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Research Article

Aspergillus terreus taxol influence on solid ehrlich tumor model

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ABSTRACT

Despite The current study aimed to elucidate the antitumor and anticancer effect of taxol extracted from Aspergillus terreus against EAC solid tumor in male Swiss albino mice. Ehrlich solid tumor was inoculated subcutaneously by the concentration of(2 × 10 6)×2.5 ml and after that fungal taxol was injected i.p. by conc. of 2mg/kg BW and continued for 30 days. Fungal taxol has a marked cytotoxic effect against MCF-7 cell line also inhibit KI67 expression in comparison with positive control mice which showed increase of serum ALT and AST activity accompanied by a decline in albumin serum level and also increase in liver tissue homogenate lipid peroxidation (MDA) level was observed, fungal taxol leads to reduction in the mice body weight and both tumor weight and volume in comparison with positive control group and histopathologically fungal taxol has improved the pathological features caused by the oxidative stress induced by EAC solid tumor.

Keywords: Ehrlich Ascites Carcinoma; Taxol; Mcf-7; Ki67; *Aspergillus Terreus*

INTRODUCTION

Clinically, paclitaxel (taxol) is the furthermost anticancer drug and it is the most widely chemotherapeutic agent against solid tumors[1]. It is well documented that paclitaxel has a great effect in tumor regression as it stabilizes the polymerization of microtubules that leads to the cell cycle arrest at the mitotic phase and as result of all these progressions DNA fragmentation occurs and apoptosis[2]. However, the exact mechanisms and the factors that govern the anticancer function of taxol are not completely understood [3].

Alternative sources of paclitaxel as producing it from fungi has been used to solve the problem of being limited supply as it requires destruction plenty of *Taxus brevifolio* bark[4].

Taxol production from *Aspergillus terreus* which is a taxol producing fungi gives a great chance to produce taxol by a great scale through fermentation process, thus our work aimed to elucidate that taxol extracted from*Aspergillus terreus* have cytotoxic and antitumor activity *in vitro* and *in vivo*.

MATERIAL AND METHODS

Preparation of Fungal Taxol:

Fugal taxol was extracted from *Aspergillus terreus* as it is the highest taxol producing endophytic fungiby the method described before[5].

In Vitro Cytotoxicity Study:

Human breast cancer cell line was used(MCF-7)[6], where known concentrations of fungal taxol were prepared in 0.1% DEMSO firstly, then for the attachment for the cells, it was seeded in 96 well plates for 24 h and later treated with the fungal taxol then after 72 h the media were removed and cell cultures were incubated with MTT reagent for the formation of formazon complex after 4 h at 37°Cand absorbance was read at 540 nm and cytotoxicity percentage was calculated

In Vivo Cytotoxicity Study:

Experimental animals: 30 adult male Swiss albino mice (8 weeks of age) obtained from the National Cancer Institute, Cairo University, their weight ranging from 20-25 g. from the same bred, supplied by diet and water ad libitum and kept for 7 days for adaptation before the begging of the experiment.

Experimental design**:**Solid tumor induction was made by injection the right hind limp subcutaneously by the concentration of (2×10^6)×2.5 mlin both group (2) which is the positive control group and group (3) which is the taxol treated group whilegroup (1) is the negative control group which isinjected by 2.5 ml saline only. Treatment with taxol started at day 7 of transplantation by taxol by the dose 2mg/kg BW[7]i.p. once daily respectively for 30 days, by the end of experiment solid form of the tumor were removed and weighted after weighting of mice from each group.All the explained procedureswere carried out as per the Faculty of Medicine, Zagazig University Animal Ethics and confirmed to follow NIH guidelines Committee guidelines (15-08-263 (IACUC)).

Sampling of Blood:

Blood samples for biochemical analysis were collected into plain tubes and centrifuged for 15 min. at 4000 r.p.m and serum then be collected and stored at -20°C till use.

Tissue Collection and Histopathological Examination:

After the end of the experiment, liver and kidney from each group were directly excised and rinsed in saline where kidneys were preserved in 10% buffered formalin while liver have been cut into 2 parts one of them kept in phosphate buffer saline for biochemical analysis while the other part preserved in 10% buffered formalinfor histopathological examination where sections of both liver and kidney tissues were stained byHematoxylin and eosin staining and examinedby microscope[8]. After weighting of the EAC solid tumor, its volume(mm^3)be calculated by the equation $A \times B^2 \times 0.5$, where A is the longest diameter and B is the shortest diameter[9], then fixed 10% neutral buffered formalin for immunohistochemical investigation.

Immunohistichemical Analysis for KI67 [10]

Slides of solid tumor were incubated with the 1ry antibodies against KI67 over night at 4°C in a humidified chamber later on, it incubated with 2ry antibodies (HRP-conjugated secondary antibodies) at 37°C for 30 min., then it has been visualized with a chromagen dye (DAB chromagen) then stained by Myers hematoxylincounter stain and examined by microscope.

