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Toxicological study on Epothilone B which isolated from Aspergillus fumigatus

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ABSTRACT

Background: The myxobacterium Sorangium cellulosum produces epothilones, which are secondary metabolites that are macrolactones that have a broad anticancer effect on a variety of cancers with a higher propensity to stabilize their microtubule arrays during cellular division. Epothilones' higher water solubility contributes to their effective anticancer activity, which significantly affects tumor cells resistant to several drugs. Because of its water solubility, epothilone has a thousand-fold better effectiveness than taxol against tumors that are resistant to it, meaning that it can be used in vivo without the need for adjuvants. **Aim:** The aim of this study is designed to evaluate the anticancer activity of Epothilones as a natural products extracted from fungi and test it's cytotoxic effect. **Methods:** extracted of epothilones from Aspergillus fumigatus were examined invitro on different cell lines And evaluate biochemical estimations of complete blood picture, liver function and kidney function were performed**. Results:** Invitro, IC_{50} of extracted epothilone B On different human lines liver cancer, colon cancer, prostate cancer, breast cancer and vero (normal cells) were found 6.32 ± 0.05 µM , 7.34 ± 0.21 µM , 7.6 ± 0.06 µM , 11.91 ± 0.24 µM and 18.77 ± 0.3 µM respectively. **Conclusion:** extracted of Epothilones has apotent antitumor activity on different cell lines, and as a drug may be safe compounds.

Introduction:

Cancer is one of the fatal and diverse group of disorders with varied biological properties caused by series of clonally selected mutation in key tumor-suppressor genes and oncogenes. It is characterized by the uncontrollably dividing proliferation of aberrant cells or tissues that have the potential to invade and damage healthy bodily tissue. WHO projections for 2011 show that cancer has a greater death rate than either heart disease or strokes.[1] According to GLOBOCAN 2012, It was discovered that there were 8.2 million cancer-related deaths and 14.1 million new cancer diagnoses globally in 2012. Over the coming decades,

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there will likely be a constant rise in the cancer burden due to ongoing changes in the world's population and epidemiology [2,3]. Radiation therapy, chemotherapy, and surgical excision are the gold standard methods for treating cancer, either separately or in combination [4]. But these treatments have serious side effects, like baldness, nephrotoxicity, depression of the bone marrow, and others that leave the patient psychologically and functionally disabled and ultimately socially isolated. Multidrug resistance (MDR) in cancer chemotherapy has adversely affected treatment success rates, making it a severe threat to universal health care. MDR is defined as resistance to some chemotherapeutic medications along with concurrent cross-resistance to other anticancer medications with different structures or modes of action. The complicated resistance mechanisms of cancer, biological activity limitations, and toxicity of MDR reversal medicines mean that existing chemotherapeutic drugs fall short of optimal cancer treatment needs [5]. Therefore, there is an urgent need to find a different, non-invasive therapeutic approach to treat cancer patients who are severely disabled in order to overcome this issue and combat the most deadly illness in the world. Natural compounds derived from bacteria and plants, such epothilones, have been crucial in the search for cancer drugs, producing a wide range of therapeutically effective compounds. In contrast, despite substantial research efforts revealing a huge number of fungi-derived natural compounds with promising anticancer action, studies of fungal metabolites and their derivatives have not produced a therapeutic cancer treatment. Numerous natural products have shown significant growth-inhibitory qualities in human cancer cell lines in vitro, and some of these chemicals have even been shown to be beneficial in treating human cancer in mice models. It is anticipated that a large number of these molecules will soon go through human clinical trials. The myxobacterium Sorangium cellulosum produces epothilones, which are secondary metabolites of macrolactones that have wide anticancer efficacy against a variety of malignancies and a stronger propensity to stabilize their microtubule arrays during cellular division [7]. Epothilones' superior water solubility contributes to their effective anticancer activity, which significantly affects tumor cells resistant to several drugs. Epothilone has a thousand-fold better activity than Taxol against cancers resistant to Taxol because of its water solubility, which eliminates the need for adjuvants in in vivo applications [8,9]. in this study we aim to evaluate the anticancer activity of Epothilone B which isolated from ***Aspergillus fumigatus fungi*** as a natural products extracted from fungi and test it's cytotoxic effect invitro and invivo IC_{50} and LD_{50} .

