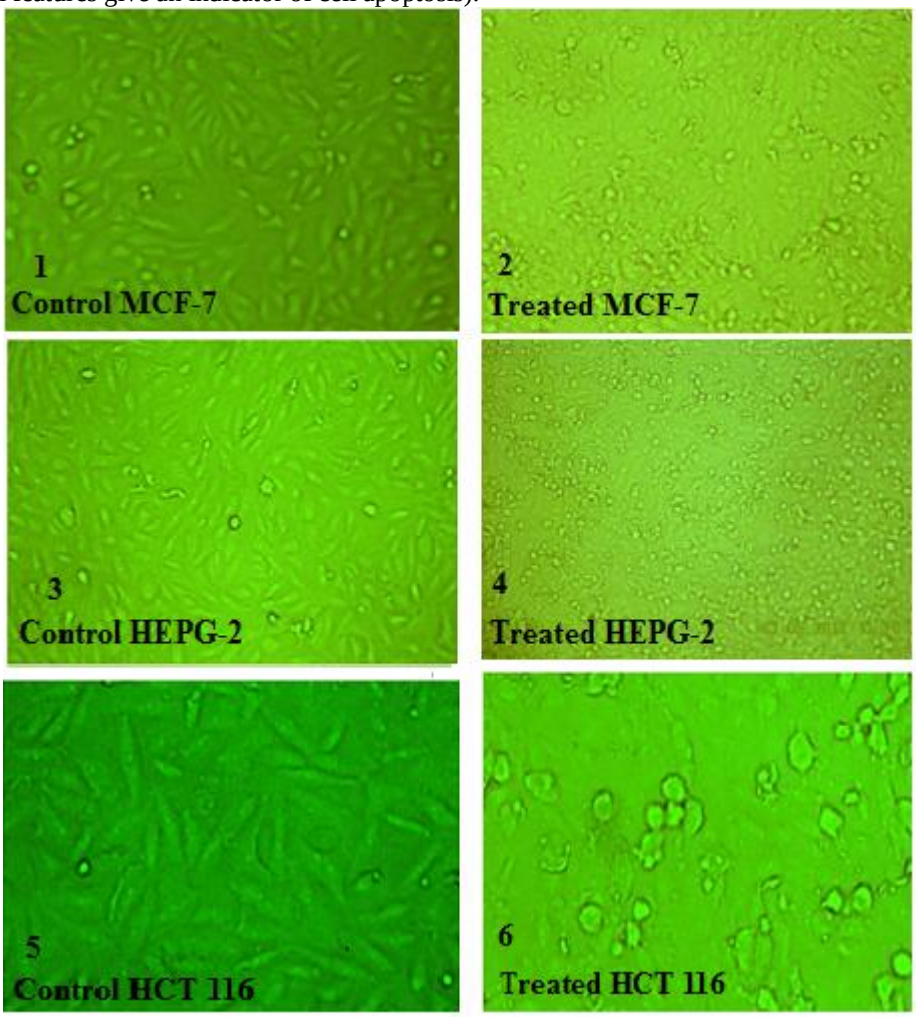


**Figure (1):** (1)MCF-7 cells (control) , (2) MCF-7 cells treated with *Balanites aegyptiaca,* (3) HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Balanites aegyptiaca*, (5) HCT116 cells (control) and (6) HCT116 cells treated with *Balanites aegyptiaca*

### 1.2. Measurement of cytotoxicity to cells by (SRB assay)

The survival fraction (% viability) was used as indicator for cell cytotoxcity HEPG-2, MCF-7, HCT116 cells were treated with different serial concentrations of *Balanites aegyptiaca* extracts for 48 hrs. Surviving fraction at each concentration and the inhibition concentration (**IC50)** which is the concentration of treatment was determined and calculated by using sigma plot program, were calculated in tables (1).