Estimation of Biochemical Parameters:

Malondialdehyde (end product of lipid peroxidation) in liver tissue homogenate was estimated by the method described before[11] using Biodiagnostic kit (Biodiagnostic Company, Giza/Egypt), serum levels of ALT, AST[12]and albumin[13]were estimated using Biodiagnostic kit (Biodiagnostic Company, Giza/Egypt).

STATISTICAL ANALYSIS

All data are expressed as mean ± standard deviation (SD) using one way of variance (ANOVA) by SPSS 14.0 version[14], *P* values < 0.05 were considered as statistically significant.

RESULTS

The extracted Taxol from *A. terreus* was 20.2 µg/g fungal dry weight,it was found that Taxol extracted from *Aspergillus terreus*have a noticeable and remarked cytotoxic effect on MCF-7 cell line with (IC50 value of 38.03 µg/ml) while on normal retina cell line as a control, it was found to be a safe compoundshowing no cytotoxicity as presented in table (1).

Our results in table (2) demonstrated thattumor volume and weight have been reduced to great extent by the influence of fungal taxol,where the tumor volume appears in positive control group by 2.86±0.25 mm^3 with reduction in fungal taxol group by 47.90 % as the tumor volume in fungal taxol group was 1.49±0.11 mm^3, also the tumor weight in positive control group was 1756.35±0.13 mg that was clearly reduced in fungal taxol group 1164.5±0.16mg by 31.69 %, also fugal taxol has reduced the elevated body weight caused by EAC solid tumor by 7.45% as the body weight was 27.37±3.21 and 25.33±2.33in positive control group and fungal taxol group respectively.

Our results in table (3) showing that fungal taxol treatment lowered the ALT and AST activity by 51.6% and 51.1% respectively in f. taxol group in comparison with positive control group, albumin level increased by 33.3% in f.taxol compared to positive control group, also it was observed that MDA level lowered by 44.17% in liver tissue homogenate of f.taxol group in comparison with positive group. It was found that a significant inhibition observed in the expression of KI67 as fungal taxol group showed few scattered immunostained nuclei with moderate intensity when compared by positive control group which revealed more than 10/ HPF immunostained nuclei with marked intensity as presented in fig. (1), indicating that fungal taxol is able to inhibit the process of more proliferation and neovascularization.

Our findings in fig. (2) and fig. (3) revealed thatfungal taxol have an improvement effect onliver and kidney sections as it overwhelms the injurious fluctuations resulted from the tumor progression.

As liver tissue sections showed normal central vein, sinusoids and hepatic parenchyma in negative control group while positive control group showed focal hepatocellular dysplasia with pleomorphic hyperchromatic nuclei and frequent mitosis and the fungal taxol group showed infiltration of hepatic lobule with non- specific inflammatory cells fig. (2).

For kidney tissue sections showed normal renal cortexin negative control group while positive control group showed few scattered aggregates of inflammatory cells while fungal taxol group showed focal degeneration of tubular epitheliumfig. (3).

Table 1:Cytotoxic activity test against human tumor cell line MCF-7 (breast cancer cell line) and normal retina cell line (RPE).

Cell line	MCF-7	RPE1
LC_{50} (µg/ml)	38.03	-

n = 10 mice per group and *p<0.01 vs. positive control group

Table 2: Effect of fungal taxol on body weight, tumor volume & tumor weight.

Groups	Body weight (g)	Tumor weight (mg)	Tumor volume (mm^3)
Positive control	27.37±3.21	1756.35±0.13	2.86±0.25
Fungal taxol	25.33±2.33	1164.5±0.16*	1.49±0.11*
Percent change	7.45%	31.69%	47.90%
Negative control	25.91±3.76	-	-

n = 10 mice per group and *p<0.01 vs. positive control group

Table 3: Effect of fungal taxol on some biochemical parameters.

Groups	ALT (U/ml)	AST (U/ml)	Albumin (mg/dl)	MDA (nmol /g)
Negative control	37.3±4.5	99.5±9.7	3.9±0.08	105.03±1.27
Positive control	105±9.3	264.9±37	2.7±.4	208.684±4.07
Fungal taxol	50.8±3*	128.3±6.4*	3.6±0.1*	116.49±1.74*
% change	51.6%	51.1%	33.3%	44.17%

