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de Oliveira Filho, J. W. G., Andrade, T. D. J. A. D. S., de Lima, R. M. T., Silva, D. H. S., Dos Reis, A. C., Santos, J. V. D. O., de Meneses, A-A. P. M., de Carvalho, R. M., da Mata, A. M. O., de Alencar, M. V. O. B., Dias, A. C. S., da Silva, F. C. C., Islam, M. T., Clark, C. C. T., Sousa, J. M. D. C. E. & Melo-Cavalcante, A. A. D. C.

Original citation & hyperlink:
de Oliveira Filho, JWG, Andrade, TDJADS, de Lima, RMT, Silva, DHS, Dos Reis, AC, Santos, JVDO, de Meneses, A-APM, de Carvalho, RM, da Mata, AMO, de Alencar, MVOB, Dias, ACS, da Silva, FCC, Islam, MT, Clark, CCT, Sousa, JMDCE & Melo-Cavalcante, AADC 2020, 'Cytogenotoxic evaluation of the acetonitrile extract, citrinin and dicitrinin-A from Penicillium citrinum', Drug and Chemical Toxicology.
https://dx.doi.org/10.1080/01480545.2020.1769642

DOI 10.1080/01480545.2020.1769642
ISSN 0148-0545
ESSN 1525-6014

Publisher: Taylor and Francis

This is an Accepted Manuscript of an article published by Taylor & Francis in Drug and Chemical Toxicology on 25/5/2021, available online: http://www.tandfonline.com/10.1080/01480545.2020.1769642

Cytogenotoxic evaluation of the acetonitrile extract, citrinin and dicitrinin-A from Penicillium citrinum

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ABSTRACT

Endophytic fungi are promising sources of bioactive substances; however, their secondary metabolites are toxic to plants, animals, and humans. This study aimed toevaluate the toxic, cytotoxic, mutagenic and oxidant/antioxidant activities of acetonitrile extract (AEPc), citrinin (CIT) and dicitrinin-A (DIC-A) of Penicillium citrinum. For this, the test substances at 0.5; 1.0; 1.5 and 2 lg/mLwere exposed for 24 and 48 h in Artemia salina, and 48 h in Allium cepa test systems. The oxidant/antioxidant test was evaluated in pre-, co- and post-treatment with the stressor hydrogen peroxide (H_2O_2) in Saccharomyces cerevisiae. The results suggest that the AEPc, CIT and DIC-A at 0.5; 1.0; 1.5 and 2 lg/mL showed toxicity in A. saline, with LC_{50} (24 h) of 2.03 lg/mL, 1.71 lg/mL and 2.29 lg/mL, and LC_{50} (48 h) of 0.51 lg/mL, 0.54 lg/mL and 0.54 lg/mL, respectively.In A. cepa, the test substances also exerted cytotoxic and mutagenic effects. The AEPc, CIT and DIC-A at lower concentrations modulated the damage induced by H_2O_2 in the proficient and mutant strains of S. cerevisiae for cytoplasmic and mitochondrial super-oxide dismutase. Moreover, the AEPc at 2 lg/mL and CIT at the two highest concentrations did not affect the H_2O_2-induced DNA damage in the test strains. In conclusion, AEPc, CIT and DIC-A of P. citri-num may exert their toxic, cytotoxic and mutagenic effects in the test systems possibly through oxida-tive stress induction pathway.

KEYWORDS

Endophytic fungi; Penicillium citrinum; mycotoxins; cytotoxicity; genotoxicity; mutagenicity

1. Introduction

Secondary metabolites, also known as natural products, are an abundant source of compound with promising biological activities (Blunt et al. 2008, 2009). Fungi, derived from marine algae represent a potential source for obtaining secondary metabolites (Wali et al. 2019) that can be widely used in human medicines (Gerwick and Fenner 2013). Several studies suggest that endophytic fungi have a variety of biotechno-logical potential, such as enzyme production, bioremediation, biodegradation, biotransformation, biocontrol, and treatment of various diseases, including cancer (Arora and Chandra 2011, Yao et al. 2011, Wen et al. 2014, Blunt et al. 2015, Zheng et al. 2016).

Among the various secondary metabolites derived from fungi, citrinin (CIT), a polyketide, was first isolated by Hetherington and Raistrick from Penicillium citrinum (Thacker et al. 1997). Species belonging to the genus Penicillium have several metabolic routes, commanded by specific genes, allowing the biosynthesis of potent secondary metabolites, being widely used as antibiotics and antifungals, in addition to other metabolites applied as immunosuppressants and antitumor agents (Visagie et al. 2014). The species P. citrinum corresponds to a filamentous fungus with extensive territorial expansion, and well known for its potential to produce sec-ondary metabolites (Bennett and Klich 2003). Studies show that the species P. citrinum produces an eccentric diversity of secondary metabolites, including the natural metabolite CIT