Material and Methods:

Substance: Epothilone B prepared and isolated from ***Aspergillus fumigatus fungi*** at Zagazig university faculty of science in our previous study.

FT-IR analyses of the putative epothilone:

The FT-IR spectra of the sample were assessed by Bruker FT-IR Spectrometer in range of 400-4000 cm^{-1} using KBr pellets, the sample was dissolved in $CDCl_3$. The chemical shifts and coupling constants are expressed in part per million (δ-scale) and hertz (Hz) [10].

The Invitro study:

Antitumor Efficiency of Epothilone B from Aspergillus fumigatus

Acquiring and Modifying Cells: The colon cancer cell line (HCT116), human hepatocellular carcinoma (HepG2), breast cancer cell line (Michigan Cancer Foundation-7) (MCF-7), and Vero normal cell line were provided by the National Cancer Institute, Cairo University, Egypt. All cells were maintained at 37 °C in DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin, following the approved cell culture protocol. The environment also included 5% CO_2 and 95% humidity [11].

Cytotoxic effect of Epothilone B on cancer cell lines:

With a few modifications, the cell cytotoxicty experiment was conducted using Mossman's methodology [12]. The ability of live cells to convert MTT into blue formazon product is how this assay assesses mitochondrial function. In summary, 96-well plates containing 10,000 cells per well were seeded, and various concentrations of epothilone B (2.5-80 µg/ml) were applied for 24 hours on a variety of cell types, including HepG2 (liver cells), Mcf 7 (breast cells), Pc3 (prostate cells), HCT 116 (colon cells) and Vero (normal cells). Following the completion of the treatment period, the culture media was removed from each well to prevent interference from the epothilone B. A fresh medium containing 10% of the culture volume of MTT solution was then added, and the mixture was incubated for three hours at 37 °C until a purple formazan product formed. Acidified isopropanol was used to dissolve the formazan product that was produced. The remained extracted epothilone B was then settled down by centrifuging a 96-well plate for five minutes at 2300 g. Afterwards, a fresh 96-well plate was filled with 100 µl of supernatant, and the absorbance was measured at 570 nm. Additionally, using the IC_{50} value to determine which chemical (drug) was most effective against HepG2, cell survival as a function of time was measured [13,14].

The invivo study:

Toxicity study (LD_{50}):

Male albino rats were used to determine the approximate median lethal dosage (LD_{50}) of Aspergillus fumigatus-derived epothilone B. Using thirty male albino rats, the rats were housed in a standard laboratory setting at 25±2°C with free access to food. Five dose levels of Epothilone B were placed in ascending concentrations in a regular progression, and all injections were administered intraperitoneally. Animal injection results were documented 24 hours after the injection, and the Meier method was used to calculate the lethal dose (LD_{50}) [15].

Animals and experimental design

Thirty healthy adult male albino rats weighing 150 grams were used in this investigation. Theodor Bilharz Research Institute in Cairo, Egypt provided the animals, which were housed in a typical environment with unlimited access to food and water. Every animal was kept in clean, plastic cages in rooms with exhaust fans and good ventilation. They were given a regular pellet feed and daily access to water. The National Research Center's Ethics Committee gave its approval for all animal procedures, and Zagazig University's institutional animal care and use committee's recommendations were followed.

(ZU-IACUC/1/F/179/2024). Six groups of five rats each were created by randomly dividing the animals.

* Control one: No therapy was given to the rats (rats received saline 300 µM saline).

and other groups were given C at different conc. By increasing dose progressively.

* Group (1) were given 25 µM Epothilone B + 275 µM saline.
* Group (2) were given 50 µM Epothilone B + 250 saline.
* Group (3) were given 75 µM Epothilone B + 225 saline.
* Group (4) were given 150 µM Epothilone B + 150 saline.
* Group (5) were given 300 µM Epothilone B through intramuscular injection for each.

Samples collection

24 hours later, the rats were put to death by ether anesthesia. Under light ether anesthesia, blood samples were taken from the retroorbital venous plexus [16]. Blood was centrifuged for ten minutes at 4000 r.p.m. to create serum. A sample of serum were kept at -20 °C until biochemical analysis. EDTA vacuum tube that is used in an automatic CBC analyzer to estimate

hematological parameters in experimental mouse groups.

Biochemical Analysis

- Determination of hematological parameters.

