

RESEARCH ARTICLE

Evaluation of Cytotoxic and Genotoxic Effects of *Euphorbia Triaculeata* Forssk. Extract

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Abstract

Objective: To evaluate the cytotoxic and genotoxic activity of *Euphorbia triaculeata* Forssk. plant extract from Jazan region, Saudi Arabia, in an in vitro cancer model, which could be beneficial in anticancer therapy against human breast cancer cell line (MCF-7), prostate cell line (PC-3), human hepatocellular carcinoma cell line (HEPG2) and normal breast epithelial cell line (MCF-10A). The human foreskin fibroblast cell line, (Hs68), was also included in the cell panel. Doxorubicin and 5-Flurouracil, broad-spectrum anticancer drugs, were used as the positive control. **Methods:** Cytotoxicity of *Euphorbia triaculeata* plant extract was investigated by employing MTT assay and the genotoxicity was assessed by using comet assay. **Results:** Both toxicity tests exhibited significant toxicity results. In the comet assay, the *Euphorbia triaculeata* extract exhibited genotoxic effects against MCF-7 DNA and PC 3 but not on HEPG2 cell lines in a time-dependent manner by increasing the mean percentage of DNA damage. *Euphorbia triaculeata* extract showed significant toxicity against cancer cells. Comparison with positive control signifies that cytotoxicity exhibited by methanol extract might have moderate activity. **Conclusion:** The present work confirmed the cytotoxicity and genotoxicity of *Euphorbia triaculeata* plant. However, the observed toxicity of this plant extract needs to be confirmed by additional studies. Based on our results, further examination of the potential anticancer properties of *Euphorbia triaculeata* plant species and the identification of the active ingredients of these extracts is warranted.

Keywords: *Euphorbia triaculeata* Forssk.- MTT assay- comet assay- cytotoxicity- genotoxicity- anticancer activity

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Introduction

Natural products, especially medicinal plants, have played a significant role in drug discovery and development of therapeutic agents. Plants contain many biologically active compound(s) which have potential for development as therapeutic agents. More than 35,000 plant species have been reported to be used in various human cultures around the world for medical purposes (Faleyimu and Oso, 2012). There is a growing scientific consensus about the impending loss of natural product drug discovery scientists (Gurib-Fakim, 2006).

Southwestern of Saudi Arabia is rich of wild plants, comprise about 70% of total flora of Saudi Arabia. One of the largest families in this part of the country is Euphorbiaceae. Many species of this family are used for the treatment of many diseases by the traditional medicine practitioners of Saudi Arabia (Al-Yahya et al., 1990) *Euphorbia* (Euphorbiaceae) is the large genus of about 2000 species throughout the world, of which about 40 species are recognized from Saudi Arabia (Chaudhary, 2001). Species of *Euphorbia* have been used in the

treatment of asthma, leukemia, warts, as a laxative and diuretic, and have antiviral, antibacterial and antifungal activities (Vijaya et al., 1995; Betancur-Galvis et al., 2002; Yu et al., 2005). As anticancer and antitumor, some *Euphorbia* species have activity for some cancers and tumor, like malignant melanoma, squamous cell carcinoma and lung cancer (Sreenika et al., 2015; Wang et al., 2012). It has been reported that some species of *Euphorbia* have potential cytotoxic effect against different cell lines (Sadeghi-Aliabadi et al., 2009). Genotoxicity and mutagenicity assays were recommended to ensure the quality and safety of natural therapeutic compounds by national and international regulatory agencies (OECD, 2014). Though the medicinal importance of plants has been proved with scientific research but care is needed while selecting the right plant for right indication. Proper authentication and identification of plants are needed to limit their side effects (Hussain et al., 2009; Katiyar et al., 2012). The use of plant-based medicines in the treatment of different ailments can be found 5000 years ago, (Anushia et al., 2009; Goldman, 2001). More than one hundred allopathic medicines are being obtained

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from plant extracts and used to treat different diseases (Nadeem et al., 2002; Li and Vederas, 2009; Atmakuri and Dathi, 2010).