(El-Neketi et al. 2013, Hu et al. 2017). Since CIT possesses good antibiotic properties, studies have endeavored to reduce its toxicity (Xu et al. 2006), rather than evaluating its antitumor effect (Du et al. 2010, Wang et al. 2013). However, CIT, as well as its co-product, a polyketide known as dicitri-nin-A (DIC-A) has been explored for their cytotoxicity (Yao et al. 2011), anti-microbial effect, genotoxic and mutagenic properties (Kumar et al. 2014). CIT has been identified as an important antitumor agent, due to its antagonistic properties, such as antioxidant and cytotoxic properties (Lesgards et al. 2014). Moreover, CIT has been reported as a neuroprotectant, as it can prevent glutamate excitotoxicity and neuronal death (Nakajima et al. 2016). Fungi belonging to the genus Penicillum have several metabolic routes, managed by spe-cific genes, which allow the biosynthesis of potent biomole-cules used as antibiotics (Penicillin and some of its derivatives), immunosuppressants (Cyclosporin), cholesterol inhibitors (Levostatin) and antitumor (Paclitaxel) (Keller 2019, Williams and Andersen 2020).

In order to understand the effects of natural substances obtained from endophytic fungi, regarding nuclear DNA damage, it is necessary to apply several non-clinical tests in eukaryotic systems, involving in vitro and in vivo studies, such as Artemia salina and Allium cepa for the evaluation of tox-icity, cytotoxicity and mutagenicity. A. salina is a minicrusta-cean that providesan important information on toxicity (Gajardo and Beardmore 2012), it is easy to culture, has low costs and is readily commercially available (Nunes et al. 2006, Shaala et al. 2015). On the other hand, A. cepa test is able to evaluate different DNA damages such as toxic, cytotoxic, gen-otoxic and mutagenic effects, which are analyzed by the number of mitotic cells (mitotic index); formation of micronu-clei and chromosomal changes (Leme & Marin-Morales 2009). Moreover, such tests can evaluate DNA repair activities (Santos et al. 2015), and more importantly, shows similar results in other test systems constituted with prokaryotic and eukaryotic test models (Fiskesjo€ 1985).

The cellular response to oxidative damage to fungal DNA is similar to the response of mammalian cells, and 30% of genes related to human diseases have functional homolo-gous genes in yeasts (De La Torre-Ruiz et al. 2015). The tests with Saccharomyces cerevisiae are important for measuring the mechanisms of changes in replication fidelity, sensitivity to DNA damage and the cellular responses (Skoneczna et al. 2015), including oxidative stress and antioxidant capacity (De Oliveira et al. 2014). Therefore, the aim of the present study was to isolate and characterize compounds (CIT and DIC-A), from acetonitrile extract (AEPc) of the P. citrinum of endo-phytic of the marine macroalgae Dichotomaria marginata and to evaluate their toxic/cytotoxic, mutagenic and oxidant/anti-oxidant effects in A. salina, A. cepa and S. cerevisiae test systems.

2. Materials and methods

2.1. Experimental design and isolation of substances

Analytical grade solvents such as methanol (CH_3OH), ethyl, n-hexane and acetonitrile (CH_3CN) were purchased from the

Synth, Dynamics, Merck. These solvents were used for extraction and fractionation. Sephadex LH-20 Sigma-Aldrich stationary phase was used for the open-column chromatographic separation and solid media were used for the growth and isolation of microorganisms: Parboiled rice (Marcon^{V_R}) and PDA (Potato Dextrose Agar-Sigma^{V_R}). ^{1}H NMR (500 MHz), ^{13}C NMR (126 MHz), HMBC, HMQC, were obtained on a Bruker Avance DRX-500 spectrometer with the solvents $CDCl_3$, DMSO-d_6 (CIL and Isotec-INC), and the not-deuterated residual solvent as internal reference. The electrospray ioniza-tion mass spectrometry was obtained in the positive mode using Orbitrap XL Hybrid with Fourier Transform (Thermo Scientific Instruments) coupled to a Thermo Instruments CLAE system (Accela PDA Detector, Accela automatic sampler and Accela pump, Thermo Instruments Scientific). The follow-ing conditions were used: capillary voltage of 4.5 kV; capillary temperature of 260 C; auxiliary gas flow rate of 10–20 arbi-trary units; carrier gas flow rate of 40–50 arbitrary units; spray voltage of 4.5 kV; mass range of 100–1000 u.m.a (maximum resolution 30 000).

2.2. Collection of Dichotomaria marginata

The red macroalgae Dichotomaria marginata was collected in December 2009 in the northern region of S~ao Paulo state, on the beach of Fortaleza, in the city of Ubatuba, Brazil (23 $24^{0}93^{0}$ and 45 $03^{0}41^{00}$W) during low tide. Dr. Nair Yokoga (Institute of Botany, S~ao Paulo, Brazil) identified D. marginata, and a voucher specimenwas deposited in the Herbarium of the Botany Institute of S~ao Paulo, Brazil (Voucher no. SP 400960).