An automatic CBC analyzer (Sesmex Kx-21) was used to measure hemoglobin (HB), red blood cells (RBCs), white blood cells (WBCs), platelets, and blood indices [17].

- Estimation of liver function (liver function tests)

***Determination of ALT and AST*:** The International Federation of Clinical Chemistry states that the kinetic approach was applied using a Spectrum kit [18].

Determination ALP, albumin and Total protein: A Diamond kit was used to measure albumin and ALP [19]. The Biuret method was used to determine total protein [20].

- Estimation of kidney function (kidney function tests): Urea and creatinine were determined colorimetrically by using a Diamond kit [19].

Statistical analysis:

The means ± SD were used to express the results, which were carried out in biological triplicates. Using a one-way ANOVA and Fisher's Least Significant Difference post hoc test, the significance and F-test were determined.

Results:

Chemical characterization of the extracted Epothilone from *A*. fumigatus

The FT-IR analysis showed the purified sample's chemical identification. From the FT-IR chart, the hydroxyl groups at 3300 cm^{-1}, CO at 1663 cm^{-1} and epoxy ring at 1200 cm^{-1}, was observed (Figure 1). The aliphatic CH, ester groups, and aromatic ring stretches were identified as the sources of the peaks at 2921, 1623, 1485, and 1099, respectively.

Invitro study:

The cytotoxic effect of extracted epothilone B on different human cancer cell lines

The MTT assay was used to assess the cytotoxicty of Vero, HepG2, Mcf 7, Pc3, and HCT 116 cells after they were exposed to extracted Epothilone B (2.5–80 µg/ml) for a full day. The isolated epothilone B significantly and dose-dependently reduced the viability of all five types of cells, according to the results. Additionally, we noticed that the following order of extracted epothilone B produced cytotoxicity. HepG2 cells < HCT 116 cells < Pc3 cells < Mcf 7 < Vero cells. Cell viability was decreased to (6.32 ± 0.05 µM) for HepG2 and (7.34 ± 0.21µM) for HCT 116, while (7.6 ± 0.06 µM) for Pc3 cells, while (11.91 ± 0.24 µM) for Mcf 7 and (18.77 ± 0.3 µM) for Vero cells at the concentrations of 2.5,5,10, 20, and 40 µg/ml, respectively ($p < 0.05$) (as shown in Figure 2).

The invivo study:

Toxicity study

Determine the median lethal dose (LD_{50}) of the Epothilone B from *Aspergillus fumigatus*. All doses of Epothilone B were found to be safe up to 300 µM extract / gm mice, as none of the mice were dead, which suggests that Epothilone B from *Aspergillus fumigatus* may be safe compound.

1-Effect of a different doses of Epothilone B (25 µM , 50 µM , 75 µM , 150 µM and 300 µM /150gm) for 24 h on plasma Hematological parameters **Table (1).**

2-liver function parameters in various studied groups:

- Effect of a different doses of Epothilone B (25 µM , 50 µM , 75 µM , 150 µM and 300 µM /150 gm) for 24 h on serum levels. (ALT, AST, ALP, Albumin and Total protein) **Table (2), Figure (3).**

3- Kidney function parameters in various studied groups:

- Effect of a different doses of Epothilone B (25 µM, 50 µM , 75 µM , 150 µM and 300 µM /150 gm) for 24 h on serum levels. (Creatinine and urea (mg/dl)) **Table (3), Figure (4).**

Discussion:

