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Cytotoxic effect of saponins extracted from Yucca on human breast cell line (HBL-100) *in vitro*.

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Abstract: The present study was conducted to investigate the cytotoxic effects of saponins extracted from Yucca (*Yucca gloriosa var. Variegata* L) on humane breast cell line (HBL-100) on diffrent exposure time *in vitro*, by using double dilution series (concentration between 2.44 – 5000 µg/ ml). The results showed that the cytotoxic effect of saponins dependent on amount of dose and exposure time. The concentration 5000 µg/ml gave higher growth inhibition (IR), were (73,78 and 96) % compared with control 100% after (24,48 and 72) hours respectively from exposure time, all inhibition rate begin decreases with decreases the concentration of saponins. However low concentrations of extract was found to induce the (HBL-100) cells growth and proliferation, it was between (114 to 147)% by treatment with (39.06 to 2.44) µg/ ml after 72 hours. Yucca saponins worked in tow direction (inhibition and proliferation), this phenomena called (Hormesis).

Key words: Yucca, saponins, HBL-100, inhibition Rate, proliferation Rate.

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Introduction:

Breast cancer is the most common cancer in Ira, it ranks the first in all the years from 1986-2011 according to the Iraqi Cancer Registry .The incidence rate about (23.01) per 100,000 female population in 2011 compared to (16.56) per 100.000 female population in 2008 (1).Chemotherapy is one of the conventional cancer treatments in addition to surgery, radiotherapy and adjuvant therapy which is using of chemotherapy and /or endocrine therapy following surgical removal of the primary tumor aimed to killing disseminated tumor cell while radiation therapy is generally used after primary tumor resection surgery. It is applied to the tumor bed and regional lymph nodes to kill any residual tumor cells which may cause replace and the development of metastases (2). All these types of treatments are costly and carry a high risk of side effects and resistance, besides of their unavailability, resulting in high morbidity and mortality rates especially in poor countries (3).The researchers have developed anti-cancer strategies to overcome such fatal

disease, and accordingly novel pharmacological paradigms have been developed which quickly and efficiently moves prospective anti-cancer drugs from the discovery phase through pharmacology testing and into therapeutic trial assessment. Some of these developments are based on natural products (4). There is a large and over expanding global population that prefers the use of natural products in treating and preventing various medical complications (5).

Yucca plant is a genus in the family Agavaceae including approximately 42 species distributed around world and two species in Iraq(6). This plant has been described as one of the plants that has been contributed an important source of drugs since long time (7). Saponins are glycosylated natural products, a wide group of structurally diverse glycosides of the plant kingdom and also of some marine organisms and insects (8,9). Several studies were reported to saponins possession anti-cancer activity from *Yucca gloriosa var. Variegata* have been guaranteed the structure-activity relationships of these saponins. The current study aimed to evaluate the cytotoxic effect of saponins extracted from Yucca on human breast cell line (HBL-100).

Materials and Methods:

Plant material:

Yucca gloriosa var. *Variegata* leaves were collected from the greenhouse of College of Science for Women, University of Baghdad. Mother plant was obtained with label showing the scientific name of the studied plant with its photograph from a local nursery in Baghdad. Leaves were rinsed gently by tap water then left under tap water for 30-60 minutes to get rid of dust and other attached materials.

Preparation of saponin extract:

The extraction of saponins from the leaves of *Yucca gloriosa* var. *Variegata* was carried out following the method of Kaur *et al*. (10). The collected leaves were dried in oven at 40°C and were pulverized by electrical blender. Dried plant material (36g) was extracted with 360 ml ethanol (70%) by heating to 50 °C for 8 hours using ordinary reflux apparatus, and then the plant material was filtered after cooling. The filtrate was evaporated at room temperature into about 40 ml. Water-saturated n-butanol (60ml) at 1:3 ratio was added to a total of 40 ml of ethanol extract, then the mixture was shaked slowly several times. The n-butanol phase was separated to the upper and lower phases, water was disregarded. The extract was left to dry in petri dishes at room temperature.

Cell line and cell culture preparation:

Human breast cell line (HBL-100) was used in the study, where cells were grown in T-25 culture flasks in humidified atmosphere with 5% CO_2 at 37°C and maintained in RPMI-1640 media. When the cell grown and form confluent monolayer were detached from the surface of flask according to the following protocols(11).