2.3. Isolation and identification of endophytic fungus

The endophyte fungus P. citrinum was isolated as previously described (Gubiani et al. 2014), from the inner tissue of the marine red macroalga D. marginata. After isolation, pure P. citrinum culture was identified by Dr. Anil Sazak (Ondokuz Mays Universities Fen Edebiyaties Fakulties Biyoloji Bolumu Kurupelit/Samsun Turkey).

2.4. Acetronitrile extract of P. citrinum (AEPc) and isolation of CIT and DIC-A

The endophytic fungus P. citrinum was grown in five Erlenmeyer flasks, each containing 90 g of rice. The medium was autoclaved four times (on four consecutive days) at
121 C for 40 min. After sterilization, the medium was inoculated and incubated at 25 C for 21 days. At the end of the incubation period, the cultures were combined, ground and extracted with CH_3OH (5 250 mL). The solvent was evaporated, producing a raw extract of CH_3OH, which was dis-solved in CH_3CN and hexane partitioned to obtain the AEPc.

A portion (900 mg) of the AEPc was fractionated by Sephadex LH-20 column chromatography (70 cm 3.0 cm) and eluted with CH_3OH: DCM (1: 1), producing 45 fractions of approximately 80 mL each. The analysis by charge-coupled device (CCD) and mass spectrophotometry (CLAE-DAD-IES-

MS) [Column C18; CH_3OH: H_2O (5–100% CH_3OH), 254 nm], allowed their clustering in 16 new fraction groups throughthe similarity of the chromatograms (FDm1Se-FDm16Se). The FDm9Se fraction (79 mg), after methanol washing, provided the CIT (30 mg) and FDm11Se (198 mg) was rechromatographed on Sephadex LH-20 column (70 cm x 3 cm) and eluted isocratically with $CHCl_3/CH_3OH$ (1/1). From the fractions, 22 subfractions were collected, which resulted in the DIC-A isolation (27 mg, in subfraction 3).

2.5. Characterization of AEPc, CIT and DIC-A

The analysis of RMN ^1H and ^{13}C revealed the substance CIT, HRESIMS m/z [M þ H] ^{þ} 251.0917 (calculated for $C_{13}H_{14}O_5$) and m/z 273.0735 [M þ Na]^þ. ^1H RMN ($CDCl_3$, 500 MHz) 8.23 (s, H-1), 4.76 (qd, J ¼ 6.8, H-3), 2.97 (qd, H-4), 1.23 (d, J ¼ 6.8, H-9), 1.35 (d, J ¼ 7.2, H-10), 2.01 (s, H-11), and DIC, HRESIMS m/z [M þ H]^þ 381.1701 [M þ H]^þ, (calculated for $C_{23}H_{24}O_5$), including m/z 403.1521 [M þ Na]^þ and m/z 783.3252 [2M þ H]^þ. ^1H RMN ($CDCl_3$, 500 MHz) 6.39 (s, H-7), 4.98 (qd, J ¼ 8.5, H-3), 4.62 (m, H-2^0), 3.16 (m, H-3^0), 3.12 (m, H-4), 2.11 (s, H-11), 1.33 (d, J ¼ 9.0, H-9^0), 1.41 (d, J ¼ 8.0, H-8^0), 1.43 (d, J ¼ 8.5, H-9), 1.31 (d, J ¼ 7.0, H-10), 2.20 (s, H-10^0). The substances were identified according to the method developed by Wakana et al. (2006) and Yao et al. (2011). The ^1H NMR spectrum of the AEPc extract shows a variety of methyl, methylenic, methylenic and olefinics hydrogen signals (Supplementary materials: Figure 1S–7S).

2.6. Brine shrimp lethality bioassay (BSLB)

The microcrustacean toxicity assay was conducted according to the method described by Meyer et al. (1982). Cysts of A. salina were incubated in artificial salted water (23 g of NaCl, 11 g of $MgCl_2.6H_2O$, 4 g of Na_2SO_4, 1.3 g of $CaCl_2.2H_2O$ or 1.3 g of $CaCl_2.6H_2O$ and 0.7 g KCl in 1000 mL of distilled water) at 25–30 C. Sodium bicarbonate (Na_2CO_3) was used as a buffering agent for theadjustment of pH 9.0. After 48 h, ten live A. salina nauplii were collected and transferred to the test tube. The final volume of each sample was 5 mL of saline and tap water (1:1). Solutions of AEPc, CIT and DIC-A were prepared in quadruplicate at concentrations of 0.5, 1.0, 1.5 and 2.0 lg/mL. After 24 and 48 h, the number of live nau-plii was counted. Artificial saline and potassium dichromate ($K_2Cr_2O_7$) were used as negative control (NC) and positive control (PC), respectively.