**Table(1):** The surviving fraction (% viability) of HEPG-2, MCF 7, HCT 116 cells post treatment with different concentrations of *Balanites aegyptiaca*.

| Concentrations µg/ml | Surviving fraction%± SE | | |
|---|---|---|---|
| | HEPG-2 | MCF-7 | HCT116 |
| 0.00 ( control) | 100 | 100 | 100 |
| 0.01 | 91.5±0.09 | 80.1±0.09 | 95.3±0.06 |
| 0.1 | 89.7±0.06 | 78.0±0.05 | 92.3±0.1 |
| 1 | 81.3±0.3 | 75.4±0.05 | 91.8±0.09 |

| | | | |
|---|---|---|---|
| 10 | 57.5±0.3 | 20.6±0.005 | 89.6±0.1 |
| 100 | 17.4±0.04 | 18.2±0.06 | 40.4±0.15 |
| **(IC50)** | 12.0±.01 | 2.3±.03 | 69.3±.1 |

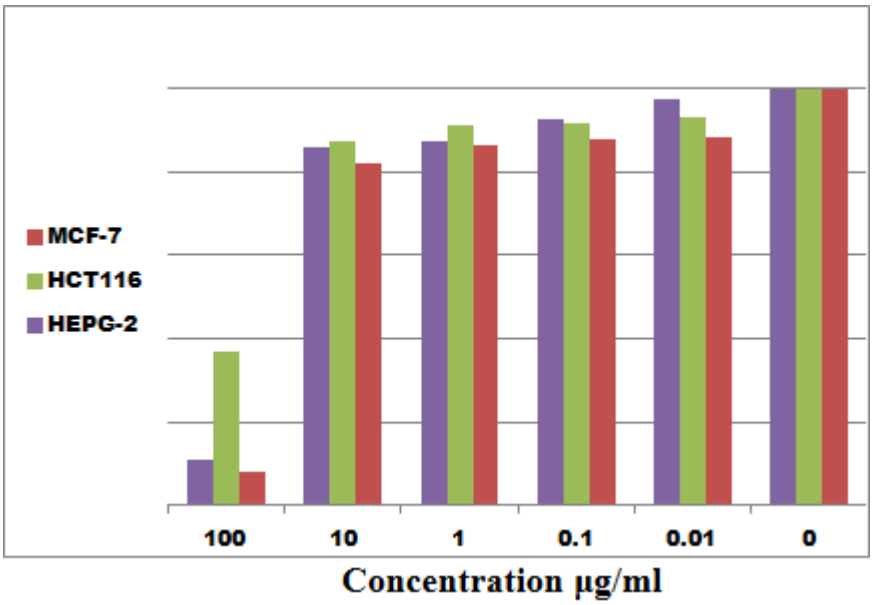


**Figure (2)**: The surviving fraction (% viability) of HEPG-2, MCF 7, HCT116 cells post treatment with different concentrations of *Balanites aegyptiaca*.