A group of deadly illnesses known as cancer are thought to be a major worldwide health threat. Numerous physical, chemical, genetic, and environmental variables promote the development and spread of cancer. As of yet, no effective anticancer medication has been discovered that claims to both treat and prevent cancer from spreading. The goal of research is to develop a more potent chemotherapy medication that targets and kills cancer cells while having minimal or no detrimental effects on healthy cells [21,22]. Natural compounds derived from plants and fungi, such as epothilones, have proven essential in the search for cancer treatments since they provide a range of compounds with potential therapeutic applications. One type of macrolide molecule that has anticancer activity and potent inhibitory effects on various cancer types is epothilones. Epothilone's enhanced water solubility contributes to its impressive action. It subsequently establishes a robust conjugation with tubulin found in the microtubule arrays of cancer cells, so inhibiting their cellular division at the G2-M phase [23]. Anticancer medications that target microtubules are among the most often given medication. Many novel drugs that target microtubules are being tested in therapeutic setting, despite that fact the processes by which disruption of microtubule activity results in the selective killing of cancer cells are yet unknown. The initiatives partly aim to address some of the issues with taxane- based treatments, such as challenges with formulation and administration and vulnerability to p- glycoprotein-induced resistance. These initiatives have produced epothilones, a promising new class of anticancer medications. Epothilones bind to and stabilize microtubules in a way that is comparable to paclitaxel, if not exactly the same, according to preclinical research, and they also work well in tumor models that are resistant to paclitaxel [24]. Based on their greater affinity to interact with β-tubulin microtubule arrays, epothilones have a unique activity that stabilizes tubulin disintegration and causes cell death. Epothilone derivatives have been recognized for their potent anticancer effect towards several types of solid tumors [25, 26, 27]. Epothilones exhibit a kinetic advantage over paclitaxel mostly because to their increased water solubility and lower tubulin binding energies, as demonstrated by molecular modeling analyses. Additionally, they have a significant impact on numerous drug-resistant cancers [28, 29]. Currently, epothilones are commonly produced using S. cellulosum as the source. In this study, We have investigated the toxicological effects of Epothilone B in five various human body cell lines. (HCT 116, HepG2, PC3, McF 7, and Vero cells) Following the extraction of Epothilone B, we characterised it and then tested it in vitro systems to see if it had any cytotoxic potential. The biotransformation of a chemical in vitro may be modest compared to that in in vivo systems due to the restricted metabolic capacity of the in vitro models. As showed in Fig. 2 it is evident that HepG2, HCT 116, Pc3, Mcf 7 and Vero cells responded differently to Epothilone B exposure. The viability values indicate that Mcf 7 and Vero cells were more sensitive to the extracted epothilone B exposure than HepG2, HCT 116 and Pc3 cells after 24 h. Decrease in cell viability after Epothilone B treatment were in following order; HepG2 cells < HCT 116 cells < Pc3 cells < Mcf 7 < Vero cells. Then we have investigated the cytotoxicity effects of Epothilone B in vivo The approximate median lethal dose (LD_{50}) of Epothilone B from *Aspergillus fumigatus* was carried out on male albino rats. using 30 male albino rats, Ascending concentrations of 5 dose levels of Epothilone B were arranged in a regular progression, and all injections were given intraperitoneally. All doses of Epothilone B were found to be safe up to 300 μM

extract/150 gm mice, as none of the mice were dead, which suggests that Epothilone B from *Aspergillus fumigatus* may is safe compounds. and then evaluate biochemical estimations of complete blood picture, liver function and kidney function were performed.

Conclusion:

In conclusion, Epothilone B was extracted and chemically verified from *A. fumigatus.* The purified Epothilone B had a potent activity towards the different cell lines "HepG-2, MCF-7, Pc3 and HCT-116", compared to the normal Vero cells. The purified Epothilone B has a strong anti-tubulin polymerizing activity by about 2 folds, higher than Taxol, ensuring the efficiency to bind with the β-tubulin and stopping their polymerization. The approximate median lethal dose (LD_{50}) of Epothilone B from *Aspergillus fumigatus* was carried out on male albino rats. All doses of Epothilone B were found to be safe up to 300 μM extract/150 gm mice, as none of the mice were dead, and we showed the effect of Epothilone B on CBC, liver and kidney functions, which suggests that Epothilone B from *Aspergillus fumigatus* may is safe compounds.