2.7. Evaluation of toxicity, cytotoxicity and mutagenicity in A. cepa

Medium size bulbs of A. cepa were obtained from the Center Supply company- CEAPI in Teresina- Piauı, 2017. The A. cepa test was carried out according to Fiskesjo€ (1985). Briefly, onions were kept in direct contact with AEPc, CIT and DIC-A for 48 h, at a temperature of 25 ± 1 C, protected from light and maintenance of the solution every 24 h. The concentra-tions tested were same as BSLB assay. The positive (PC) and negative control (NC), were composed of copper sulfate ($CuSO_4.5H_2O$) at 6 lg/mL and dechlorinated water, respectively. After treatment, the roots were removed and fixed for 24 h in Carnoysolution. The roots were washed with distilled water (3 baths of 5 min each), root hydrolysis was performed with 1 N HCl at 60 C for 11 min and the bath was repeated. The basophilic structures were stained with Schiff reagent for 2 h and then rinsed with the aid of running tap water. For assembling the slides, the root meristematic region was removed with a scalpel and a drop of 2% acetic carmine was added to stain the nuclear regionand covered with the cover slip. A slight pressure was exerted on the coverslip in order to scatter the meristematic cells throughout the slide.

The toxicity of the test samples and controls was evaluated by determining the root growth inhibition in milli-meters of each onion. Cytotoxicity (mitotic index, MI) and mutagenicity (chromosomal changes, CA) were evaluated by counting 5000 meristematic cells (experimental unit: 1000 cells/slide, total of 5 slides per treatment) by using a light microscope DM 500 (400x). Genotoxicity includes aneugenic effects (C-metaphases, metaphases with chromosomal adhe-sions, chromosomal losses, multipolar anaphases and poly-ploid metaphases) or clastogenic effects (chromosomal fragments, chromosomal bridges and other changes). In add-ition, the presence or absence of MN, which may result from aneugenic or clastogenic effects, was also evaluated along with the other chromosomal alterations (CA) (Mazzeo et al. 2011).

2.8. Oxidant/antioxidant evaluation in S. cerevisiae

The S. cerevisiae strains used for oxidant and/or antioxidant test included the proficient cytoplasmic superoxide dismutase (SodWT), three simple deficient strains (Sod1D, Sod2D and Cat1D) and two double deficient strains (Sod1D/Sod2D and Sod1D/Cat1D) (Table 1). These strains were proficient and deficient in Superoxide dismutase (Sod) and catalase (Cat) and were kindly provided by the Toxicological Genetics Research Group from the Federal University of Rio Grande do Sul (UFRGS). For the assay, S. cerevisiae strains were replicated in solid YEPD medium (1% yeast extract, 2% glucose, 2% peptone and 2% agar) and stored under appropriate condi-tions according to Andrade et al. (2011). Cells were seeded from the center to the edge of a petri dish in a continuous cycle, to both sides of the plate containing, in the center, a sterile filter paper disk, in which was added 10 lL of the test substances.

The S. cerevisiae strains (Sodwt, Sod1D, Sod2D, Sod1DSod2D, Cat1D, Sod1DCat1D) were seeded in petri dishes from the center to the margin, where a disk of sterile filter paper was placed into the center of the plate and 10 lL of AEPc, CIT and DIC-A (at concentrations of 0.5–2.0 lg/mL) were added to three different treatment conditions (pre-, co-and post-treatment). In pretreatment, the concentrations of the tested samples were first added to a filter paper disk in the center of the YEPD plate and 2 h later the oxidizing agent (30% hydrogen peroxide, H_2O_2) was added. In co-treatment, the samples and H_2O_2 were simultaneously added. In post-treatment, the H_2O_2 was first added and the sample

Table 1. Strains of S. cerevisiae.

Description	Genotype	Origin
EG103 (SOD WT)	MATa leu2-3,112 trp1-289 ura3-52 GALþ	Edith Gralla, L Angeles
EG118 (Sod1D)	Sod1:URA3 all other markers as EG103	
EG110 (Sod2D)	Sod2:TRP1 all other markers as EG103	
EG133 (Sod1DSod2D)	Sod1:URA3 sod2::TRP1 double mutant all other markers as EG103	
EG223 (Cat1D)	EG103, except cat1: TRP1	
EG (Sod1DCat1D)	EG103, except sod1: URA3 and cat1:: TRP1	

concentrations were added 2 h later. After 48 h of incubation, in an oven at 30 C, the growth inhibition halo was measured in millimeters (mm) from the margin of the filter paper disk to the cell growth initiation. All assays were performed in triplicate. 20 lL of 30% H_2O_2 was used as a stressor.