**2.3.SDS polyacylamide Gel Electrophoresis**.

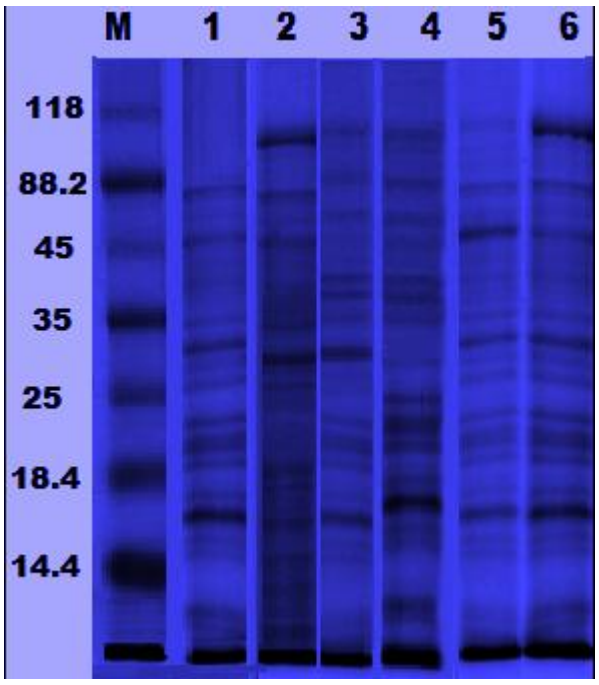


**Figure (3): Effect of** *Balanites aegyptiaca* **on protein pattern.**

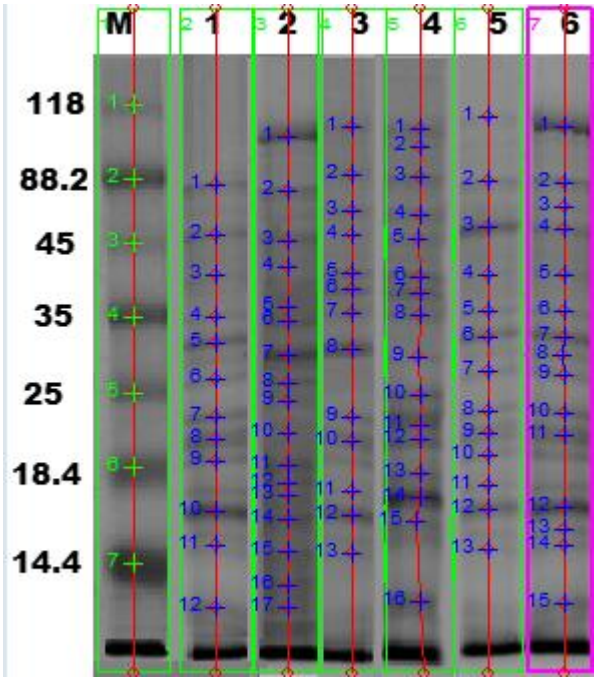


**Figure (4):** Sodium Dodecyl Sulphate polyacrylamid Gel electrophoresis (SDS-PAGE) of total proteins in HEPG-2, MCF-7 and HCT116 cells line treated for 48 hrs. (M) marker , (1) MCF-7 cells (control) , (2) MCF-7 cells treated with *Balanites aegyptiaca*, (3) HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Balanites aegyptiaca*, (5) HCT116 cells (control) and (6) HCT116 cells treated with *Balanites aegyptiaca*.

The results of scanning of the SDS-PAGE (SDS-poly acrylamide gel electrophoresis) of HEPG-2, MCF-7 and HCT116 cells in response to the treatment with *Balanites aegyptiaca* were expressed as variations in molecular weights (Mw), number of bands, bands area, bands distance , bands width and bands highest were recorded in tables (2,3 and 4) .

Table (2): Show distance, width, height, area, and molecular weight of bands of non treated MCF-7 cells (control) and treated with *Balanites aegyptiaca*.

| | Control MCF-7 | | | | | Treated MCF-7 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Bands** | **Dist** | **Width** | **Height** | **Area** | **Mol. weight** | **Dist** | **Width** | **Height** | **Area** | **Mol. weight** |
| **1** | **90** | **12** | **35** | **287** | **84.15** | **66** | **18** | **65** | **800** | **105.11** |
| **2** | **115** | **12** | **35** | **357** | **50.4** | **93** | **10** | **46** | **368** | **80.1** |
| **3** | **135** | **25** | **30** | **689** | **40.68** | **118** | **12** | **45** | **482** | **46.35** |
| **4** | **156** | **10** | **29** | **253** | **35** | **131** | **9** | **37** | **330** | **41.76** |
| **5** | **169** | **14** | **44** | **441** | **31.58** | **151** | **18** | **52** | **850** | **36.35** |
| **6** | **187** | **11** | **31** | **244** | **26.84** | **158** | **12** | **53** | **583** | **34.47** |
| **7** | **206** | **15** | **40** | **390** | **22.86** | **175** | **16** | **67** | **870** | **30** |
| **8** | **217** | **14** | **44** | **525** | **20.9** | **189** | **9** | **52** | **421** | **26.32** |
| **9** | **228** | **11** | **37** | **312** | **18.94** | **198** | **13** | **48** | **549** | **24.29** |
| **10** | **253** | **18** | **66** | **798** | **16.57** | **214** | **12** | **46** | **503** | **21.43** |
| **11** | **270** | **10** | **28** | **254** | **15.15** | **230** | **11** | **54** | **556** | **18.58** |
| **12** | **301** | **18** | **41** | **462** | **12.26** | **239** | **8** | **54** | **390** | **17.73** |
| **13** | **-** | | | | | **245** | **7** | **52** | **341** | **17.23** |
| **14** | **-** | | | | | **257** | **14** | **57** | **712** | **16.23** |
| **15** | | | | | | **273** | **11** | **53** | **523** | **14.9** |
| **16** | | | | | | **290** | **11** | **56** | **562** | **13.42** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **17** | | | | | | **301** | **8** | **52** | **396** | **12.14** |

Table (3): Show distance, width, height, area, and molecular weight of bands of non treated HEPG-2 cells (control) and treated with *Balanites aegyptiaca*.

