Trop J Nat Prod Res, March 2021; 5(3):478-484 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Cytotoxicity in Swiss Albino Mice by Extract of *Aspergillus tubingensis* (CRE4) Endophyte Isolated from *Catharanthus roseus*

Eman A.M. Ali[1]*, Tahany M.A. Abdel-Rahman[1], Azza H.M. Hassan[2], Hedia-Allah Ebrahim[1]

[1]*Botany and Microbiology Department, Faculty of Science, Cairo University, 12613, Giza, Egypt*
[2]*Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*

ARTICLE INFO

Article history:
Received 26 November 2020
Revised 28 February 2021
Accepted 12 March 2021
Published online 01 April 2021

ABSTRACT

Cancer represents a significant portion of deaths around the world. The increasing rate of death requires finding new sources of anticancer drugs. Some endophytic fungi represent an important source of bioactive compounds with anticancer activity. The current research aimed to study the *in vivo* cytotoxicity of extract from the endophytic fungus *Aspergillus tubingensis* strain CRE4 isolated from *Catharanthus roseus*. *A. tubingensis* strain CRE4 was identified using molecular techniques by sequencing of the internal transcribed spacer (ITS) region with accession number MG407714. Ethyl acetate extract was prepared from the fungus and was studied for its *in vivo* cytotoxicity in Swiss albino mice at doses of 50 and 100 mg/kg after 14 and 28 days from injection with Ehrlich ascites carcinoma (EAC). The body weight and the tumour growth of the Swiss albino mice decreased when treated with *A. tubingensis* extract. However, the mean survival time and the life span of the mice bearing tumour were increased as compared to positive control groups. Moreover, *A. tubingensis* extract improved the biochemical and haematological parameters of tumour bearing mice. The histopathological study showed that in the *A. tubingensis* extract treated groups, extensive apoptotic cells were noticed in the tumour. The highest improvement was detected in tumourous mice treated with 100 mg/kg of fungal extract after 28 days from EAC injection as compared to other treated groups. In conclusion, *A. tubingensis* strain CRE4 (MG407714) exhibited effective cytotoxicity on mice bearing Ehrlich Ascites Carcinoma in a dose-dependent manner.

Keyword: *Catharanthus roseus*, Endophytic fungi, *Aspergillus tubingensis*, Cytotoxicity, Albino mice.

Introduction

Catharanthus roseus (L.) G. Don. plant belongs to family Apocynaceae. *C. roseus* is considered the most important plant in the medicinal field, due to its constitution of many different types of alkaloids. It produces more than 120 alkaloids, where 70 of which are pharmacologically active. These alkaloids are used as antidiabetic, antimicrobial, antitumour and antihypertensive agents. Moreover, it is used in treatment of other public diseases all over the world. Extraction of active metabolites is of high cost and low yield.[1, 2, 3] Different optimization methods have been done on *C. roseus* to increase the production of these active compounds. Other investigations have focused on screening of the endophytes isolated from different part of the plant.[4] Extraction of microbial metabolites is less expensive and easier.[2]

Endophytes are defined as microorganisms that live inside plant tissues without causing harm to the hosts.[5] Many pharmaceutical compounds were isolated and identified from endophytic fungi.[6] Endophytic fungal metabolites have been tested as antimicrobial, antioxidant, antidiabetic, anticancer, antiviral agents, immunomodulatory and insecticidal compounds. The searching and screening for endophytic microbial metabolites has been increasing because they have the ability to produce secondary metabolites similar to those of their hosts such as alkaloids, flavonoids, terpenoids, saponins, phenolic acids, benzopyrones and others.

Endophytes produce metabolites with anticancer activities. Kharwar *et al*.[7] reported that about 100 compounds are used as anticancer agents and classified in the nineteen various classes of chemicals acting against different cancer cell lines. These compounds were isolated from about 50 fungal species which are located in different groups of endophytes. Many reports stated that the endophytic fungi are able to produce the same bioactive compounds of their host plants.[8, 9]

Aspergillus tubingensis belongs to the genus *Aspergillus*, section Nigri.[10] Its morphological characters and habitat resembles to *Aspergillus niger*.[11] *A. tubingensis* is used in industrial and biotechnological fields. It produces mycotoxins, amylases and some organic acids like ascorbic and citric acids.[12, 13]

The present research was planned to study the *in vivo* cytotoxicity of ethyl acetate extract of the endophytic fungus *Aspergillus tubingensis* strain CRE4 isolated from *Catharanthus roseus* using cancer-bearing albino mice.

Materials and Methods

Isolation of the fungus from plant tissues

The plant roots were separated and washed with tap water. Surface sterilization was then carried out using ethanol (70%) for 1 min followed by sodium hypochlorite (4%) for 3 min then finally with ethanol (70%) for 1 min. Samples were rinsed twice with sterile distilled water, dried with sterile filter paper and then were cut into small pieces with sterile scalpel.[14] Root pieces were then inoculated on Martin's agar medium containing chloramphenicol to suppress bacterial growth and incubated at 25-27°C. Martin's agar medium

*Corresponding author. E mail: emanali@sci.cu.edu.eg
Tel: +2 01222643685

Citation: Ali EAM, Abdel-Rahman TMA, Hassan AHM, Ebrahim HA. Cytotoxicity in Swiss Albino Mice by Extract of *Aspergillus tubingensis* (CRE4) Endophyte Isolated from *Catharanthus roseus.* Trop J Nat Prod Res. 2021; 5(3):478-484. doi.org/10.26538/tjnpr/v5i3.11