The comet assay is a versatile and sensitive method for measuring single-strand and double-strand breaks in DNA (Collins et al., 2008). Among the variety of methods developed for detecting DNA damage, comet assay or single cell gel electrophoresis (SSGE) assay is often used since it is fast, convenient and easy to apply. It is particularly attractive as a method to undertake in vivo and in-vitro studies (Singh et al., 1988; Olive et al., 1990). Researchers focused on this technique because of its low cost and sensitivity. The sensitivity of the method allows detecting the DNA damage on the individual cell level and it has the capability to measure genotoxic potential, quantitatively in prokaryotic and eukaryotic cells.

Since there are no previous studies on cytotoxicity or genotoxicity on *Euphorbia triaculeata* extract, so, the aim of the present study was to determine the in vitro anticancer and genotoxic activity of methanolic extract of *Euphorbia triaculeata* plant against human breast cancer cell line (MCF-7), prostate (PC-3) cell line, human hepatocellular carcinoma cell line (HEPG2) and normal breast epithelial cell line (MCF-10A). The human foreskin fibroblast cell line, Hs68, was also included in the cell panel. The cytotoxic and genotoxic potency of the plant extract was studied in comparison to two known anticancer drugs, 5-Fluorouracil (5-FU) and Doxorubicin (DOX).

Materials and Methods

Collection of plant material

Our target plant, *Euphorbia triaculeata* Forssk. was collected from different locations from South region of Saudi Arabia and was identified by one of the present authors (Dr. Yahya Masrahi, Department of Biology, Faculty of Science, Jazan University, Saudi Arabia).

Extraction of plant material

The washed plants were dried in the shade at room temperature for seven days. Then, they were crushed into final powder. One hundred powder was placed once in the Soxhlet hot extractor using 70% methanol (500 ml) as solvent for two hours at 40 °C, then the plant extract (PE) solution was filtered by Whatman filter paper No. 3. The PE was concentrated to dryness in rotary evaporator under reduced pressure at 45°C. Then, the resulting extract was stored, protected from light in a refrigerator at -20°C in a glass container until use. The plant extract was dissolved at a concentration of 10mg/ml-1 in DMSO.

Determination of anticancer activities

Cell culture

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, and Sanford, ME, USA). Human breast cancer cell line (MCF-7), prostate cell line (PC-3), human hepatocellular carcinoma cell line (HEPG2) and normal breast epithelial cell line (MCF-10A) were included. The human foreskin fibroblast cell line, Hs68, was also included in the cell panel. These cell lines

were obtained from the National Cancer Institute, Cairo University. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml of penicillin, and 100 µg of streptomycin/ml in a humidified incubator with 5% CO₂ at 37°C.

Cell viability assay

For determination of cytotoxic activity of extracts, viable cell numbers were determined using the trypan blue exclusion method. Trypan blue dye exclusion was added to all cultures in a ratio of 1:1. Then, extract treatments and preparations were examined under the standard light microscope at 100X magnification. In principle, viable cells exclude an acid dye such as trypan blue; its uptake is indicative of irreversible membrane damage preceding cell death. The ratio of live cells to dead cells (cell viability) was also determined. Standard curves were prepared and 50% cytotoxic concentrations of extracts (CC50), which caused a 50% decrease in cell viability, were derived.

Trypan blue exclusion assay was performed to assess the effect of the methanolic extract of *Euphorbia triaculeata* on the viability of MCF-7, PC-3, HEPG2, MCF-10A, and Hs68 cells. Approximately 7.5×10⁴ cells/ml was seeded in a six-well tissue culture plate and different concentrations of the extract was added after 24 h. For the determination of growth rate, smaller aliquots were collected in 0.5 ml tubes, trypan blue (0.4%) was added to the cell suspension, and the number of cells (viable-unstained and non-viable-blue) was counted using a hemocytometer. The media were not changed during the induction period. Each experiment has repeated a minimum of three times.

MTT assay

The methanolic extract of *Euphorbia triaculeata* was subjected to a screening system for evaluation of its anticancer activity against breast carcinoma (MCF-7), prostate (PC-3), hepatocellular carcinoma (HEPG2), normal breast epithelial (MCF-10A) and foreskin fibroblast cell line, Hs68, in comparison to the known anticancer drugs: 5-FU and DOX.