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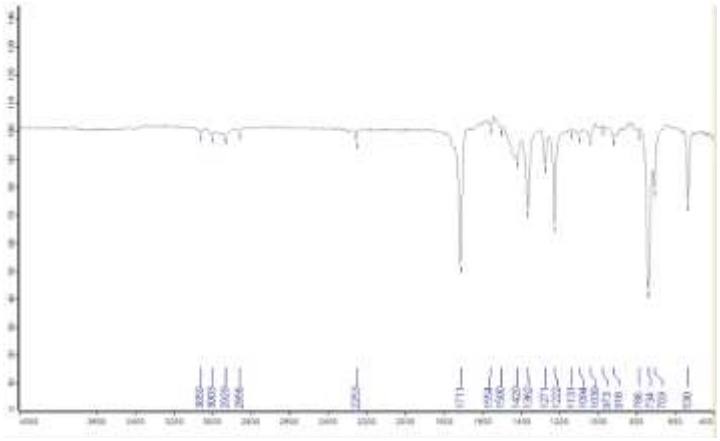
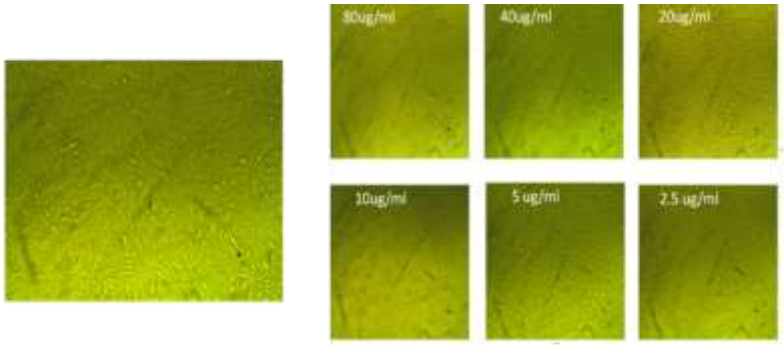
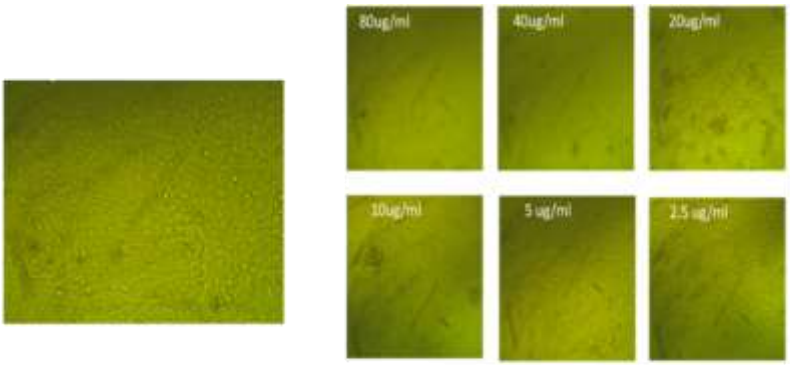


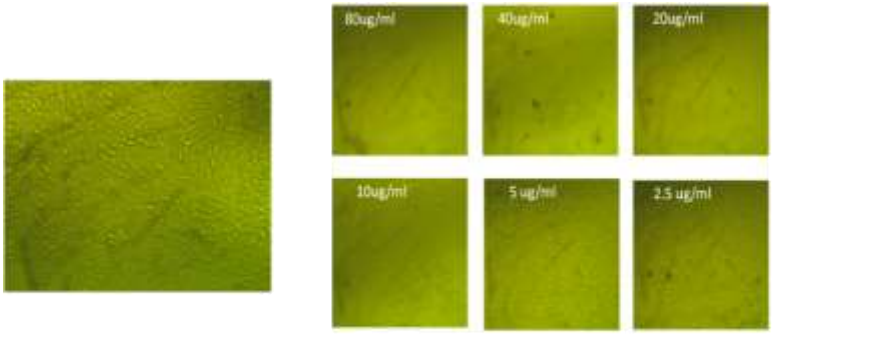
Figure (1). Chemical validation of the extracted epothilone B from Aspergillus fumigatus. A, FT-IR spectra of the purified Epothilone.



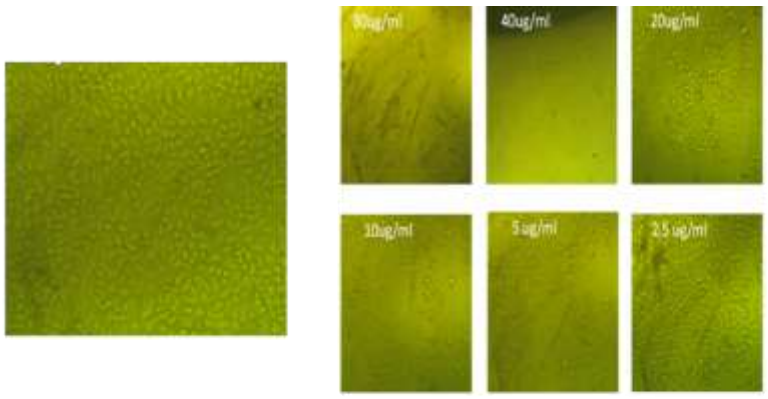
Con. Vero cells The effect of sample on vero cells at different con.