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

composition is as follow (g/l distilled water): peptone (5), dextrose (10), Mg $SO_4.7H_2O$ (0.5), KH_2PO_4 (1) and agar (18). The isolated endophytic fungus was identified morphologically and microscopically according to Moubasher.[15]

Molecular identification
Molecular identification of the endophytic fungus *Aspergillus tubingensis* strain CRE4 isolated from *Catharanthus roseus* was performed. DNA extraction was carried out according to the protocol of Gene JET genomic DNA purification Kit (Thermo K0721). The amplification reaction was carried out using Maxima® Hot Start PCR Master Mix (Thermo K1051) using the universal primers ITS1 and ITS4. PCR was carried out during thermal cycling conditions with initial denaturation at 95ºC for 10 min (1 cycle), followed by 35 cycles of denaturation at 95ºC for 30 sec, annealing at 57ºC for 1 min and extension at 72 ºC for 1 min: 30 sec and ended by final extension cycle at 72ºC for 10 min. PCR product was cleaned up using Gene JET™ PCR Purification Kit (Thermo K070). PCR product sequencing was done at the German company GATC Biotech using ABI 3730xl DNA sequencer by using forward and reverse primers with the new 454 technology. BLAST program available at NCBI GenBank databases (National Center for Biotechnology Information, website: www.ncbi.nlm.gov/blast) was used to align DNA strand forward and reverse sequence. The finalized sequence was blasted against the collection of non-redundant NCBI nucleotide sequence database. Accession number of the identified fungus was obtained.

Preparation of A. tubingensis extract
Aspergillus tubingensis was cultured on Cazpex dox´s broth medium for 21 days at 25°C. The culture filtrate was extracted with ethyl acetate using a separation funnel. The ethyl acetate layers were collected, concentrated, evaporated to dryness, and then stored for subsequent analysis.

In vivo cytotoxic assay of A. tubingensis extract
A total of 48 Swiss male albino mice (8-10 weeks old), weighting 20-25 g, were used in this study. These mice were maintained under standard laboratory conditions (temperature 22 ± 2°C, light/dark cycles of 12/12 h and fed with rodent standard diet) in the animal house of the National Cancer Institute, Cairo University, Egypt. The study was approved by the Institutional Animal Care and Use Committee (CU-IACUC) with approval number CUlS7217.

Transplantation of Ehrlich Ascites Carcinoma
Ehrlich Ascites Carcinoma (EAC) cells were obtained from National Cancer Institute, Cairo University, Egypt. EAC cells were collected from Swiss mice bearing EAC. For the experiment, EAC cells were prepared by diluting 2 mL of tumour cells suspension with 18 mL of saline solution (pH 7.4) under sterile conditions.[16]

Experimental protocol
Swiss mice were divided into 4 groups, each group containing 12 mice. 0.2 mL of Ehrlich suspension cells was injected subcutaneously for solid tumour formation into all mice groups except those in negative control group. Each group was treated with fungal extract after 48h from injection of EAC until 28 days, except positive control group which was treated with saline solution. The first group was the negative control (normal mice), while the second one was the positive control (EAC-mice + 0.2 mL of saline solution/ day for 28 days intraperitoneally). Furthermore, the third group represented treated mice as follows; EAC-mice + 50 mg/kg body weight of fungal extract (injected as 0.2 mL of fungal extract/ day intraperitoneally to reach a total concentration of 50 mg/kg after 28 days), and the fourth one was another treated group as follows; EAC-mice + 100 mg/kg body weight of fungal extract (injected as 0.2 mL of fungal extract/ day intraperitoneally to reach a total concentration of 100 mg/kg after 28 days).[17] At the 14th day from EAC injection, three mice from each group were sacrificed for haematological and histological studies. Sacrificing was done after fasting mice for 18h. At the 28th day from EAC injection, three other mice from each group were also sacrificed for measuring the same parameters. The remaining mice in each group were maintained for measuring the mean survival time and life span.

Tumour growth response
The antitumour activity of the fungal extract was determined by the change in body weight and the change in diameter of the tumour after 10, 20 and 28 days.[18]

Determination of mean survival time
The Mean Survival Time (MST) of standard and groups treated with the fungal extract was determined by noticing the mortality of Swiss albino mice as shown in Eq. (1).[16]

Mean Survival Time (MST) = (Days of 1st death + Days of last death)/2 …… Eq. (1)

Percentage of increase in life span (%ILS)
The effect of *A. tubingensis* extract on percentage of increase in life span (%ILS) of animals was calculated depending on the MST values,[16] as displayed in Eq. (2).

%ILS = {(MST of treated group- MST of control group) / MST of control group} ×100. …… Eq. (2)

Haematological and biochemical parameters
Blood was collected from the major blood vessels of mice using ether as anesthetic. Determination of haemoglobin (Hb) levels, red blood cell counts (RBC),[19] white blood cell counts (WBC),[20] were done. Lishman stained blood smear method was used to determine WBC differential counts.[21] Also, serum alanine transaminase (ALT), serum aspartate transaminase (AST),[22] cholesterol and triglycerides,[23] were measured.