Cell viability was determined colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to evaluate the antiproliferative activities of the tested extracts against the cancer cell lines. The assay depends on the cleavage of the tetrazolium salt (MTT) into formazan blue by the mitochondrial enzyme succinate dehydrogenase. The conversion takes place only in living cells and the amount of formazan produced is proportional to the number of viable cells present. Thus, the MTT assay is potentially useful for assaying antiproliferative activities of materials. Exponentially growing cells were plated in triplicate in 96-well sterilized plates at a density of 1×10⁴ cells/well. After 24 h, cells were treated with escalating doses of the extract under investigation and incubated in 5% CO₂ atmosphere with high humidity. After 48 and 72 h of the extract exposure, the cells were incubated with MTT (0.5 mg/ml) for another 4 h at 37°C. The blue MTT formazan precipitate was

then solubilized in detergent (50% final concentration of N, N-dimethylformamide and 10% of sodium dodecyl sulfate) and incubated for an additional 2 h. Absorbance was measured at 570 nm on a multiwell ELISA plate reader. The mean absorbance of medium control was blank and was subtracted. IC50 values were estimated after 72 h exposure to the extract. The absorbance of control cells was taken as 100% viability and the values of treated cells were calculated as a percentage of control. The 5-fluorouracil and doxorubicin anticancer drugs were used as positive control, and cells without samples were used as negative control. The relation between surviving fraction and extract concentration was plotted to get the survival curve of the three cancer cell lines with the specified extract. DMSO is the vehicle used for dissolution of products (Hartmann, 2003).

Determination of DNA damaging effects of the extract Alkaline comet assay

The genotoxic effect of the extracts was determined by alkaline comet assay. For the comet assay, 100, 150, 200 and 300 µg/ml extract concentrations, which have the high cytotoxic effect, were used. Cells were seeded at a density of 5×10^4 cells/well into a sterile 24-well plate and allowed to adhere overnight. After 24 h incubation at 37°C under a humidified 5% CO₂ to allow cell attachment, the cells were treated with 100, 150, 200 and 300 µg/ml concentrations of the methanolic extract and incubated for 72 h under the same conditions. Subsequently, the cells were trypsinized and centrifuged. After centrifugation, the cells were suspended in 150 µl of molten 0.5% low melting point agarose (LMPA) in phosphate buffered saline (PBS) without calcium and magnesium. Then 150 µl aliquots of the cell suspension were rapidly spread on three slides pre-coated with 85 µl of 1% normal melting agarose (NMA) and cover-slipped. After the solidification of agarose for 5 min at 0°C, the coverslips were gently removed and the third layer of 75 µl LMPA was added. The slides were then placed in a tank filled with lysis solution (2.5 M NaCl, 0.1 M EDTA, pH 10, 10% DMSO and 1% Triton X-100 both freshly added) at 4°C. After 1 h, the slides were removed from the lysis solution and incubated in fresh electrophoresis buffer (0.3 M NaOH and 1 M EDTA, pH>13) for 20 min at room temperature for unwinding of DNA. Electrophoresis was then carried out at room temperature in the same electrophoresis buffer for 30 min at 0.7 V/cm and 300 mA. Finally, after electrophoresis, the slides were gently washed twice for 5 min in fresh neutralization buffer (0.4 M Tris-HCL, pH 7.59, followed by dehydration in absolute methanol. Then the slides were stained with 75 µl 1X ethidium bromide and cover slipped.