2.9. Statistical analyzes

A. saline data were normalized, logarithmically transformed and subjected to non-linear regression analysis, in order to determine the concentration that causes death of 50% of the microcrustaceans (LC_{50}). The results for A. cepa test were expressed as mean ± standard deviation (SD) and analyzes performed using two-way analysis of variance (ANOVA), followed by the Tukey test for multiple comparisons. S. cerevisiae data were expressed as mean ± SD, with ANOVA analysis, followed by the Bonferroni test for multiple comparisons. All analyzes were performed using the GraphPad Prism software (version 6.0), considering $p < .05$, with a 95% confi-dence level.

3. Results and discussion

3.1. Effects AEPc, CIT and DIC-A on BSLB

The AEPc and the metabolites CIT and DIC-A showed toxicity in A. salina with LC_{50} for 24 h 2.03 lg/mL (with 95% CI: 1.59–2.60 lg/mL); 1.71 lg/mL (95% CI: 1.41–2.06 lg/mL) and 2.29 lg/mL (95% CI: 1.69–3.12 lg/mL), respectively. At 48 h, the LC_{50} values of AEPc, CIT and DIC-A were 0.51 lg/mL (95% CI: 0.39–0.69 lg/mL); 0.54 lg/mL (95% CI: 0.39–0.73 lg/mL) and 0.54 lg/mL (95% CI: 0.34–0.85 lg/mL), respectively. The LC_{50} values for the PC at 24 h was 1.05 lg/mL (95% CI: 0.93–1.18 lg/mL) and, at 48 h, it was 0.7 lg/mL (95% CI: 0.59–0.85 lg/mL). The evaluation of lethality in A. salina has been suggested as a toxicological test for a wide variety of substances, including crude extracts, isolated and synthetic compounds (Sangian et al. 2013, Islam et al. 2017). Compounds with a low LC_{50} may have potential antitumor activity (Nunes et al. 2008, Arcanjo et al. 2012). CIT is evident to induce dermal toxicity and apoptosis in the skin of rats, possibly due to its oxidative stress induction capacity (Kumar et al. 2011).

DIC-A (also named as penicitrinone-A) is a co-product of CIT (dimer CIT) (Clark et al. 2006, Wakana et al. 2006). DIC-A with an IC_{50} value 58.4 ± 4.0 (Nong et al. 2013) is known to inhibit the protein tyronine phosphatase 2 (Shp2), which is a protein associated with breast cancer and other types of neo-plasms such as leukemia, lung, liver and gastric carcinoma. In addition, the Shp2 is involved in several cancer-related processes, including metastasis, apoptosis, DNA damage, cell proliferation and anti-cancer drug resistance (Zhang et al. 2015).

In this study, AEPc, CIT and DIC-A induced toxicity in A. salina at 24 and 48 h at all concentrations (0.5–2.0 lg/mL) as observed by the reduction of survival rate of the nauplii when compared to the NC group. No statistical differences were observed between the substances and concentrations, as well as in relation to the PC group. The PC group significantly reduced survival rate in comparison to the test substances, especially at higher concentrations (Figure 1(A)). Similar data were observed at 48 h exposure, except for CIT and DIC-A at higher concentrations, where the nauplii survival ratewas statistically different from the PC group (Figure 1(B)).

3.2. Cytotoxic and mutagenic effects of AEPc, CIT and DIC-Ain A. cepa

To date, CIT has been extensively studied for its toxic proper-ties (Blasko et al. 2013), including nephrotoxicity, hepatotox-icity (Bennett and Klich 2003, Flajs and Peracia 2009, EFSA, 2012, Shi and Pan 2012), cytotoxicity and genotoxicity (Chang et al. 2009). In our study, AEPc, CIT and DIC-A induced cytotoxicity at all concentrations, where these sub-stances increased the number of cells in interphases and reduced the number of dividing cells in A. cepa meristematic cells (Table 2).

Toxic and cytotoxic alterations are related to the effects of anti-neoplastic drugs, since they induce apoptosis (Xiao et al. 2003, Jordan and Wilson 2004). In this sense, CIT, at 20–100 lM cencentrations, have been reported to induce apoptosis in several human cell lines, including leukemic cells (Yu et al. 2006, Chan 2007). However, at 50 lM, CIT was not cytotoxic but affected microtubules organization, which is a risk factor for carcinogenesis (Gayathri et al. 2015, Yu et al. 2015). CIT cytotoxicity may be associated with the mitochon-drial dysfunction and the influx of calcium ions, leading to increase in membrane permeability, as observed in renal and hepatic cell membranes (Chagas et al. 1995, Da Lozzo et al. 1998). The mitotic index (MI) from A. cepa meristematic cells reliably identifies cytotoxic effects, when the MI shows a reduction of 50% in comparison to the NC group, it is consid-ered a sublethal effect (Mesi and Koplicu 2013).