n = 10 mice per group and *p<0.01 vs. positive control group

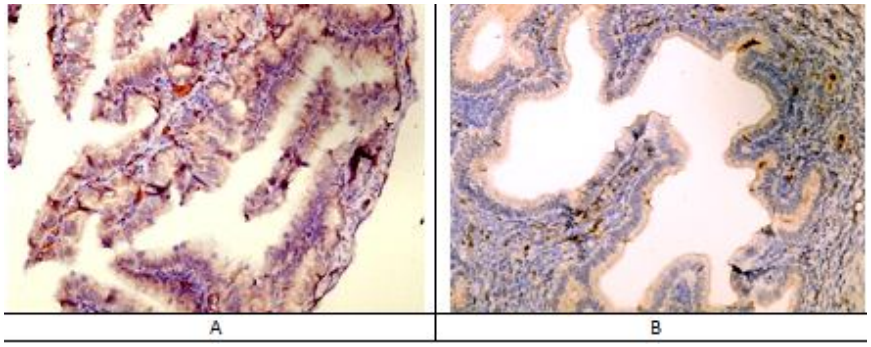

Figure 1: Photomicrograph of mice EAC solid tumor showing (A) positive control and (B) fungal taxol groups respectively (Hematoxylin counter stain x400). Showing that fungal taxol inhibited expression of KI-67 in tumor tissues as compared to positive control animals.

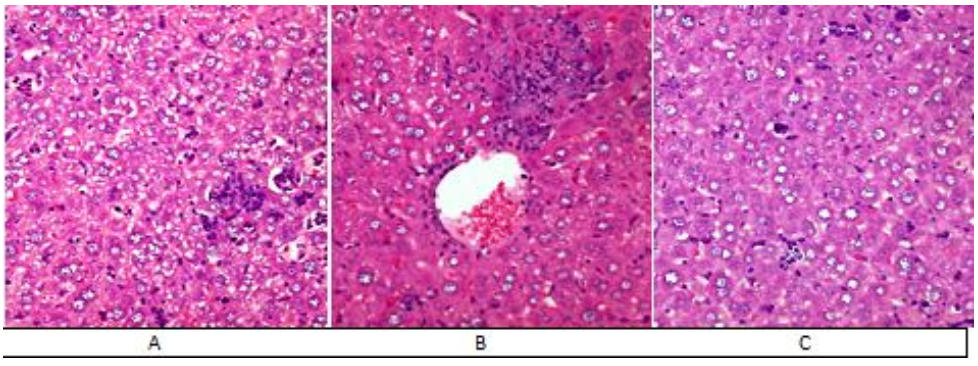

Figure 2: Photomicrograph of mice liver showing (a) negative control, (B) positive control and (C) fungal taxol groups respectively (H&Ex400). Where fungal taxol overwhelms the injurious fluctuations resulted from the tumor progression.

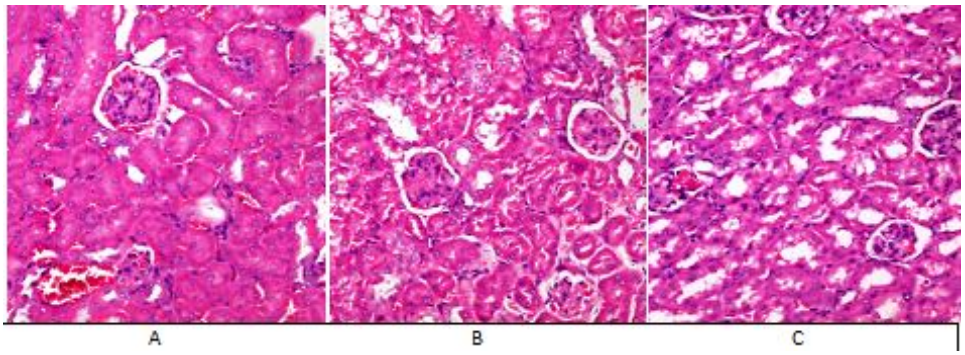

Figure 3: Photomicrograph of mice kidney showing (a) negative control, (B) positive controland (C) fungal taxol groups respectively (H&Ex400). Where fungal taxol causes improvement in the pathologically of kidney as a whole .

DISCUSSION

Paclitaxel extracted from the bark of*Taxus brevifolio* tree was investigated as anticancer agent against EAC solid tumor bearing mice and paclitaxel was known as effective drug against ovarian, breast and lung cancers,which causes cytotoxicity to cancerous cells and induces apoptosis in it[15]in consent with this our findings indicates that fungal taxol showed to a great extent that it have anticancer properties both *in vivo* and *in vitro* as cleared on experimental animal tumor model (Ehrlich solid tumors) and (MCF-7) human breast cancer cell line. Taxol have been reported to havean apoptotic effects on diverse number of cancer cell lines.Our results in showed marked cytotoxic effect onbreast cancer cell line (MCF-7) which consonantKimet al.[16]who stated that viability of breast tumor MCF-7 cells was suppressedby paclitaxeltreatment accomplished by AMPK signaling throughout the regulation of FOXO3a and EF1α, in another study the cytotoxic and anticancer properties of the venom (*A. amoreuxi*) appeared on cancer cells were demonstrated through the induction of apoptosis in MCF-7 cell line which verified its competence to induce apoptosis in cancerous cells [17].