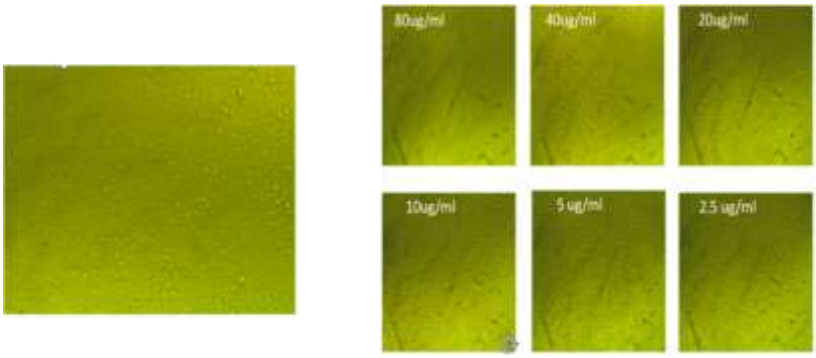
Cont. HePG2 cells effect of sample on HePG2 cells at different conc.



Cont.Pc3 cells effect of sample on Pc3 cells at different conc.



Con. HCT 116 cells effect of sample on HCT 116 cells at different conc.



Con. Mcf7 cells effect of sample on Mcf7 cells at different conc.

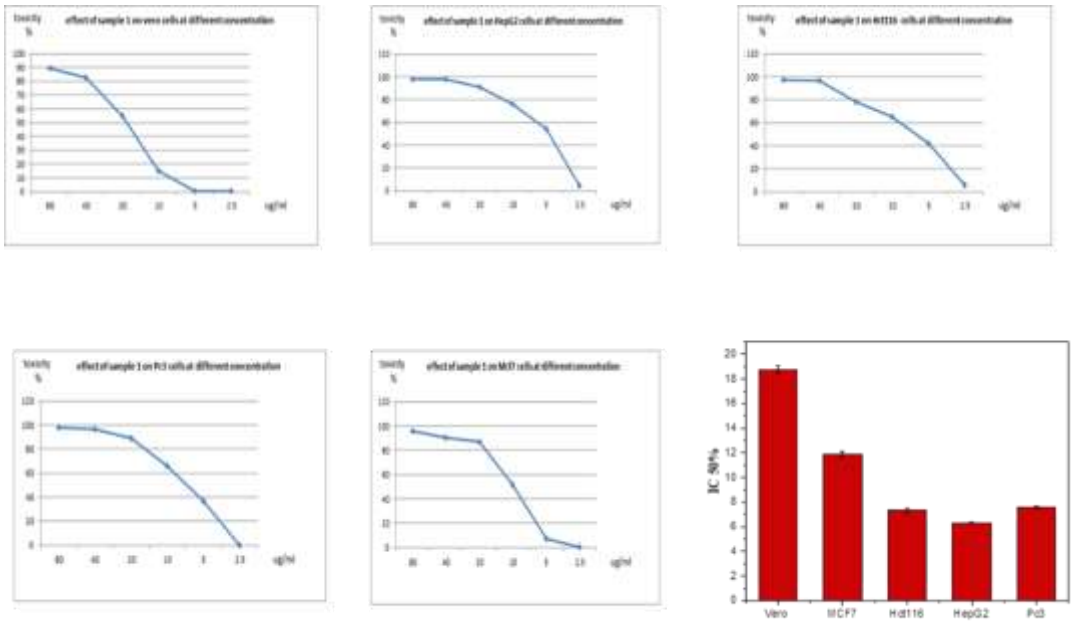


Figure (2). The extracted epothilone B is cytotoxicity against various cancer cells treated for a whole day in varying concentrations (2.5–80 µg/ml). A) The extracted epothilone B is cytotoxic potential against prostate cancer cells (Pc3). B) The extracted epothilone B is cytotoxic efficacy against breast cancer cells (MCF-7). C) The extracted epothilone B is cytotoxic potential against colon cancer cells (HCT 116). D) The extracted epothilone B is cytotoxic potential against liver cancer cells (HepG2). E) The extracted epothilone B is cytotoxic potential against normal cells (vero.) H) The extracted epothilone B is cytotoxicity against different cell lines. panel is the calculated IC_{50} at different times. *Significant difference as compared to the controls ($p < 0.05$).