The mutagenic effects of the isolates of P. citrinum was evaluated by cytogenetic damage, including MN formation and increased in c-metaphase CA, bridges, loose chromo-somes and anaphasic delays, leading to a significant increase in CA. AEPc, CIT and DIC-A, at all tested concentrations induced significant CA parameters. AEPc and DIC-A also

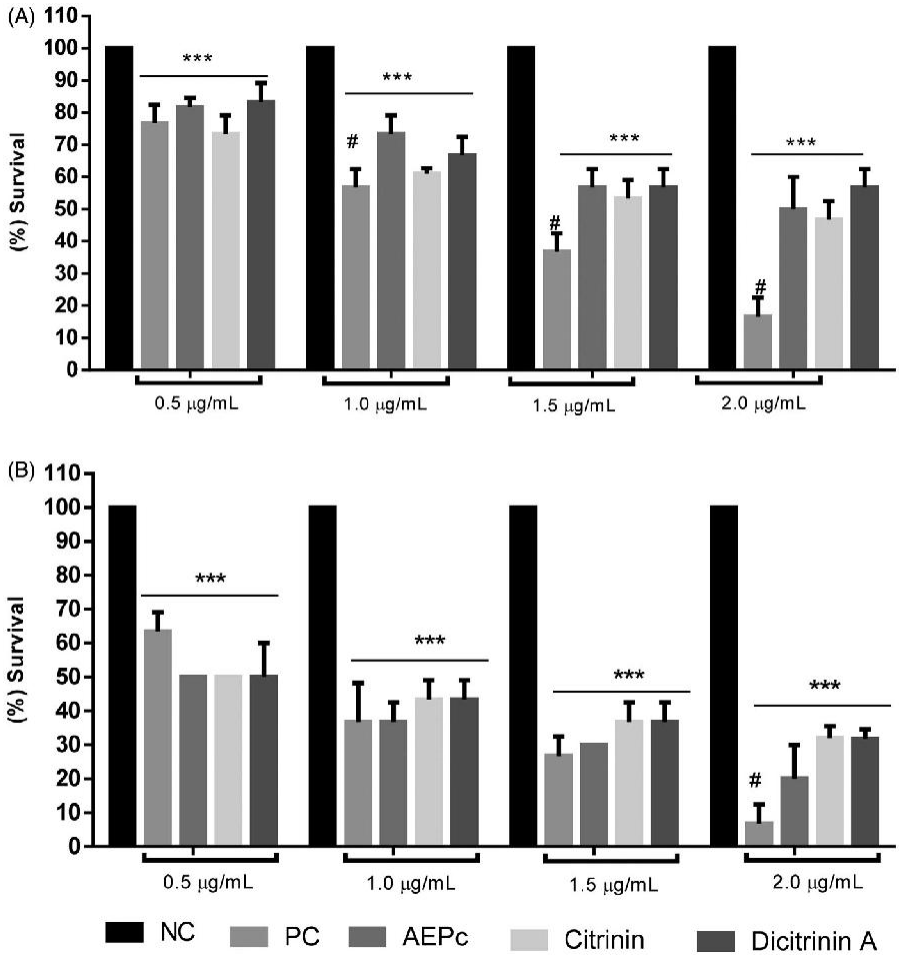

Figure 1. A. salina survival (%) to P. citrinum isolates: AEPc, CIT and DIC-A.

Table 2. Cytotoxicity of AEPc, CIT and DIC-A from P. citrinum in A. cepa meristematic cells after 48 h of exposure.

Treatments	Conc. (mg/mL)	Interphase	Prophase (P)	Metaphase (M)	Anaphase (A)	Telophase (T)	Mitotic index (%)
NC	0	439.4± 9.1	458.8± 18.9	43.4± 7.1	29.8 ± 3.1	28.6 ± 3.0	56.0 ± 0.9
PC	6.0	876.8± 15.6	62.6± 14.6	29.0± 5.4	18.8 ± 3.3	13.6 ± 2.3	12.4 ± 1.5
AEPc	0.5	585.2± 28.5	357.6± 22.8	24.4± 12.0	15.6 ± 7.1	16.8 ± 3.3	41.3 ± 2.1
	1.0	545.2± 23.1	399.0± 18.2	25.0± 7.5	17.0 ± 3.7	13.4 ± 1.9	45.4 ± 1.8
	1.5	561.2± 11.9	361.4± 6.8	29.0± 6.4	23.4 ± 3.1	25.0 ± 1.2	43.8 ± 1.2
	2.0	597.6± 12.2	329.2± 8.8	24.2± 2.9	25.2 ± 2.1	23.8 ± 2.1	40.2 ± 1.2
CIT	0.5	559.2± 28.0	367.0± 24.8	22.8± 5.9	23.4 ± 8.5	27.2 ± 4.4	44 ± 2.4
	1.0	613.2± 35.9	332.2± 38.7	27.5± 4.0	14.8 ± 6.4	13.2 ± 1.78	38.7 ± 3.6
	1.5	589.2± 56.6	367.2± 38.3	16.0± 5.5	11.2 ± 1.8	17.2 ± 7.1	41.2 ± 4.1
	2.0	623.0± 13.5	326.4± 17.5	22.6± 5.7	10.6 ± 2.5	17.4 ± 1.6	37.7 ± 1.3
DIC-A	0.5	569.8± 96.6	359.2± 57.0	21.4± 2.8	23.6 ± 2.8	26.0 ± 2.1	43 ± 5.9
	1.0	591.8± 19.6	344.4± 21.8	18.6± 1.7	21.6 ± 1.3	23.6 ± 1.5	40.8 ± 2.0
	1.5	603.2± 14.3	327.2± 13.9	23.4± 2.0	24.4 ± 2.3	21.8 ± 1.6	39.7 ± 1.4
	2.0	626.4± 19.3	305.4± 19.0	20.0± 2.5	23.0 ± 3.1	25.2 ± 2.2	37.4 ± 1.9