Our results demonstrated that fungal taxol significantly decreased the tumor volume and Weight, in addition to the body weight which supports the anticancer activity of it.

This is at one with Singh et al. [18]revealed that anticancer activity of *Morinda citrifolia* demonstrated by the decrease in theviable cells count, tumor volume and tumor weight along with the increase in the life span of animals. Moreover the cytotoxic potential of 2-quinolone derivatives was observedin a solid tumor model as a result of the immune system activation which is verified by the decrease in the tumor volume and weight that may reflected the cell cycle arrest and the generation of apoptosis or necrosis in cancerous cells[19].

KI-67 is a nuclear protein appears inside the nucleus during cell division in all stages of cell cycle except G0 and considered as a good marker for cell proliferation[20].The data in our experiment clearly indicatedthat fungal taxol inhibited expression of KI-67 in tumor tissues as compared to positive control animals. Immunohistochemical studies for KI67 experiments revealed the presence of it in positive cells, which are indicative of cell proliferation in untreated tumor tissues. However upon Extract of *Vernonia condensata* treatment, a significant decrease in tumor cell population stained with KI67 was observed that proved it Inhibits tumor progression in addition to increasing the life span of tumor-allograft bearing mouse [21].

A clear correlation was observed between free radical generation and the development of cancer as excessive free radical generation in oxidative stress causes damages in macromolecules such as lipids, proteins and carbohydrates[22]. The lipids undergo peroxidation at the primary site and the products of lipid peroxidation generate more free radicals, which migrate along with circulation to the other sites[23]. One of the well known documented markers for carcinogenesis is elevated malondialdehyde (MDA) levels.

Meanwhile liver is the main organ for many metabolic reactions as drug activation, and detoxification, lipid peroxidation determination was done with liver homogenates. In the present study a significant elevation in liver MDA level was observed in EAC bearing mice (positive control group) which supports the role of oxidative stress in cancer progression and treatment with fungal taxol reduced this elevation .Consistent with our study, using *A. australis* venom in the treatment of EAC bearing mice showed a significant decrease in oxidative stress biomarker level achieved in mice liver cells[24].Kumeret al.[19]stated that elevated MDA level was observed in EAC inoculated mice which supports the role of

oxidative stress in cancer progression which is suppressed by the treatment with 2-quinolone derivatives.

To evaluate the hepatoprotective effects of fungal taxol biochemical parameters was reviewed as well as histopathological analysis of experimental animals. It is well known that number of agents can lead to the induction of several liver diseases which can be aggravate into hepatocellular damage evidenced by the elevations in the serum activities of ALT, AST and decrease in Albumin serum level[25].

EAC bearing mice showed cellular degeneration caused by carcinogenesis,EAC cells causes hepatotoxicity that was reversed by the protective effect of fungal taxol to great extent
In case of tumor bearing mice, activities of ALT, ASTwere found to be increase more drastically with decline of albumin level as a result of acute and permanent toxicities persuaded by EAC cells. After treatment with fungl taxol in the EAC bearing mice these values have been meliorated to normal values. From this itfollows thatfungal taxol treatment prevented the damage generated by EAC. In another study EAC inoculation in mice showed great abnormalities in the histology of nearly all the studied organs whichwere reduced by PEBM supplementation that indicates its antiproliferative and hepatoprotective effect in addition to the histopathology of kidney tissues from PEBM treated mice did not show any cellular degeneration [26].

In a study conducted to examine the antioxidant effect of grape seeds against Ehrlich solid tumor induced oxidative stress, hepatic dysfunction and pathological changes in the liver of albino mice were cleared by the increase of (ALT) and (AST), elevation in (MDA) level accompanied by a decline in (GSH), (SOD) and (CAT) and albumin levels in blood and liver. Histopathologically and ultrastructurally, liver of EAC bearing group showed hepatic degeneration with sinusoidal and lymphocytic infiltration, increase of collagen fibers, irregular nuclei, altered mitochondria and increase of secondary lysosomes.Therefore normalization of liver DNA and protein content alonge withreduced MDA level with increase in the antioxidant parameters accompanied with improvement in the pathologically of examined hepatic lesions,revealed the potent antioxidant properties of grape seeds by protecting the liver from the oxidative stress caused by the introduction of EAC [27].

CONCLUSION

Paclitaxel is a widespread used chemotherapy but unfortunately it requires the devastation of enormousnumber of *Taxus brevifolio* bark and the goal is that to explore another way to produce taxol which was done by fermentation of *Aspergillus terreus*that gives taxol by large scale andfungal taxol has proved by many ways that it have agreat anticancer activity and it may undergo the apoptotic pathway and further studies should be carried on to elucidate further its mode of action.

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