Histological study
Skin biopsies from two mice in each group were taken, and were fixed for 24h in 10% formalin, then washed with tap water and dehydrated in serial dilutions of alcohols. The Specimens were purified in xylene established in paraffin at 56°C by using air of hot oven for 24h. Paraffin Melissa wax tissue blocks were prepared and partitioned at 4 µm thickness using sledge microtome. This tissue sections were composed on glass slides and were deparaffinized then, haematoxylin and eosin stain was used, and the slides were finally investigated under light microscope.[24]

Statistical analysis
The results were expressed as mean ± standard deviations (mean ± SD). Data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package of Social Science (SPSS) program, version 20. The presented mean values were separated using Duncan's Multiple Range test, at $p \leq 0.05$.[25]

Results and Discussion

Molecular identification of Aspergillus tubingensis
Aspergillus tubingensis strain CRE4 isolated from *C. roseus* was identified using molecular techniques with accession number MG407714. Its relation with other fungal species is displayed in Figure (1) by phylogenetic tree.

In vivo cytotoxicity assay
Tumour Growth Response, Mean Survival Time and % Increase in Life Span.
The data in Table (1) showed that the 50 and 100 mg/kg doses of fungal extract reduced the body weight of treated mice bearing tumour as compared to the untreated positive control group. The mean survival time (MST) of mice bearing tumour increased in treated groups as compared to the positive control group. Increases in the life span of tumour bearing mice treated with fungal extracts were observed as compared to the positive control group. This indicated that the fungal extract of *A. tubingensis* possesses an antitumour activity which increased the life span and survival time of mice bearing EAC in a dose dependent manner. Mice groups treated with fungal extracts showed a decrease in the tumour growth as compared to the positive control along the period of experiment with maximum decrease after 28days from EAC injection. Ehrlich ascites carcinoma (EAC) is one of the most common tumours used in

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

experimental studies. EAC is an undifferentiated carcinoma, highly transplantable, originally hyperdiploid, rapidly proliferated, 100% malignant, without regression, has shorter life span and does not have tumour-specific transplantation antigen (TSTA). EAC looks like human tumours which are very sensitive to chemotherapy because they have a rapid growth rate and are undifferentiated.[26] Abdel-Azeem et al.[27] studied the anticancer activity of extracts of endophytic fungi isolated from medicinal plants in Egypt. They recorded a significant decrease in tumour volumes of tumour-bearing mice after treatment with fungal extracts. Zein and Sabry,[17] studied the anticancer activity of taxol produced by the endophytic fungus *Aspergillus terreus* against male Swiss albino mice bearing EAC. The tumour volume and the mice body weight were reduced in comparison with positive control group. Hereher et al.[28] studied the antitumour activity of mushroom polysaccharides using mice bearing EAC, where significant reduction in tumour volume was observed in treated mice as compared with positive control.

Effect of fungal extract on biochemical parameters of mice bearing tumour

The results in table (2) show that at both the 14th and the 28th days, positive control groups exhibited an increase in the serum level of ALT, AST, cholesterol and triglyceride as compared to negative control group. However, mice groups treated with 50 and 100 mg/kg of fungal extract showed a significant decrease in the serum level of ALT, AST, cholesterol and triglyceride as compared to positive control group. This indicted the protective effect of the fungal extract against the tumour caused by EAC development. In the same vein, an increase in serum AST and ALT activities was detected in positive control Swiss albino mice which then decreased after treatment with fungal taxol extracted from the endophyte *Aspergillus terreus*.[17] Similarly, Wani et al.[29] reported that ethyl acetate extract of *Chaetomium cupreum* at a dose of 200 mg/kg decreased AST, ALT, cholesterol and triglycerides in EAC-bearing Swiss albino mice. Also, a highly significant decrease in AST and ALT was noticed in mice bearing EAC treated with mushroom polysaccharides compared with positive control.[28]

Haematological parameters

Table (3) shows the effect of *A. tubingensis* extract on haematological parameters after 14 days from injection with EAC. The results showed that Hb content, RBC count and platelets (PLT) in the EAC positive control group significantly decreased as compared to negative control group. Treatments with fungal extract at doses of 50 and 100 mg/kg significantly increased the three parameters compared to positive control group ($p \leq 0.05$). The total WBC significantly increased in positive control as compared to normal group, while the groups treated with fungal extracts reduced them, with more significant reduction at the dose of 100 mg/kg, as compared to positive group. Differential WBC count in positive group showed an increase in neutrophils, lymphocytes and basophiles, whereas they significantly decreased in the two groups treated with fungal extract as compared to positive control group. The results in table (4) show the haematological parameters after 28 days from EAC injection. It was found that Hb content, RBC and PLT in the EAC positive control group showed more reduction after 28 days compared to those after 14 days from EAC injection. WBC and neutrophils showed an increase in count after 28 days as compared to those after 14 days from EAC injection. In general, the groups treated with different concentrations of fungal extract showed a significant improvement of haematological parameters as compared with positive control. The present results match the findings of Wani et al.[29], as they stated that 200 mg/kg of *Chaetomium cupreum* extract increased haemoglobin content and red blood cell count, while decreased white blood cells count in EAC-bearing mice. Additionally, Choudhury et al.[30] found that the total counts of RBC, WBC and differential counts of WBC changed by EAC inoculation in Swiss albino mice, and were repaired when treated with MT81 in a dose-dependent manner.

Histopathological study

In the present study, histopathological alterations of tumour tissues were demonstrated at the 14th and the 28th days from EAC injection. Tissues of normal -ve control mice are displayed in Figure (2). It shows normal muscle bundles surrounded by normal adipose tissue.