Table (1): Hematological parameters in all studied groups

	Parameter	control	25 µM/150gm	50 µM/150gm	75 µM/150gm	150 µM/150gm	300 µM/150gm
Complete Blood Count	RBCs($x10^6$)	8.75±0.1	8.81±0.14	8.83±0.16	8.85± 0.18	8.86 ± 0.19	8.89 ± 0.21
	Hb	13.5±0.32	12.9±0.31	13.9±0.34	13.6 ± 0.33	12.95 ± 0.29	13.2 ± 0.35
	PLT($x10^3$)	670±2.85	673±2.83	675±2.85	677 ± 2.86	678± 2.88	681 ± 2.91
	WBCs($x10^3$)	8.1±0.1	8.3±0.13	8.5±0.14	8.7 ± 0.16	8.9 ± 0.17	9.1 ± 0.19

Table (2): Effect of epothilone B on liver function parameters (ALT, AST, ALP, Alb and T.P) in the serum of rats with different doses

Parameter	Control	25 µM/150gm	50 µM/150gm	75 µM/150gm	150 µM/150gm	300 µM/150gm
ALT (U/L)	28±2	30±2.2	31±2.33	33±2.38	35±2.41	39±2.52
AST (U/L)	51±1.52	53±1.56	55±1.7	59±1.9	62±2.1	71±2.5
Alb (g /dl)	4.1±0.97	3.9±0.9	3.6±0.87	3.3±0.83	3.1±0.8	2.9±0.77
Total protein (g/dl)	6.8±1.3	7.1±1.5	7.3±1.7	7.6±1.8	8±2	8.3±2.3