For each treatment concentration, 100 randomly selected cells from each of three slides were evaluated for DNA damage visually using a 40X objective on a fluorescent microscope. Tail parameters were then calculated automatically using the Komet 4.0 image analysis system (Kinetic Imaging, Liverpool, UK) fitted with an Olympus BX50 fluorescence microscope that was equipped with an excitation filter of 515–560 nm and a

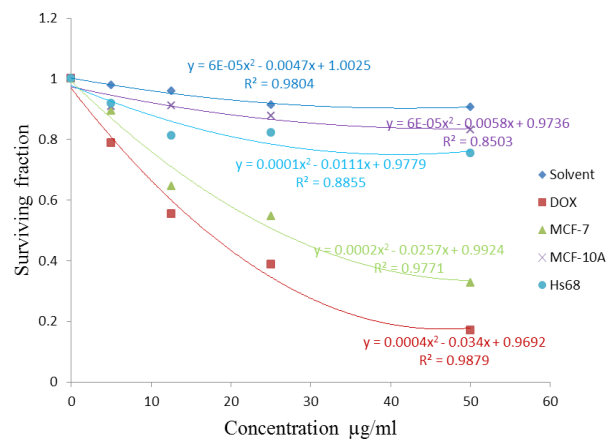


Figure 1. The Cytotoxic Activity of the *Euphorbia Triaculeata* Extract Against Breast Carcinoma MCF7 Cancer Cell Line

barrier filter of 590 nm. Tail parameters used in this study were the tail moment (TM), the tail DNA % (TD), and the tail length (TL). The TM was defined by the percentage of DNA in the tail multiplied by the length between the center of the head and tail, which was defined by Olive et al., (1990).

Statistical Analysis

Data were expressed as mean \pm SD. Mean values were compared with one-way ANOVA followed by Scheffe's test as a post hoc one. $p < 0.05$ was considered significant. All data were analyzed using SPSS version 23. The fold change of control was calculated according to the equation $\text{Fold change} = (\text{treated} - \text{control})/\text{control}$.

Results

Tail parameters

Tail parameters studied included the % DNA in tail, Tail length and Tail Moment

MCF-7 cell line

For MCF-7 cell line, all tail parameters were affected by treatment with *Euphorbia* extract in a time-dependent

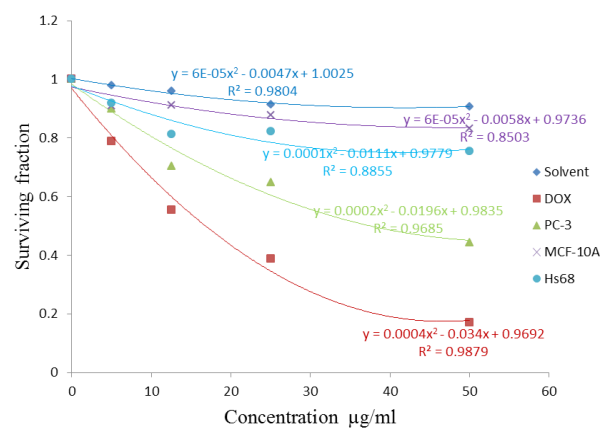


Figure 2. The Cytotoxic Activity of the *Euphorbia Triaculeata* Extract Against Prostate Carcinoma PC-3 Cancer Cell Line

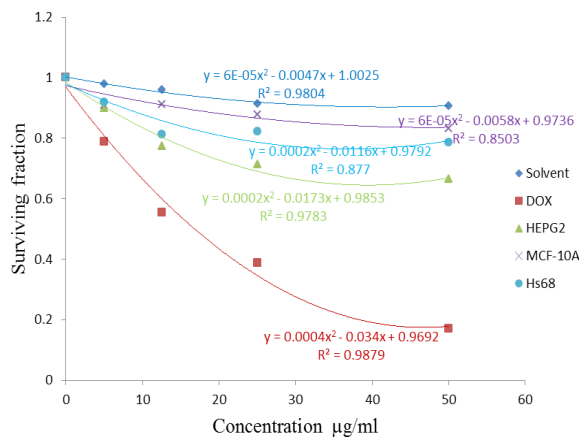


Figure 3. The Cytotoxic Activity of the *Euphorbia Triaculeata* Extract Against Hepatocellular Carcinoma HEPG-2 Cancer Cell Line

manner. The % DNA in tail increased significantly after 24hrs of treatment with the 1.58-fold increase than the control. Prolonged effect of *E. triaculeata* extract increased the % DNA in the tail in such a way that the fold increase than the control was 1.71 and 1.88 for 48 hrs and 72hrs, respectively. For tail length, a very similar condition was observed where a significant increase was detected due to treatment with *E. triaculeata* extract for different times and the fold-increase than the control were 3.39, 3.82 and 4, respectively for 24, 48 and 72hrs treatments, respectively. The tail moment was significantly increased due to treatment and the fold increase than the control was 10.32, 12.09 and 13.43, respectively (Table 1).