Figure (3 A&B) shows tissues of Ehrlich tumour positive control groups at the 14th and the 28th days, respectively. At the 14th day, frequent mitosis was observed (arrows) where the neoplastic cells appeared large with large vesicular hyperchromatic and karyomegalic nuclei. At the 28th day, mitotic activity of neoplastic cells was detected. Tissues of mice treated with 50 mg/kg of fungal extract showed proliferation of fibroblasts which invade the tumour stroma, associated with abundant apoptosis and/or necrosis after 14 days from EAC injection. On the other hand, marked improvement was demonstrated in this group after 28 days with extensive apoptosis of neoplastic cells associated with few and sparse viable cells (Figure 4 A&B). Remarkable improvement was observed in mice treated with 100 mg/kg of fungal extract after 14 days from EAC injection compared to those of +ve control and 50 mg/kg treated ones, as the central necrosis and/or apoptosis were large and extensive (Figure 5A).

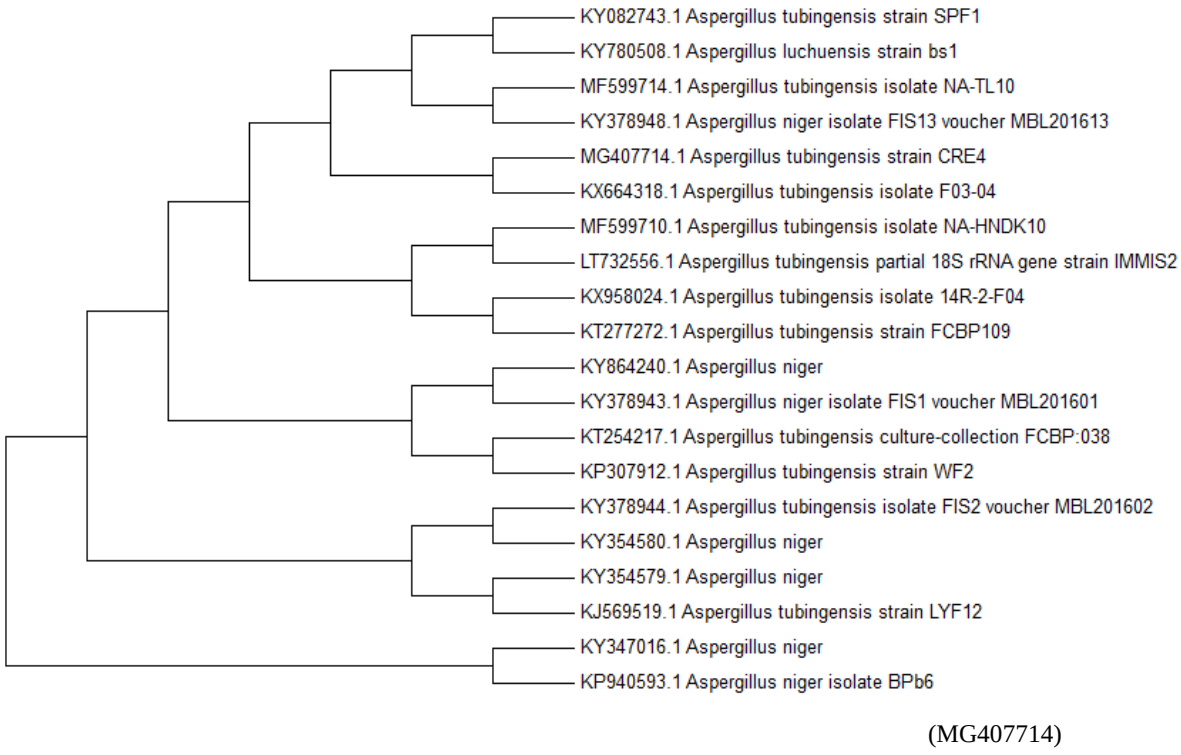

Figure 1: Phylogenetic analysis of *A. tubingensis* strain CRE4

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The best remarkable improvement was demonstrated in the tumourous mice treated with 100 mg/kg of fungal extract at the 28th day compared to other treated groups (Figure 5B). Apoptosis of neoplastic cells was massive and diffuse with no viable cells demonstrated in this group. In general, histopathological alterations demonstrated at the 28th day showed significant and remarkable improvement in mice tissues as compared to those demonstrated at the 14th day. The improvement was recorded in a dose-dependent manner.Ehrlich solid tumours are structures like organs in their heterogeneity and their complication. Into these tumours, there are differences in oxygen pressure, nutrient flux and pH which may participate in the resistance of tumour to chemotherapy due to unequal drugs distribution into the matrix of the tumour.[31, 32]In tumour histopathology tissue, positive control group of solid tumour showed sheets of tumour cells of various shapes and proliferation. 250 mg of powdered freeze dried of *Morinda citrifolia L.* exhibited little proliferation with some of apoptosis and necrosis, while 500 mg of dried powdered freeze of *Morinda citrifolia L.* showed more necrosis and apoptosis.[33] Zein and Sabry[17] stated that histopathologically, taxol produced from the endophyte *Aspergillus terreus* enhanced the pathological features caused by the oxidative stress induced by EAC solid tumour. Choudhury et al.[30] stated that the structural analogue of the toxin MT81displayed prominent antineoplastic activities against EAC cells in Swiss albino mice. Cell apoptosis was higher in EAC-bearing mice treated with *Chaetomium cupreum* extract at a dose of 200mg/kg as compared with doses of 100mg/kg and 50mg/kg.[29]