Cell growth preparation and cytotoxicity assays:

For cell growth and cytotoxicity assays, 200µl of cells suspension were

seeded in 96-well microtitration plates at density 1×10^4 cells/ml and incubated for 24 hr in humidified atmosphere incubator at 37°C**.** Then the medium was discard and added 200 µl from two fold serial dilution of twelve concentrations from saponins extract , was between (2.44 to 5000 µg/ml) in serum free media (SFM).For exposure time 24, 48 and 72 hr. Three replicates wells were used for each concentration of extract and 200µl of maintenance medium added to 8 wells represented as negative control. At the end of the exposure period the plates were stained by 100µl of 0.5% crystal violet solution and incubated at 37C° for 20 min, the stain was washed gently with tap water until the dye was removed. The plate was left at room temperature to dry. The optical density of each well was read by using a micro-ELISA reader at 492 nm transmitting wavelength (Freshney, 1994), the percentage of inhibition rate (I.R) was calculated according to the following equation (12):

Proliferation rate (P.R) was calculated according to the following equation (13):

IR=A-B/A X 100

PR= B/A X 100

IR= Inhibition Rate
PR= Proliferation Rate
A =Optical density of control
B= Optical density of test

Results:

The toxic effect of the saponins extracted from yucca was studied on the human breast cell line (HBL-100) with concentrations ranging from (2.44 - 5000) µg/ml by two fold dilution method.

The results showed in figure (1) that the inhibitory effect after 24 hours of treatment was clear at the highest concentrations used (5000) µg/ml, gave the highest percentage of Inhibitory Rate (73%) Compared to control (100%), while the inhibitory rate decreased at the concentration between (2500-39.06) µg/ml there were ranged between (34 – 18)%. The subsequent concentrations gave an increase in the proliferation rate, (105%) compared to control (100%) at the lowest concentrations used (2.44) µg/ml.

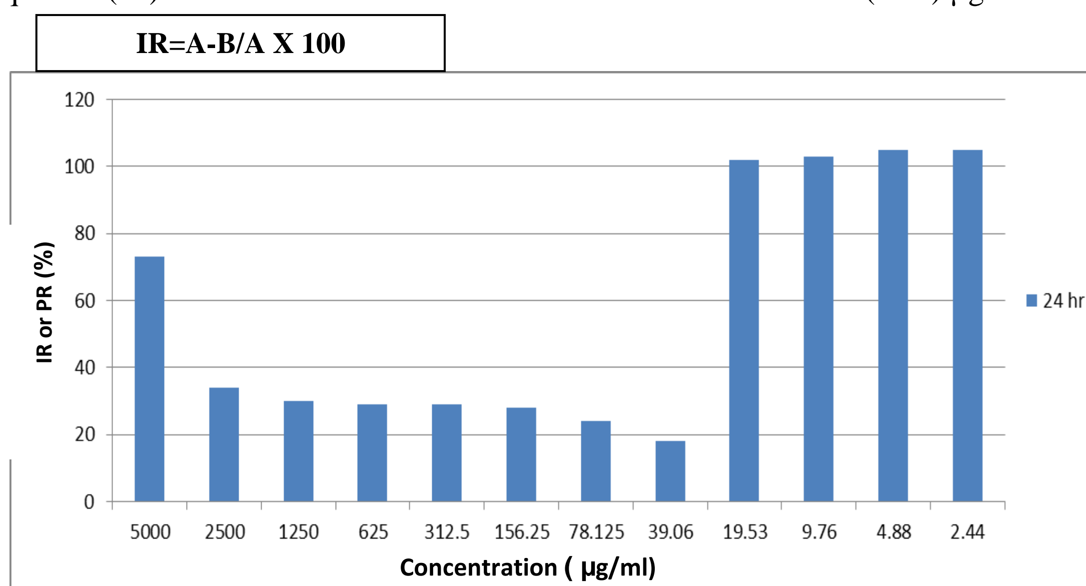
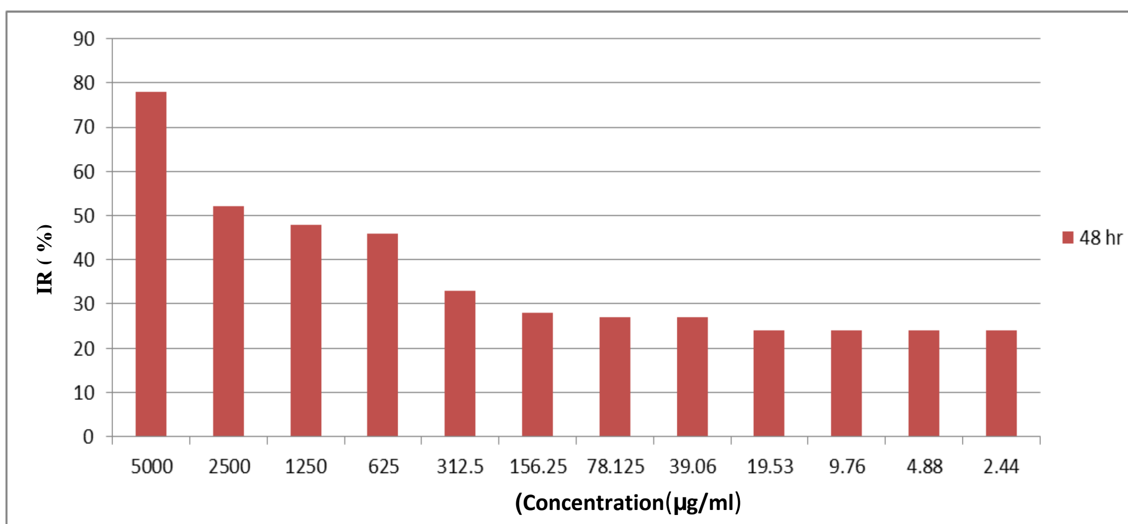