Varules are mean ± SD of 5000 cells (n ¼ 5 bulbs / group). $p < .05$; $p < .01$; $p < .001$ compared to the NC group; ANOVA with Tukey Post-test, by multiple comparisons.

increased the number of MN formation by the two highest concentrations (Table 3).

Micronucleus (MN) formation is an irreversible nuclear alteration process, and it is the result of the final process of DNA changes. This parameter is commonly used to detect DNA damage after exposure to mutagenic agents (Fernandes et al. 2007). There are reports of CIT cytotoxicity and geno-toxicity in embryonic cells, as observed by the induction of different mechanisms, including reactive oxygen species (ROS) formation, nitric oxide (NO) production, Bax and Bcl-2 overexpression, mitochondrial membrane loss, cytochrome C release, caspase-9/3 activation, p21, kinase 2 and c-JUN-terminal activation. In another study, CIT induced an increase in the frequency of MN, in concentrations 30 mM, from in vitro tests performed on V79 cells (Follmann€ et al. 2014). Moreover, CIT also causes apoptosis due to HSP90 inactivation and Ras and Rad 1 degradation (Chan 2008).

Polyketides are evident to induce apoptosis and MN formation (Yu et al. 2006, Chan 2007, Donmez€-Altuntas et al. 2007). CIT induces DNA damage via ROS formation through mitogen-activated protein kinase (MAPK) activation (Chan et al. 2007, Farrugia and Balzan 2012). In rats, CIT at high

Table 3. Mutagenic effects of AEPc, CIT, DIC-A on meristematic cells of A. cepa, after 48 h exposure by micronucleus formation and chromosomal aberrations.

		Types of chromosomal alterations (CA)					
Treatment	Conc. (mg/mL)	Micronucleus	c-metaphase	Bridges	Loose chromosomes	delays	Total
NC	0	0.6 ± 0.4	0.8 ± 0.2	0.2 ± 0.0	0.8 ± 0.1	1.0 ± 0.4	3.4± 0.5
PC	6.0	3.4 ± 1.1	4.8 ± 0.8	5.0 ± 2.3	10.2 ± 0.8	10.2 ± 0.8	33.6± 3.7
AEPc	0.5	0.8 ± 0.0	3.4 ± 0.5	2.8 ± 1.3	1.8 ± 1.0	3.0 ± 2.2	15.4± 2.9
	1.0	2.2 ± 1.0	3.0 ± 0.7	2.6 ± 1.0	3.0 ± 1.4	3.4 ± 0.7	15.0± 1.3
	1.5	3.0 ± 0.8	4.6 ± 1.5	3.6 ± 0.8	3.6 ± 1.0	3.8 ± 1.0	17.8± 4.6
	2.0	4.4 ± 1.1	4.0 ± 1.0	4.4 ± 2.6	4.6 ± 1.5	3.0 ± 0.8	16.8± 1.8
CIT	0.5	1.8 ± 1.1	2.6 ± 1.9	1.8 ± 1.4	1.2 ± 0.7	4.2 ± 2.4	12.6± 2.9
	1.0	1.9 ± 1.1	4.2 ± 1.1	3.2 ± 2.1	0.6 ± 0.4	7.6 ± 1.6	19.2± 1.3
	1.5	2.2 ± 1.2	3.6 ± 1.5	2.0 ± 1.0	4.6 ± 1.6	7.2 ± 2.3	19.6± 4.6
	2.0	3.6 ± 1.5	4.2 ± 0.8	4.2 ± 1.3	5.6 ± 1.1	5.8 ± 0.8	21.6± 1.8
DIC-A	0.5	0.6 ± 0.3	3.2 ± 0.8	3.8 ± 0.8	3.2 ± 1.1	2.2 ± 1.3	13.8± 1.9
	1.0	0.2 ± 0.4	2.8 ± 1.3	3.8 ± 1.3	4.0 ± 1.5	1.2 ± 0.4	12.0± 3.2
	1.5	0.2 ± 0.4	4.2 ± 1.3	3.8 ± 0.8	4.8 ± 1.3	2.0 ± 0.7	15.0± 1.4
	2.0	2.4 ± 0.5	4.4 ± 1.1	4.8 ± 1.4	6.2 ± 1.6	2.0 ± 1.0	18.0± 2.3