| | **Control** HEPG-2 | | | | | **Treated** HEPG-2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Bands** | **Dist** | **Width** | **Height** | **Area** | **Mol. weight** | **Dist** | **Width** | **Height** | **Area** | **Mol. weight** |
| **1** | **61** | **9** | **46** | **376** | **109.14** | **62** | **11** | **17** | **147** | **108.34** |
| **2** | **85** | **12** | **43** | **438** | **89.81** | **71** | **8** | **7** | **25** | **101.09** |
| **3** | **103** | **13** | **40** | **453** | **66.6** | **86** | **10** | **6** | **27** | **89.01** |
| **4** | **115** | **10** | **36** | **346** | **50.4** | **105** | **12** | **8** | **42** | **63.9** |
| **5** | **134** | **11** | **49** | **466** | **40.95** | **117** | **11** | **6** | **34** | **47.7** |
| **6** | **142** | **10** | **52** | **436** | **38.78** | **136** | **8** | **13** | **53** | **40.41** |
| **7** | **154** | **8** | **43** | **315** | **35.54** | **144** | **9** | **15** | **72** | **38.24** |
| **8** | **172** | **16** | **63** | **728** | **30.79** | **155** | **14** | **2** | **5** | **35.27** |
| **9** | **206** | **11** | **42** | **359** | **22.86** | **176** | **11** | **4** | **12** | **29.74** |
| **10** | **218** | **18** | **44** | **688** | **20.72** | **195** | **8** | **12** | **48** | **24.82** |
| **11** | **243** | **14** | **33** | **418** | **17.4** | **210** | **16** | **23** | **202** | **22.15** |
| **12** | **255** | **16** | **58** | **679** | **16.4** | **217** | **10** | **18** | **117** | **20.9** |
| **13** | **274** | **11** | **35** | **339** | **14.82** | **234** | **10** | **7** | **33** | **18.15** |
| **14** | **-** | | | | | **246** | **16** | **28** | **212** | **17.15** |
| **15** | **-** | | | | | **258** | **14** | **4** | **43** | **16.15** |
| | | | | | | **298** | **22** | **21** | **243** | **12.93** |

Table (4): Show distance, width, height, area, and molecular weight of bands of non treated HCT116 cells (control) and treated with *Balanites aegyptiaca*.

| | **Control** HCT116 | | | | | **Treated** HCT116 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Bands** | **Dist** | **Width** | **Height** | **Area** | **Mol. weight** | **Dist** | **Width** | **Height** | **Area** | **Mol. weight** |
| **1** | **56** | **12** | **28** | **251** | **113.17** | **61** | **16** | **60** | **640** | **109.14** |
| **2** | **88** | **13** | **27** | **253** | **86.85** | **89** | **12** | **29** | **219** | **85.5** |
| **3** | **111** | **14** | **51** | **502** | **55.8** | **101** | **11** | **17** | **152** | **69.3** |
| **4** | **135** | **7** | **27** | **169** | **40.68** | **112** | **12** | **22** | **215** | **54.45** |
| **5** | **153** | **9** | **26** | **185** | **35.81** | **135** | **10** | **17** | **142** | **40.68** |
| **6** | **166** | **14** | **39** | **342** | **32.37** | **153** | **12** | **14** | **129** | **35.81** |
| **7** | **183** | **13** | **19** | **176** | **27.89** | **166** | **15** | **28** | **237** | **32.37** |
| **8** | **203** | **7** | **24** | **142** | **23.39** | **175** | **9** | **12** | **78** | **30** |
| **9** | **214** | **15** | **30** | **357** | **21.43** | **185** | **13** | **13** | **79** | **27.37** |
| **10** | **225** | **10** | **23** | **171** | **19.47** | **204** | **13** | **28** | **193** | **23.22** |
| **11** | **240** | **9** | **20** | **141** | **17.65** | **215** | **13** | **25** | **247** | **21.25** |
| **12** | **252** | **17** | **45** | **481** | **16.65** | **251** | **16** | **45** | **410** | **16.73** |
| **13** | **272** | **12** | **21** | **185** | **14.98** | **262** | **9** | **8** | **45** | **15.82** |
| **14** | **-** | | | | | **270** | **10** | **3** | **6** | **15.15** |
| **15** | **-** | | | | | **299** | **16** | **26** | **256** | **12.48** |

**2.4. DNA Fragmentation**

DNA fragmentation is a characteristic feature of apoptosis. Increased DNA fragmentation was apparent in MCF-7 , HEPG-2 and CHT 116 cells after treatment with IC50 values of *Balanites aegyptiaca* for 48 h. (Fig. 9).