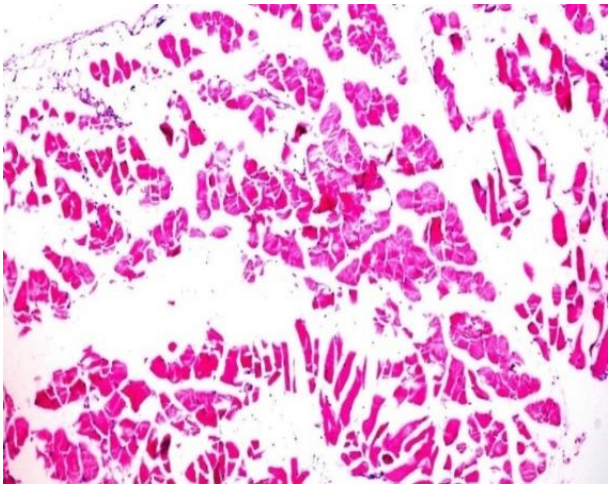

Figure 2: T.S. of muscle bundles surrounded by normal adipose tissue of normal negative control mice (H&E, 100X).

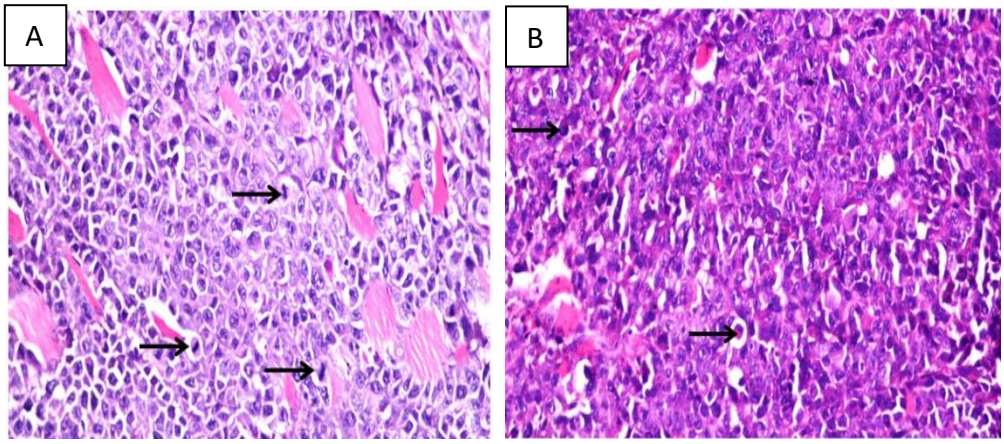

Figure 3: T.S. of Ehrlich tumour bearing mice showing Ehrlich Ascites Carcinoma (EAC) cells (A) at the 14th day from EAC injection showing frequent mitotic figures (arrows); note: The neoplastic cells appeared large with large vesicular hyperchromatic and karyomegalic nuclei; (B) at the 28th day from EAC injection showing mitotic activity of neoplastic cells (H&E, 400X).

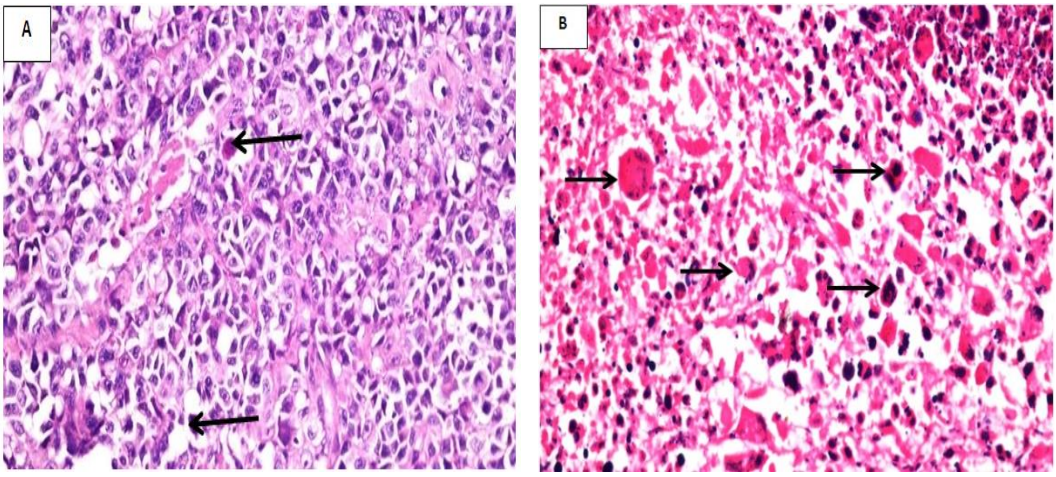

Figure 4: T.S. of Ehrlich tumour in mice group treated with 50 mg/kg of *Aspergillus tubingensis* extract (A) at the 14th day from EAC injection showing apoptotic bodies (arrows); (B) at the 28th day from EAC injection showing extensive apoptosis (arrows) (H&E, 400X).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