PC-3 cell line

For PC3 cell line, all tail parameters were affected by treatment with *Euphorbia triaculeata* extract in a time-dependent manner as seen in MCF-7 cell line but the effect of treatment was not so strong as revealed from the fold increase than the control. As for the % DNA in

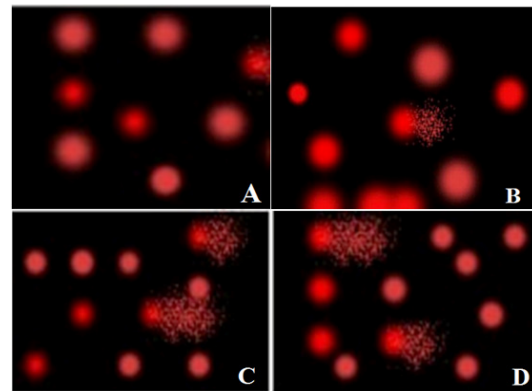


Figure 4. Photomicrographs of Stained DNA For Alkaline Comet Assay For 24 H Showing DNA Damage in Comet Tail of Different Cell Lines Under The Effect of *E. Triaculeata* Extract (25 µg/ml). A: untreated showing no DNA damage, B: HEPG2 C: MCF-7 cells and D: prostate cell line (PC 3)

tail, it increased significantly after 24hrs of treatment with the 0.79-fold increase than the control. Prolonged effect of *E. extract* increased the % DNA in the tail in such a way that the fold increase than the control was 1.05 and 1.31 for 48 hrs and 72hrs, respectively. For tail length, a significant increase was detected due to treatment with *E. triaculeata* extract for different times and the fold-increase than the control ranged between 2.16 – 2.75 for 24, 48 and 72hrs treatments, respectively. The tail moment was also significantly increased due to treatment and the fold increase than the control was 4.63, 6.55 and 7.76, respectively (Table 2).

HEPG2 cell line

Table 3 summarized the effect of *Euphorbia triaculeata* extract on HEPG2 cell line. No significant effect was detected for all tail parameters and the percent change from the control was nearly zero. Thus, HEPG2 cell line was the highly resistant in all cell lines studied.

Table 1. Tail Parameters of DNA Damage by the Comet Assay in MCF-7 Cells Treated with *Euphorbia Triaculeata* Extract (25µg/ml) and Doxorubicin (0.39µg/ml).

Treatments	% DNA in tail	Fold change	Tail length	Fold change	Tail Moment	Fold change
Control	16.87 ± 3.49 a	0.00	24.01 ± 3.17a	0.00	4.03 ± 0.89a	0.00
<i>E.tri.</i> extract (24h)	43.56 ± 10.11b	1.58	105.5 ± 11.02c	3.39	45.63 ± 10.33b	10.32
<i>E. tri.</i> extract (48h)	45.78 ± 9.91b	1.71	115.7 ± 12.03d	3.82	52.73 ± 11.61c	12.09
<i>E. tri.</i> extract (72h)	48.51 ± 9.41c	1.88	120.1 ± 14.69d	4.00	58.15 ± 12.89d	13.43
Doxorubicin	44.51 ± 8.87bc	1.64	95.62 ± 13.91b	2.98	42.40 ± 9.85b	9.52

The different letters indicate statistically significant difference between mean values according to Scheffe's test after one-way ANOVA

Table 2. Tail Parameters of DNA Damage by the Comet Assay in PC3 Cells Treated with *Euphorbia Triaculeata* Extract (25µg/ml) and Doxorubicin (0.39 µg/ml)