Figure (1): Cytotoxic effect of saponins extracted from Yucca on humane breast cell line (HBL-100) after 24 h exposure time.

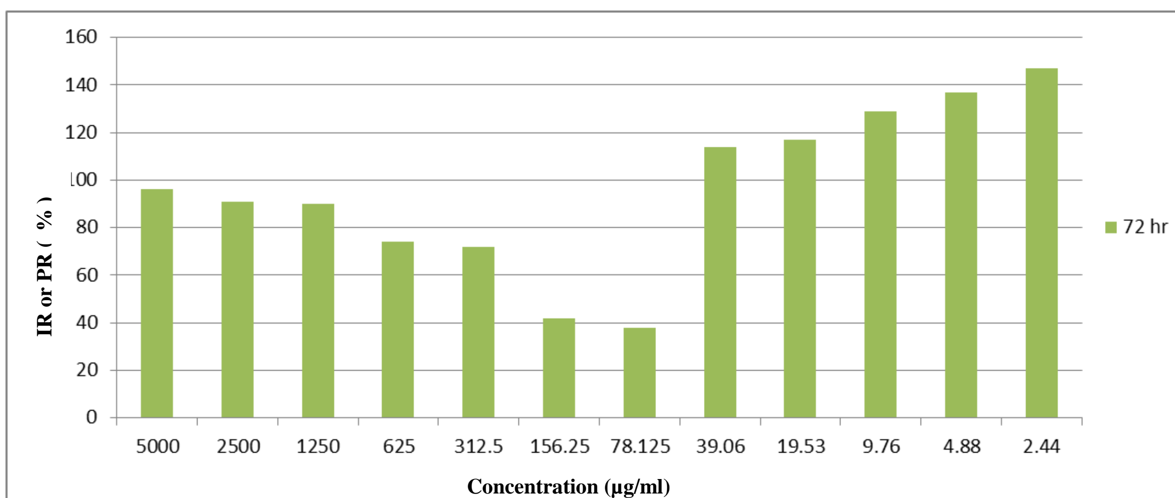
After 48 hours of treatment with saponins, the highest inhibitory rate (78%) was observed when using the concentration (5000) µg/ml compared to the control (100%),(the viability (22%). Followed by a gradual decrease "with a decrease in concentration reached to 24% at the lowest concentrations (2.44) µg/ml (Figure 2).



Figure(2): Cytotoxic effect of saponins extracted from Yucca on humane breast cell line.

(HBL-100) after 48h exposure time:

The Inhibitory Rate increased to 96%, (Viability was 4%), at the highest concentration after 72 hours exposure with yucca saponins reached to (38%) at the concentration (78.115) µg/ml, while the lower concentrations gave a increase in the percentage of Viability of the cells, the Proliferate Rate was increased in reverse with decline the concentration, ranging from (114 - 147%) for concentrations ranging between (39.06 - 2.44) µg/ml compared to control (100%), (Figure 3).