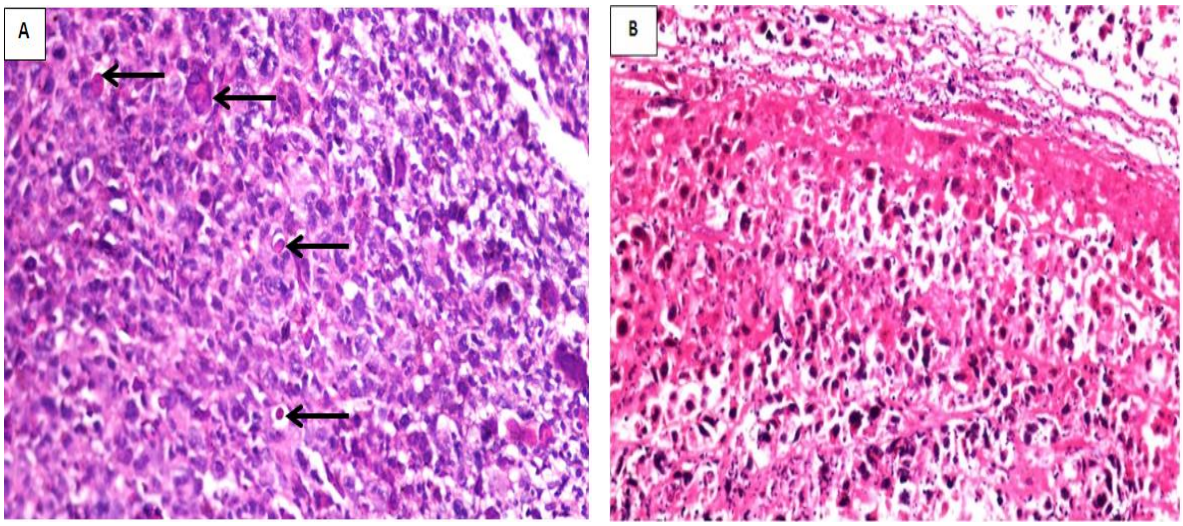

Figure 5: T.S. of Ehrlich tumour in mice group treated with 100mg/kg of *Aspergillus tubingensis* extract (A) at the 14th day from EAC injection showing abundant apoptosis (arrows); (B) at the 28th day from EAC injection showing massive apoptosis (H&E, 400X).

Table 1: Effect of *A. tubingensis* strain CRE4 extract on body weight, mean survival time, life span and tumour growth in mice

Group	Body weight (g)	Mean survival time (days)	Life span (%)	Tumour growth (mm^3)		
				After 10 days	After 20 days	After 28 days
Normal mice (-ve control)	$20.7^a \pm 0.25$	30^b	---	$0^a \pm 0.0$	$0^a \pm 0.0$	$0^a \pm 0.0$
EAC-mice (+ve control)	$26.2^c \pm 0.25$	21.5^a	---	$40^c \pm 0.24$	$41^c \pm 0.3$	$42^c \pm 0.3$
EAC-mice + 50 mg/kg fungal extract	$24.3^b \pm 0.37$	31.5^b	46.5^a	$27^b \pm 0.19$	$16^b \pm 0.16$	$12^b \pm 0.09$
EAC-mice + 100 mg/kg fungal extract	$23.4^b \pm 0.2$	33^c	53.4^b	$26^b \pm 0.17$	$15^b \pm 0.1$	$11^b \pm 0.09$

EAC: Ehrlich Ascites Carcinoma. Different letters in the same column indicate significant difference at $p \leq 0.05$ according to Duncan's multiple range test, while the same letters indicate insignificance.

Table 2: Effect of *A. tubingensis* strain CRE4 extract on biochemical parameters after 14 and 28 days from EAC injection

Group	14th day from EAC injection				28th day from EAC injection			
	ALT (U/L)	AST (U/L)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	ALT (U/L)	AST (U/L)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Normal mice (-ve control)	$26^a \pm 0.5$	$104^a \pm 0.5$	$68^a \pm 0.5$	$104^b \pm 0$	$26^a \pm 0.5$	$104^a \pm 0.5$	$68^a \pm 0.5$	$104^c \pm 0$
EAC-mice (+ve control)	$34^c \pm 1$	$138^d \pm 0$	$97^d \pm 0.5$	$140^c \pm 1$	$38^d \pm 0.5$	$186^d \pm 1$	$99^d \pm 0.5$	$142^d \pm 1.5$
EAC-mice + 50 mg/kg fungal extract	$25^a \pm 0.5$	$134^b \pm 0.5$	$87^b \pm 0.5$	$88^a \pm 0.5$	$28^b \pm 0.5$	$154^c \pm 1.5$	$84^c \pm 1$	$82^a \pm 0.5$
EAC-mice + 100 mg/kg fungal extract	$29^b \pm 0.5$	$136^c \pm 0.5$	$93^c \pm 0.5$	$105^b \pm 0.5$	$32^c \pm 0.57$	$133^b \pm 0.57$	$76^b \pm 1.1$	$87^b \pm 0.57$

ALT: Alanine transaminase, AST: Aspartate transaminase. Different letters in the same column indicate significant difference at $p \leq 0.05$ according to Duncan's multiple range test, while the same letters indicate insignificance.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Effect of *A. tubingensis* strain CRE4 extract on haematological parameters at the 14th day from EAC injection