Treatments	% DNA in tail	Fold change	Tail length	Fold change	Tail Moment	Fold change
Control	17.11 ± 3.88 a	0.00	23.46 ± 3.52 a	0.00	4.02 ± 1.11 a	0.00
<i>E.tri.</i> extract (24h)	30.59 ± 7.10 b	0.79	74.13 ± 8.32 b	2.16	22.63 ± 5.74 b	4.63
<i>E.tri.</i> extract (48h)	35.02 ± 7.21 c	1.05	87.03 ± 12.87c	2.71	30.37 ± 7.48 c	6.55
<i>E.tri.</i> extract (72h)	39.57 ± 10.13 d	1.31	88.06 ± 18.61c	2.75	35.22 ± 12.63 d	7.76
Doxorubicin	46.32 ± 11.52 e	1.71	99.14 ± 10.08 d	3.22	46.36 ± 13.44 e	10.53

The different letters indicate statistically significant difference between mean values according to Scheffe's test after one-way ANOVA

Table 3. Tail Parameters of DNA Damage by the Comet Assay in HEPG2 Cells Treated with *Euphorbia Triaculeata* Extract (25µg/MI) and Doxorubicin (0.39 Mg/MI)

Treatments	% DNA in tail	Fold change	Tail length	Fold change	Tail Moment	Fold change
Control	16.12 ± 3.73a	0.00	21.21 ± 3.43a	0.00	3.41 ± 0.90a	0.00
<i>E.tri.</i> extract (24h)	15.77 ± 3.97a	-0.02	20.77 ± 4.22a	-0.02	3.25 ± 1.02a	-0.04
<i>E.tri.</i> extract (48h)	15.62 ± 4.90a	-0.03	20.62 ± 5.89a	-0.03	3.21 ± 1.47a	-0.06
<i>E.tri.</i> extract (72h)	16.78 ± 4.89a	0.04	22.96 ± 5.89a	0.08	3.84 ± 1.58a	0.13
Doxorubicin	42.45 ± 8.94b	1.63	94.05 ± 7.70b	3.43	40.02 ± 9.63b	10.77

The different letters indicate statistically significant difference between mean values according to Scheffe's test after one-way ANOVA.

IC50 for the different cell lines

The response parameter calculated was the IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability. The extract of *Euphorbia triaculeata* showed cytotoxicity to the breast carcinoma cell line (MCF7) with IC50 values of 26 µg/ml versus 17.5 µg/ml for doxorubicin (Figure 1). For prostate cell line (PC 3), IC50 value was 48 µg/ml (Figure 2) whereas the extract showed nearly no cytotoxicity to the hepatocellular carcinoma cell line with IC50 values was more than the highest concentration used (50 µg/ml). Figure 3 showed the activity against HEPG2 cell line in comparison to the reference drugs. Figure 4 illustrates the gel electrophoresis results of the different cell lines.

Discussion

Cancer and other chronic diseases share some common pathogenic mechanisms, such as DNA damage, oxidative stress, and chronic inflammation. These diseases can be controlled by resistant to mutagens/carcinogens and/or to inhibit progression of the disease by administering chemopreventive agents (Aly and Mahmoud, 2013). Chemotherapy and surgery are standard methods for treatment of these diseases, although not been fully effective. Most of the anti-tumor drugs currently used in chemotherapy are toxic to normal cells and cause toxicity for immune cells. So, it is important to minimize curing doses to the least amount possible as well as trying to minimize the side effects of these drugs. Therefore, the identification of new anticancer drug with low side effects on the immune system has become an essential goal in many studies of immunopharmacology.

In this investigation, the methanolic extract of *Euphorbia triaculeata* was evaluated for its in vitro cytotoxic and genotoxic activity against human breast cancer cell line (MCF-7), prostate (PC-3) cell line, human hepatocellular carcinoma cell line (HEPG2), normal breast epithelial cell line (MCF-10A) and human foreskin fibroblast cell line, (Hs68). Doxorubicin and 5-Fluorouracil, which are two of the most effective anticancer agents, were used as reference drugs.

Our results showed that the methanolic extract of *Euphorbia triaculeata* exhibited a moderate to strong growth inhibition between 0-50 µg/ml concentrations in comparison to the reference anticancer drugs. The relationship between surviving fraction and extract concentration was plotted to obtain the survival curve of each of the cell lines.