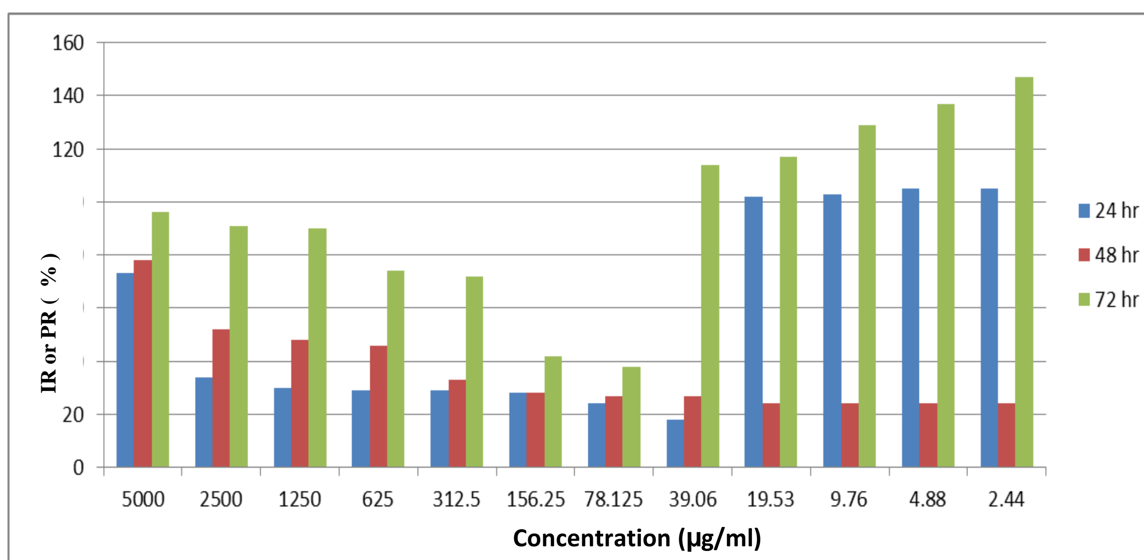


Figure(3): Cytotoxic effect of saponins extracted from Yucca on humane breast cell line

(HBL-100) after 72 h exposure time:

Figure (4) shows a comparison between the three time periods (24,48,72) hours for the effect of saponins in human breast cells(HBL-100). The study found that the best period of time for treatment to kill highest number of cells as possible was 24 hours, Inhibitory rate was high compared with 48 and 72 hours. The inhibition rate was (96%) at (5000) µg/ml of saponins, which is high compared to 73 and 78% after 24 and 48 hours, respectively. Also, at the concentrations that followed, 2500 µg/ml gave 91% the Inhibitory Rate, (viability 9%), compared to (34 and 52%) inhibition rate after (24 and 48) hours, and so for the other concentrations.

The stimulation of growth and the increase in cell growth rate taken after 72 hours from exposure time for yucca saponins. Figure (4) shows a significant increase in cell viability to 147% at 2.44 µg/ml compared to control (100%). But was (105%) after (24) hours of treatment at the same concentration used, and there was no increase in the viability of HBL-100 cells after 48 hours of treatment.



Figure(4):Comparative cytotoxic effect of saponins extracted from Yucca on humane breast cell line (HBL-100) after different exposure time.

Discussion:

The saponins from the genus *Yucca* have been proven to be antitumor, antimicrobial and anti-inflammatory activity which the anticancer activity have been demonstrated in number of studies on the genus Yucca particularly by Ibrahem, 2012 (14) in Iraq which showed the activity of steroidal saponins as anticancer from *Y. aloifolia*. However, Sobia *et al*. (15) indicated the antioxidant activity of the extract from *Y. aloifolia*. In this study, saponins extracts inhibited human breast cell line, it has been suggested that activity of these compounds by induction apoptosis and cell cycle (G1),(16,17). In anthor research, it is has been reported that the triterpene saponins (saxifragifolin B and saxifragifolin D) isolated from the plant *Androsace*

umbellate Merr (Primulaceae), possess the ability of inhibition of cancer cell growth and also induction of apoptosis (18). Also, cytotoxic activity of saponins have been investigated in this work, another study, indicated the cytotoxic effect of triterpene saponins from the leaves of *Aralia elata* (Araliaceae) which these compounds showed significant cytotoxic activity against HL60 and A549 cancer cells (19). Different steroidal saponins from various plants as *Dioscorea zingiberensis* Wright (DZW) (Dioscoreaceae) and *Allium chinense* had more cytotoxic effect against to murine colon carcinoma cell line C26 and breast carcinoma cell line. The proliferation inhibitory effect of ZS was associated with its apoptosis-inducing effect by activation of caspase-3 and caspase-9 and specific proteolytic cleavage of poly (ADP-ribose) polymerase (20,21).

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