Group	Hb (g/dl)	RBC (106/cmm)	PLT (103/cmm)	WBC (103/cmm)	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophiles (%)	Basophiles (%)
Normal mice (-ve control)	$14^{c} \pm 0.15$	$7.5^{c} \pm 0.15$	$673^{b} \pm 0.5$	$3.25^{a} \pm 1$	$51^{a} \pm 0.5$	$43^{b} \pm 0.5$	$5^{c} \pm 0$	$1^{a} \pm 0$	$0^{a} \pm 0$
EAC-mice (+ve control)	$8.8^{a} \pm 1.5$	$5.4^{a} \pm 0.15$	$650^{a} \pm 1$	$5.25^{c} \pm 1.5$	$63^{c} \pm 1$	$49^{c} \pm 0.5$	$2^{a} \pm 0.5$	$1^{a} \pm 0$	$2^{b} \pm 0$
EAC-mice + 50 mg/kg fungal extract	$11.9^{b} \pm 0.45$	$6.6^{b} \pm 0.01$	$653^{a} \pm 1$	$4.25^{b} \pm 0.57$	$58^{b} \pm 1$	$37^{a} \pm 1$	$4^{b} \pm 0.5$	$1^{a} \pm 0$	$0^{a} \pm 0$
EAC-mice + 100 mg/kg fungal extract	$11^{b} \pm 0.25$	$6.1^{b} \pm 0.01$	$685^{c} \pm 0.57$	$3.45^{a} \pm 0.5$	$60^{b} \pm 0.5$	$38^{a} \pm 0.5$	$2^{a} \pm 0.5$	$1^{a} \pm 0$	$0^{a} \pm 0$

Hb: haemoglobin; RBC: red blood cells; PLT: platelets count; WBC: white blood cells. Different letters in the same column indicate significant difference at $p \leq 0.05$ according to Duncan's multiple range test, while the same letters indicate insignificance.

Table 4: Effect of *A. tubingensis* strain CRE4 extract on haematological parameters at the 28th day from EAC injection

Group	Hb (g/dl)	RBC (10^6/cmm)	PLT (10^3/cmm)	WBC (10^3/cmm)	Neutrophile (%)	Lymphocyte (%)	Monocyte (%)	Eosinophiles (%)	Basophiles (%)
Normal mice (-ve control)	$14^{c} \pm 0.1$	$7.5^{c} \pm 0.15$	$673^{c} \pm 0.5$	$3.25^{a} \pm 1$	$51^{c} \pm 0.5$	$43^{b} \pm 0.5$	$5^{d} \pm 0.5$	$1^{a} \pm 0$	$0^{a} \pm 0$
EAC-mice (+ve control)	$8.2^{a} \pm 0.1$	$3.3^{a} \pm 0.00$	$544^{a} \pm 0.57$	$6.25^{d} \pm 0.5$	$66^{d} \pm 0.5$	$49^{d} \pm 0.5$	$2^{a} \pm 0.5$	$1^{a} \pm 0$	$2^{c} \pm 0$
EAC-mice + 50 mg/kg fungal extract	$10.7^{b} \pm 0.0$	$3.9^{b} \pm 0.01$	$551^{b} \pm 1$	$4.45^{c} \pm 1.1$	$47^{a} \pm 1$	$35^{a} \pm 0.5$	$4^{c} \pm 0.5$	$1^{a} \pm 0$	$0^{a} \pm 0$
EAC-mice + 100 mg/kg fungal extract	$10^{b} \pm 1.3$	$3.9^{b} \pm 0.01$	$564^{c} \pm 1$	$4.25^{b} \pm 1.1$	$49^{b} \pm 0.5$	$47^{c} \pm 0.5$	$3^{b} \pm 0.5$	$1^{a} \pm 0$	$1^{b} \pm 0$

Hb: haemoglobin; RBC: red blood cells; PLT: platelets count; WBC: white blood cells. Different letters in the same column indicate significant difference at $p \leq 0.05$ according to Duncan's multiple range test, while the same letters indicate insignificance.