Our results indicated that the cytotoxic effect

strengthens with increasing the concentration of the extract due to the activity of the mitochondrial enzyme, succinate dehydrogenase, which cleaves the tetrazolium ring and converts the MTT to an insoluble purple formazan. The amount of formazan produced is directly proportional to the number of viable cells (Sylvester, 2011). Polyphenol compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase enzyme to prevent the development of cancer cells (Chahar et al., 2011). The mechanism of action of anticancer activity of phenolics could be by disturbing cell division during mitosis at the telophase. It was also reported that phenolics reduced the amount of cellular protein and mitotic index, and the colony formation during cell proliferation of cancer cells. The presence of a 4-carbonyl group of the flavonoid molecule also contributes to the anticancer activity. In addition, the presence of 2, 3-double bond in flavonoid molecules correlates with mitochondrial damage and cancer cell death (Plochmann et al., 2007). The main objective of this assay is to check the cytotoxicity brought about by the extract and find the toxicity levels in terms of IC50 dose when alive and dead cell percentages are equal, which is considered as the optimum dose for the various assays. It has been shown that the methanolic extract possesses antiproliferative activity at a lower concentration. The methanolic extract of *Euphorbia triaculeata* was cytotoxic to two cell lines, which was clearly observed when viewed under inverted microscope. MTT assay was used to evaluate cytotoxicity based on the metabolic reduction of MTT. Thus, the methanolic extract of *Euphorbia triaculeata* is non-toxic to the normal cells and also has both anticancer and anti-proliferative activities against the cancerous cells.

The methanol extract of *Euphorbia triaculeata* showed genotoxic activity in an incubation time-dependent manner. The exhibited genotoxic effects were also comparable to those of the positive control doxorubicin hydrochloride tested in this study. In the group treated only with the extract, the DNA was completely damaged and the amounts of tail DNA were significantly increased over times of incubation.

The balance between the therapeutic and toxicological effects of a compound is a very important measure of the usefulness of a pharmacological drug. Therefore, the determination of the potential mutagenic effect of *Euphorbia triaculeata* extract which exhibited cytotoxic activity is mandatory (Munari et al., 2010). Consequently, comet assay was used in this study for further evaluation

on the potential mutagenic effect of *Euphorbia triaculeata* extract. In the comet assay, it was possible to quantify and to distinguish cells with different rates of DNA damage, thus the analysis of the average values of the scores for each treatment group was very important (Serpeloni et al., 2008). The comet assay has been established as a simple, rapid, cheap, flexible and, most importantly, sensitive method to detect DNA damage, which is also able to detect DNA damage in individual cells. In this assay, cells are embedded in agarose, lysed in an alkaline buffer, and subjected to an electric current. Relaxed and broken DNA fragments stream further from the nucleus than intact DNA, so the extent of DNA damage can be measured by the length of the stream (Tice et al., 2008).

In the present study, the treatment of MCF-7 cells with a concentration of 25 µg/mL of *Euphorbia triaculeata* extract for 72h caused an increase in DNA damage by approximately 48.16% compared to the unchallenged control (1.6-fold increase than the control). These data confirm the genotoxic effect of *Euphorbia triaculeata* extract. Therefore, there are genotoxic compounds in this plant that must be evaluated in future studies.

Chemoprevention and dietary modification studies are underway to identify promising candidates for reduced cancer risk. There is little toxicological information available regarding the safety evaluation on *Euphorbia triaculeata* plant. The present study documents that *Euphorbia triaculeata* plant extract display cytotoxic activity and marked genotoxicity in the comet assay. However, the observed toxicity of *Euphorbia triaculeata* extracts needs to be confirmed by additional studies. After detailed in vivo and in-vitro evaluation and through toxicologic studies, *Euphorbia triaculeata* plant methanol extract may find use as a pharmacological agent in specific dosages, especially in rural communities, where conventional drugs are unaffordable or unavailable and health facilities are inaccessible.

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Conflict of interest

No conflict of interest regarding this study.

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