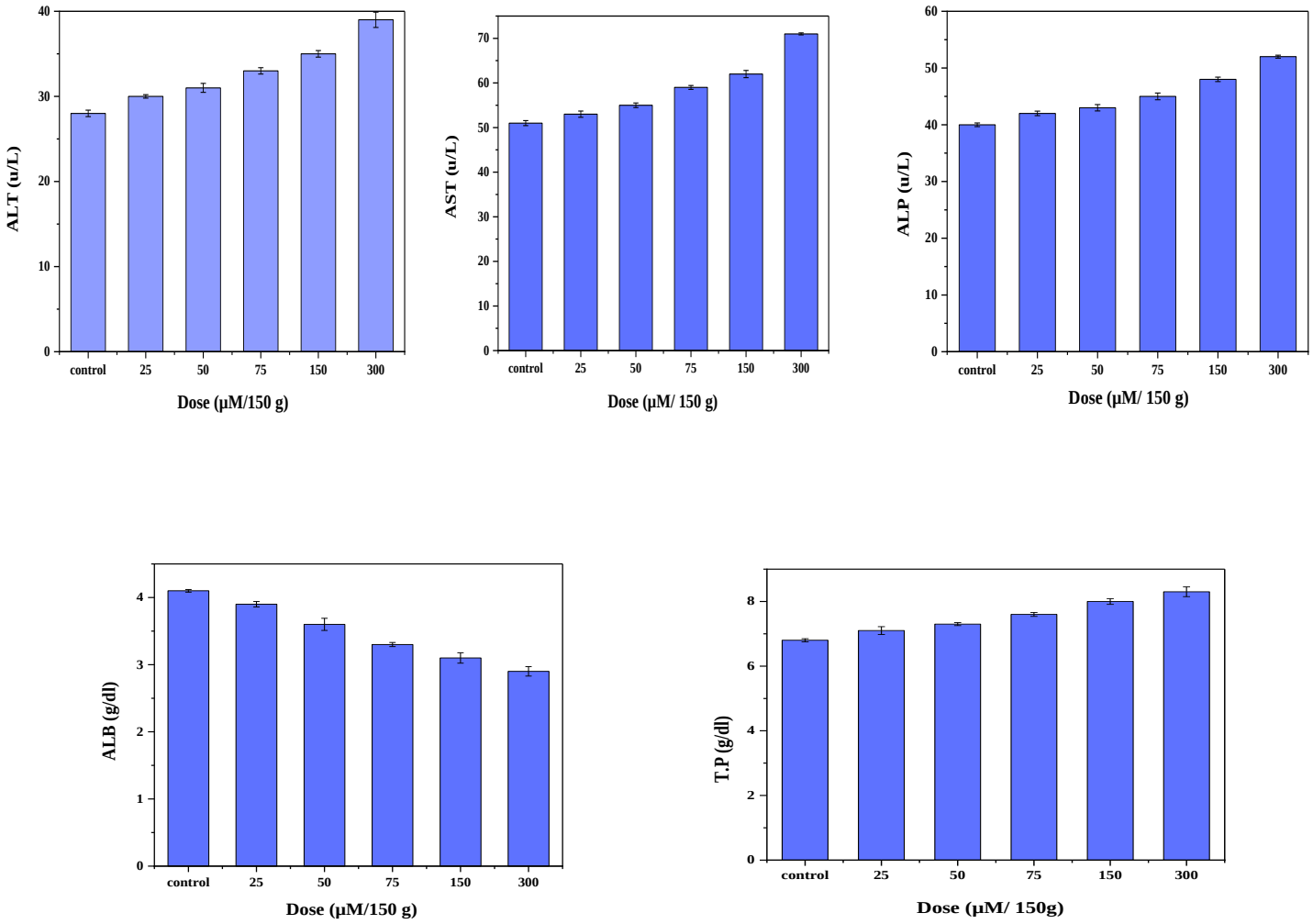
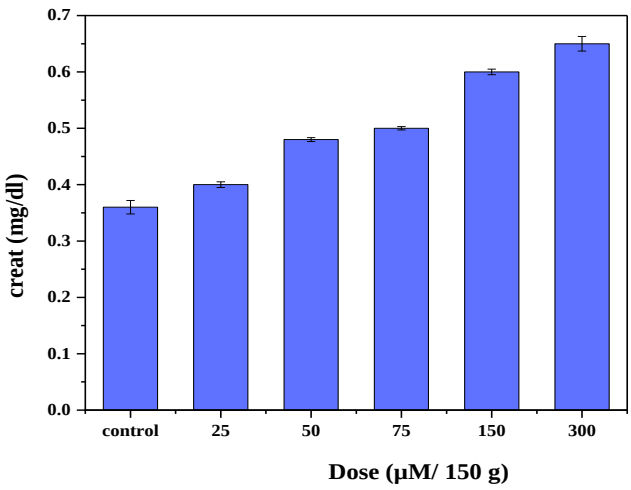


Figure (3): Effect of Epothilone B on (ALT, AST, ALP, Alb and T.P) level in the serum on different conc.

Table (3): Effect of Epothilone B on Kidney function parameters (creatinine- urea) on rats with different doses

Parameter	Control	25 µM/150gm	50 µM/150gm	75 µM/150gm	150 µM/150gm	300 µM/150gm
Creatinine (mg/dl)	0.36±0.12	0.4±0.1	0.48±0.13	0.5±0.14	0.6±0.2	0.65±0.23
Urea (mg/dl)	31.8±2.1	32±2.13	32.5±2.3	33.2±2.41	33.8±2.6	34±2.69



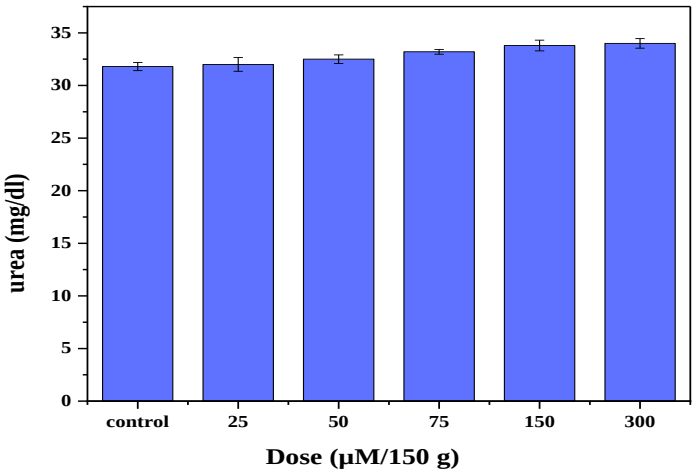


Figure (4): Effect of Epothilone B on creatinine and Urea level in the serum on different conc.