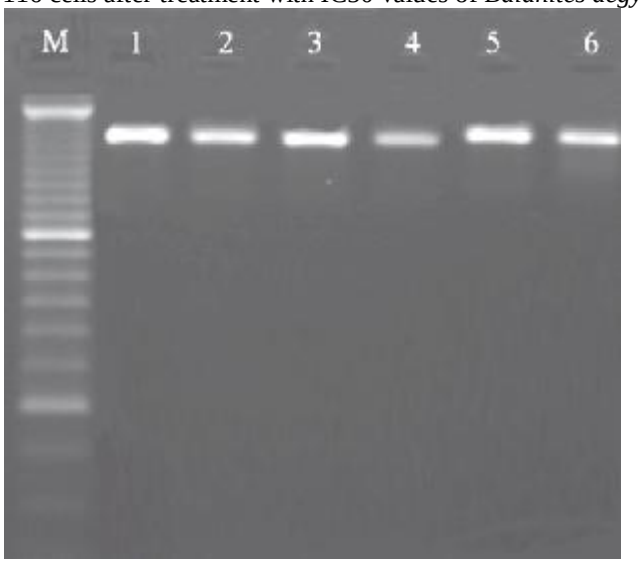


**Figure (5)**: DNA fragmentation (M) Marker (1) non treated MCF-7 cells (control) , (2) MCF-7 cells treated with *Balanites aegyptiaca*, (3) non treated HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Balanites aegyptiaca*, (5) non treated HCT116 cells (control) and (6) HCT116 cells treated with *Balanites aegyptiaca*.

2.5. **Semi-quantitave RT-PCR analysis**

The expression profile of MCF-7, HEP-2 and HCT 116 cells treated with IC50 values of *Balanites aegyptiaca* for 48 h. was examined by RT-PCR. RNA was extracted from the cells and the expression of genes; (P53 and Bax genes) were detected using semiquantitive analysis RT- PCR (Figure 6, 7).

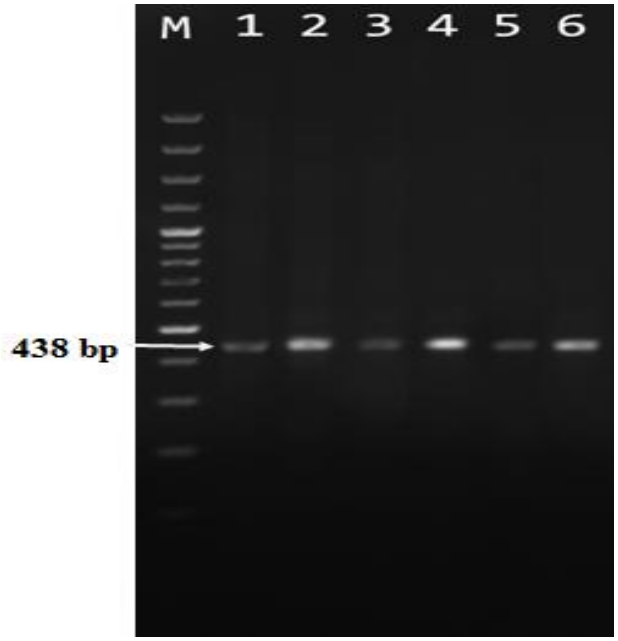


**Figure (6)**: Expression level of P53 gene (M) Marker (1) non treated MCF-7 cells (control), (2) MCF-7 cells treated with *Balanites aegyptiaca*, (3) non treated HEPG-2 cells (control), (4) HEPG-2 cells treated with *Balanites aegyptiaca, (*5) non treated HCT116 cells (control) and (6) HCT116 cells treated with *Balanites aegyptiaca*.