Conclusion

In conclusion, *A. tubingensis* strain CRE4 (MG407714) displayed potential cytotoxic effect in a dose-dependent manner on mice bearing Ehrlich Ascites Carcinoma.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Ayob FW and Simarani K. Endophytic filamentous fungi from a *Catharanthus roseus*: Identification and its hydrolytic enzymes. Saudi Pharm J. 2016; 24:273-278.
2. Yin H and Sun H. Vincamine-producing endophytic fungus isolated from *Vinca minor*. Phytomed. 2011; 18:802-805.
3. Barrales-Cureño HJ, Reyes CR, García IV, Valdez LGL, De Jesús AG, Ruíz JAC, Herrera LMS, Caballero MCC, Magallón JAS, Perez JE, Montoya JM. Alkaloids - Their Importance in Nature and Human Life. In: Kurek J (Eds.). Alkaloids of pharmacological importance in *Catharanthus roseus*. IntechOpen. 2019; 9-26p.
4. Akpotu MO, Eze PM, Abba CC, Umeokoli BO, Nwachukwu CU, Okoye FBC, Esimone CO. Antimicrobial activities of secondary metabolites of endophytic fungi isolated from *Catharanthus roseus*. J Health Sci. 2017; 7(1):15-22.
5. Kandel SL, Joubert PM, Doty LS. Bacterial endophyte colonization and distribution within plants. Microorg. 2017; 5:77.
6. Manganyi MC and Ateba CN. Untapped Potentials of Endophytic Fungi: A Review of Novel Bioactive Compounds with Biological Applications. Microorg. 2020; 8:1934.
7. Kharwar RN, Mishra A, Gond SK, Stierle A, Stierle D. Anticancer compounds derived from fungal endophytes: their importance and future challenges. Nat Prod Rep. 2011; 7:1208-28.
8. Venieraki A, Dimou M, Katinakis P. Endophytic fungi residing in medicinal plants have the ability to produce the same or similar pharmacologically active secondary metabolites as their hosts. Hell Plant Prot J. 2017; 10:51-66.
9. Kuriakose GC, Palem PPC, Jayabaskaran C. Fungal vincristine from *Eutypella* spp-CrP14 isolated from *Catharanthus roseus* induces apoptosis in human squamous carcinoma cell line -A431. BMC Compl Altern Med. 2016; 16:302.
10. Samson RA, Visagie CM, Houbraken J, Hong S-B, Hubka V, Klaassen CHW, Perrone G, Seifert KA, Susca A, Tanney JB, Varga J, Kocsubé S, Szigeti G, Yaguchi T, Frisvad JC. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Stud Mycol. 2014; 78:141-173.
11. Oisewacz H. Industrial Applications. New York: Springer-Verlag Berlin Heidelberg; 2002; 264-265p.
12. Olarte RA, Horn BW, Singh R, Carbone I. Sexual recombination in *Aspergillus tubingensis*. Mycologia. 2015; 107(2):307-312.
13. Watanabe T, Tanaka M, Masaki K, Fujii T, Lefuji H. Decolorization and semi-batch continuous treatment of molasses distillery waste water by *Aspergillus tubingensis* DCT6. Water Sci Technol. 2009; 59(11):2179-85.
14. Nath A and Joshi SR. Ultrastructural effect on mastitis pathogens by extract of endophytic fungi associated with ethnoveterinary plant, *Hibiscus sabdariffa* L. J Microsc Ultrastruct 2015; 3(1):38-43.
15. Moubasher AH. Soil fungi in Qatar and other Arab countries. Qatar: University of Qatar ,The Center for Scientific and Research; 1993.
16. Sreelatha S, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. Food Chem Toxicol 2011; 49(6):1270-1275.
17. Zein N and Sabry A. *Aspergillus terreus* taxol influence on solid ehrlich tumour model. Int J Res Pharm Sci. 2017; 7(3):31-36.
18. Gupta M, Mazumder UK, Rath N, Mukhopadhyay DK. Antitumor activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma. J Ethnopharmacol. 2000; 72:151-156.
19. Armour FE, Blood FR, Belden DA. The manual for laboratory work in mammalian physiology. Chicago: The University of Chicago Press; 1965.
20. Wintrobe MM, Lee GR, Boggs DR, Bithel TC, Athens JW, Foerester J. Clinical hematology.(5th ed.). Philadelphia: Les and Febiger; 1961; 326 p.
21. Dacie JV and Lewis SM. Practical hematology. (2nd ed.). London: Churchill Ltd; 1958; 38-48p.
22. Reitman S and Frankel AS. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Pathol. 1957; 28:53-56.
23. Ruskin RS and Aruna SR. *In-vitro* and in-vivo antitumor activity of *Catharanthus roseus*. Int Res J Pharm Appl Sci. 2014; 4(6):1-4.
24. Banchroft JD, Stevens A, Turner DR. Theory and Practice of Histological Techniques. (4thed.). New York, London, San Francisco, Tokyo: Churchil Livingstone; 1996.
25. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955; 11:1-42.
26. Ozaslan M, Karagoz ID, Kilic IH, GuldurME. Ehrlich ascites carcinoma. Afr J Biotechnol. 2011; 10(13):2375-2378.
27. Abdel-Azeem AM, Zaki SM, Salem FM, Khalil WF, Saleh SY. Environmental technology and engineering techniques: basic concepts and health. In: Khalaf MN, Smirnov MO, Kannan P, Haghi AK (Eds.). Potency of endophytic fungi isolated from medicinal plants in Egypt as a resource for anticancer compounds. Apple Academic Press; 2020; 113-131p.
28. Hereher F, ElFallal A, Tosonb E, Abou-Dobaraa M, Abdelaziz M. Pilot study: Tumor suppressive effect of crude polysaccharide substances extracted from some selected mushroom. Beni-Suef Univ J Basic Appl Sci. 2018; 7:767-775.
29. Wani NA, Khanday WI, Ali SA, Tirumalea S. Biological evaluation of ethyl acetate extract of *Chaetomium cupreum* against Ehrlich ascites carcinoma cells in Swiss albino mice. Egypt Pharm J. 2020; 19(3):274-282.
30. Choudhury SM, Gupta M, Majumder UK. Antineoplastic activities of MT81 and its structural analogue in ehrlich ascites carcinoma-bearing Swiss albino mice. Oxid Med Cell Longevity. 2010; 3(1):61-70.
31. Trédan O, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst. 2007; 99(19):1441-1454.
32. Smith JA, Ngo H, Martin MC, Wolf JK. An evaluation of cytotoxicity of the taxane and platinum agents combination treatment in a panel of human ovarian carcinoma cell lines. Gynecol Oncol. 2005; 98(1):141-145.
33. Singh H, Banerjee S, Karan S, Chatterjee TK. Evaluation of anticancer activity of Andaman freeze dried powdered *Morinda citrifolia* L. fruit against Ehrlich Ascites Carcinoma (EAC) cell induced liquid and solid tumor in Swiss Albino mice. JPR:BioMedRx: An International J. 2013; 1(6):567-573.