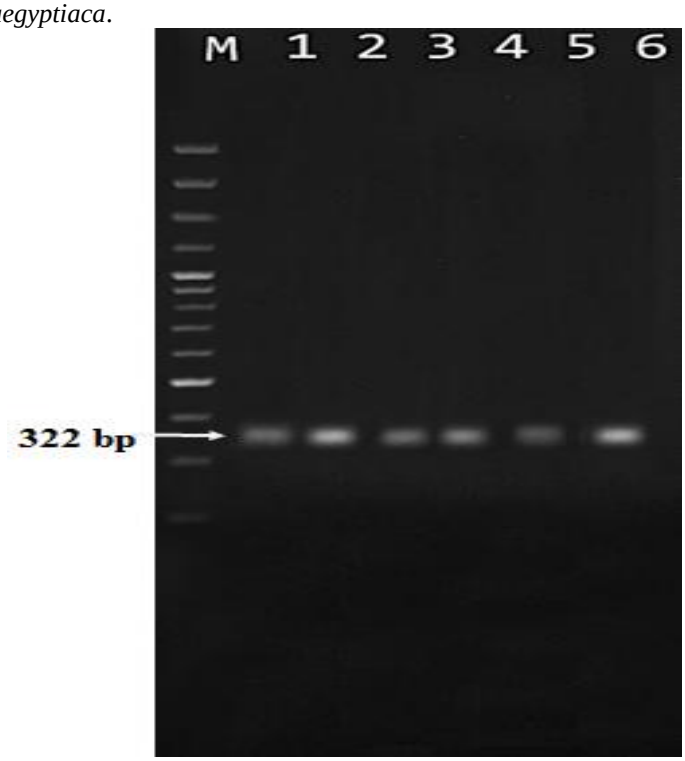


Figure (7) Expression level of bax gene (M) Marker (1) non treated MCF-7 cells (control) , (2) MCF-7 cells treated with *Balanites aegyptiaca*, (3) non treated HEPG-2 cells (control) , (4) HEPG-2 cells treated with *Balanites aegyptiaca, (*5) non treated HCT116 cells (control) and (6) HCT116 cells treated with *Balanites aegyptiaca*.

**3-Discussion**

The present study is aimed to evaluate anticarcinogenic activity of methanolic extract of *Balanites aegyptiaca* against MCF-7 (Human breast cancer cell line), HePG-2(human hepatocellular carcinoma cell line), and HCT 116(colon carcinoma cell line) cancer cells. Methanolic and butanol (BE) extracts and of two new saponins isolated from *Balanites aegyptiaca* showed significant anti-inflammatory, antinociceptive activity in the carrageenin-induced edema in the rat. **Speroni *et al;* 2005**. It has been shown that saponins are cytotoxic and are associated with anti-cancer activity. **Sparg *et al.*, 2004**. The diosgenyl saponins, which are steroidal glycosides and bear diosgenin as aglycone, are often found as the major components in traditional oriental medicines recently shown to exert cytotoxic activity against several human cancer cells. **Moalic *et al.*, 2001**, exerting their anti-tumor effect by inducing apoptosis in cancer cells. **Cheung *et al.*, 2005.** Our result illustrated activity of *Balanites aegyptiaca* as antitumor by evaluate IC50 of *Balanites aegyptiaca* and we observed that the effect of *Balanites aegyptiaca* in MCf-7 highly than HEPG-2 & HCT 116 and this showed in morphological changes in cells. The induction of apoptosis in tumor cells is considered very useful in the management therapy, as well as in the prevention of cancer. The effectiveness of radiotherapy and chemotherapy may rely on their abilities to induce apoptosis in tumor cells. **Atsushi *et al.*, 2001**.

The apoptosis pathway is regulated by several factors such as p53 and members of the Bcl-2 protein family. Wild-type p53 protein physiologically acts as a DNA-binding transcription factor and may drive apoptosis as a result of DNA-damaging events. **Atsushi et al., 2001**. The previously mentioned data come in accordance to the result presented in the presented study determined the changes in P53 expression in response to treatment with *Balanites aegyptiaca* . In addition, we can also observe in our study DNA damage in cancer cells after treatment with *Balanites aegyptiaca*. Bax protein has been established as a tumor suppressor, because Bax inactivation leads to rapid tumor growth and to a decrease in the extent of spontaneous apoptosis of tumor cells. **Atsushi *et al.*, 2001**. The Bax gene is localized in the cytoplasm of living cells after apoptotic stimulation, Bax translocates to the outer mitochondrial membrane where it oligomerizes and induces the formation of protein-conducting pores that release apoptogenic proteins. **Tsujimoto, 2003**. Bax gene aproapoptic member of the Bcl-2 family is the best characterized mediator of p53 dependent apoptosis. The Bax gene plays a direct role in increasing mitochondrial membrane permeability, leading to release of apoptogenic protein. Also Bax gene also my play important role in apoptosis. **Theodorakis *et al*., 2002.**

Here in this work the results indicated that, the expression of apoptotic gene Bax was investigated and the results demonstrated that, there is increasing in the expression of apoptotic gene Bax in MCF-7, HEPG-2, HCT 116 cells after treatment with *Balanites aegyptiaca*. Also appearance of proteins in treated cells with *Balanites aegyptiaca* is related to the excess of p53& bax genes expression.

A mixture of steroidal saponins: balanitin-6 (28%) and balanitin-7 (72%), isolated from B. aegyptiaca demonstrated appreciable anticancer effects in human cancer cell lines in vitro by using against A549 lung cancer cells and U373 glioblastoma cell lines. A mixture displayed highly antiproliferative activity. It indicated that balanitin 6/7 mixture has anticancer activities. **Gnoula *et al*; 2008.** Steroidal saponins were reported to be involved in the induction of antiproliferative effect through release of apoptosis- inducing factor and modulation of caspase activity in different human cancer cells. These activities were reported to be mediated through reactive oxygen species production. **Corbiere *et al.*, 2004; Huo *et al.*, 2004**. It is reported that bark aqueous extract of *B. aegyptiaca* used in treatment of both AIDS and Leukemia. An oral administration of the aqueous extract (30% w/v given at 100 ml every 8 hours for 30 days) for the treatment of HIV patients have shown excellent results. The same was given to patients with leukemia and a good increase in platelets and a normal blood differential reading after one month was noted. **Hamid. *et al;* 2000**.

**References**

1. Freshney, R.I. (2000). Culture of Animal Cells: A Manual of Basic Technique, 4th Ed.
2. Ali BH, Bashir AK, Rasheed RA, (2001). Effect of the traditional medicinal plants Rhazyastricta, Balanites aegyptiaca and Haplophylum tuberculatum on paracetamol-induced hepatotoxicity in mice, Phytother Res; 15(7):598-603.
3. Annan K, Dickson R. (2008),Evaluation of wound healing actions of Hoslundia Opposita vahl, Anthocleista nobilis G. Don. and and Balanites aegyptiaca L. J Sci Technol;28:26, 33.
4. Arbonnier M. (2004),Trees, Shrubs and Lianas of West African Dry Zones. PP 573.

5. Association of Official Analytical Chemists (AOAC) (1970): Official Methods of Analysis in:- The Association of Official Analytical Chemists. 11th Ed. Washington D.C.
6. Atsushi, K.; Mutsuo, F.; Manabu, M.; Yuji, O.;Shiro, S. and Shohei, O. (2001): Expression of Bax and apoptosis-related proteins in human esophageal squamous cell carcinoma including dysplasia. Canadian Academy of Pathology, 14: 741-747.
7. Beneytout JL, Nappez C, Leboutet MJ, Malivvand G, 1995. A plant steroid, diosgenin a new megakaryocytic differentiation induce of HEL cell. Biochem Biophys Res Commun, 207:398–404. Berne MR, Levy NM, 1998. Physiology. 4th ed. Mosby, New York. Pp 910-929.
8. Chapagain P Bishnu, Yehoshua Hariv, Wiesman Zeev.(2009), Desert date (Balanites aegyptiaca) as an arid lands sustainable bioresource for biodiesel. Bioresource Technology; 100 : 1221 – 1226.
9. Cheung, J.Y., Ong, R.C., Suen, Y.K., Ooi, V., Wong, H.N., Mak, T.C., Fung, K.P., Kong, S.K., (2005); Polyphyllin D is a potent apoptosis inducer in drug-resistant HepG2 cells. Cancer Lett. 217, 203–311.
10. Corbiere, C., Liager, B., Terro, F., Beneytout, J.L.,( 2004): Induction of antiproliferative effect by diosgenin through activation of p53, release of apoptosis-inducing factor and modulation of caspase-3 activity in different human cancer cells. Cell Res.14, 188–196.
11. Elfeel AA, Warrag EI. (2011):Uses and conservation status of Balanites aegyptiaca (L.)Del. (Hegleig Tree) in Sudan: Local people perspective. Asian Journal of Agricultural Sciences 3(4): 386 – 390.
12. Gnoula GM, Megalizz V, De Neve N, Sauvage S, Ribaucoure F, Guissou P, Duez P, Dubois J, IngrassiaL, Lefrance F, Kiss R, Mijatovic T. Balanitin-6 and -7 (2008): Diosgenyl saponins isolated from Balanites aegyptiaca Del. display significant anti-tumor activity in vitro and in vivo International Journal Of Oncology; 32: 5-15.
13. Gutti B, Bamidele SS, Bagaje IM. (2012):Characterization and composition of Balanites aegyptiaca seed oil and its potential as biodiesel feedstock in Nigeria. Journal of Applied Phytotechnology in Environmental Sanitation , 1(1): 29-35.
14. Hamid OA, Wahab ME, Abdu ZZ, Idris SM. (2001): Balanites aegyptiaca extracts for treatment of HIV/AIDS and leukemia.
15. Hanan AA, Ayman A, Farghaly MM, Abd El Aziz MA. (2010):Phytochemical investigation and medicinal evaluation of fixed oil of Balanites aegyptiaca fruits (Balantiaceae). Journal of Ethnopharmacology; 127 (2): 495 – 501.
16. Huo, R., Zhou, Q.L., Wang, B.X., Tashiro, S.I., Onodera, S., Ikejima, T., 2004. Diosgenin induces apoptosis in HeLa cells via activation of caspase pathway. Acta Pharmacol. Sin. 25, 1077–1082.
17. Kamel MS: (1998):furostanol saponin from fruits of Balanites aegyptiaca. Phytochemistry 48: 755-757.
18. Koko WS, Galal M and Khalid HS: (2000),Fasciolicidal efficacy of Albizia anthelmintica and Balanites aegyptiaca compared with albendazole. J Ethnopharmacol 71: 247-252.
19. Koko WS, Abdalla HS, Galal M and Khalid HS: (2005):Evaluation of oral therapy on Mansonial Shistosomiasis using single dose of Balanites aygyptiaca fruits and praziquantel. Fitoterapia 76: 30-34.
20. Lumpkin, O. (1982): Mobility of DNA in gel electrophoresis. Biopolymers, 11:2315-2316.
21. Lerman, L. and Frisch, H. (1982): Why does the electrophoretic mobility of DNA in gels vary with the length of the molecule? Biopolymers,5:995-997.
22. Moalic, S., Liagre, B., Corbiere, C., Bianchi, A., Dauca, M., Bordji, K., Beneytout, J.L., 2001. A plant steroid, diosgenin, induces apoptosis, cell cycle arrest and COX activity in osteosarcoma cells. FEBS Lett. 506, 225–230.
23. Morsy AMA, 2008. Environmental and biochemical assessment of some wild plants growing south of the eastern desert. Ph.D. thesis, Dept of Biochem., Fac. of Agric., Ain Shams Univ., pp. 1–161.
24. Nappez C, Liagre B, Beneytout JL, 1995. Changes in lipoxygenase activities in human erythroleukemia (HEL) cells during diosgenin induced differentiation, Cancer Lett, 96:133-140.
25. Natinal Research Council. Lost Crops of Africa: Volume III, Fruits, Development, Security and Cooperation. (2008).
26. Obidah W, Nadro MS, Tiyafo GO, Wurochekke AU. (2009):Toxicity of Crude Balanites aegyptiaca Seed Oil in Rats. Journal of American Science , 5(6):13-165.
27. Patil SV, Salunke BK, Patil CD, Salunkhe RB, Gavit P, Maheshwari VL. Potential of extracts of the tropical plant Balanites aegyptiaca (L) Del.(2010): (Balanitaceae) to control the mealy bug, Maconellicoccus hirsutus (Homoptera: Pseudococcidae). Crop Protection , 29: 1293 – 1296.
28. Speroni E, Cervellati R, Innocenti G, Costa S, Guerra MC, Dall'Acqua S and Govoni P: (2005): Anti-inflammatory, anti-nociceptive and antioxidant activities of Balanites aegyptiaca (L.) Delile. J Ethnopharmacol 98: 117-125.

29. Sarker SD, Bartholomew B and Nash RJ: (2000):Alkaloids from Balanites aegyptiaca. Fitoterapia 71: 328-330.
30. Speroni E, Cervellati R, Innocenti G, Costa S, Guerra MC, Dall Acqua S, et al. (2005): Anti-inflammatory, anti-nociceptive and antioxidant activities of Balanites aegyptiaca (L.) Delile. J Ethnopharmacol, 98:117–25
31. Sparg, S.G., Light, M.E., van Staden, J., (2004): Biological activities and distribution of plant saponins. J. Ethnopharmacol. 94, 219–243.
32. Speroni, E., Cervellati, R., (2005): Anti-inflammatory, anti-nociceptive and antioxidant activities of Balanites aegyptiaca (L.) Delile. J. Ethnopharmacol. 98, 117– 125.
33. Theodorakis, P.; Lomonosova, E. and Chinnadurai, G. (2002): Cretical requirement of Bax for manisifestation of apoptosis induced by multiple stimuli in human epithelial cancer cells. Cancer Research, 12:337-346.
34. Tsujimoto, Y. (2003): Cell death regulation by Bcl-2 protein family in the mitochondria. J.Cell.Physiol, 195. 158-167