AEPc: Acetonitrile extract of Penicillium citrinum; CIT: Citrinin; DIC-A: Dicitrinin A; NC: Dechlorinated water; PC: Copper sulfate. Values are mean ± SD of 5000 cells (n ¼ 5 bulbs/group), ANOVA with Tukey Post-test, by multiple comparisons; $p < .05$; $p < .01$; $p < .001$ compared to the NC group.

doses was seen to increase mRNA expression for Ccna2, Ccnb1 and E2f1 transcription factors, leading to cell cycle modifications, CA and genotoxicity (Liu et al. 2003, Knasmuller€ et al. 2004, Bouslimi et al. 2008, Folkmann et al. 2009, Chang et al. 2011, Kuroda et al. 2013). In addition, the induction of MN, mediated by CIT, and several other dam-ages caused to DNA were observed in HepG2 cells (Knasmuller€ et al. 2004). A good candidate for an antitumor agent should have the ability to induce cytotoxic, genotoxic and mutagenic effects in neoplastic cells, generating blocking effects of the neoplastic process. CIT is capable of causing clastogenic effects in in vivo and in vitro test systems (Liu et al. 2017).

Chromosomal aberrations (CA) during the cell cycle induced by chemical compounds are indicative of mutagenic-ity and cytotoxicity (Tacar et al. 2013). However, an increase in CA, such as c-metaphases and loose chromosomes, by any test substance is not necessarily due to direct clastogenic effects, rather to an effect resulting from the breakdown of the cell division machinery during mitosis, which is controlled by the cellular cytoskeleton and microtubules (Eleftheriou et al. 2012). Chromosomal abnormalities can lead to apop-tosis. In a study, an administration of 0.9 mg/kg (i.p.) or 0.1 mg/kg of CIT in rats was found to reduce CA and double strand breaks as well as aneuploidy due to cell cycle arrest (Jeswal 1996, Yu et al. 2006). On the other hand, a genotoxic effect of CIT on Vero cells was also reported the by Yu et al. (2006). In this study, we have seen that numerous cells in interphase as well as CA such as chromosomal fragments, anaphase bridges, delayed chromosomes and MN formation by the treatment of AEPc, CIT and DIC-A in A. cepa meristem-atic cells (Figure 2).

There are reports that CIT exerted nephropatoxic and hep-atotoxic effects on cultured cell lines and animal models (Arai and Hibino 1983, Aleo et al. 1991, Kogika et al. 1993). CIT induced nephrotoxicity by mechanisms associated with cell cycle arrest and microtubule formation, as evidenced in HEK293 cells, and also altered the expression of p53 and p21 proteins during the cell cycle, interfering in the cell division process (Chang et al. 2009).

3.3. Oxidant/antioxidant and DNA repair capacities of AEPc, CIT and DIC-A in S. cerevisiae

AEPc, CIT and DIC-A modulated the oxidative effects of H_2O_2-induced oxidative stress in proficient and mutated S. cerevi-siae at all concentrations (Table 4).

AEPc, CIT and DIC-A, in most of the concentrations, modu-lated the effects of H_2O_2, but did not reduce its oxidative damages when compared to the NC group. Antioxidant effects were observed in the two lowest concentrationsof AEPc against all the test strains of S. cerevisae, and at all con-centrations for the double mutant Sod1DCat1D when com-pared to the PC group. Conversely, CIT and DIC-A exerted no antioxidant effects at 2 lg/mL (Table 5). It may be due to their oxidative effects at this concentration (data not shown). According to Iwahashi et al. (2007), CIT inhibits S. cerevisiae growth only at concentrations higher than 100 lg/mL, but in our study, CIT was found to show an oxidative stress at 2 lg/mL.

According to Arora and Chandra (2011) isolated com-pounds of P. citrinum demonstrated potent antioxidant activ-ities (in vitro). However, another study indicates that CIT induces oxidative damage and lipid peroxidation at concen-trations above 15 mg/kg in rats (Kumar et al. 2014). In add-ition, genotoxicity was also observed by DNA fragmentation and apoptosis (Kumar et al. 2014). Previous studies have reported that ROS mediate DNA damage in rat skin with an increased expression of p53, p21/waf1 and Bax and cell cycle arrest in G0/G1 and G2/M (Kumar et al. 2011).

The results demonstrate that the compounds tested have similar characteristics of antineoplastic agents, however, at low concentrations, the tested compounds are antioxidant. Several studies have reported that antioxidants such as nat-ural phenolic compounds can act as cytotoxic agents (Kashif et al. 2015, Perveen & Al-Taweel 2017, Csepregi et al. 2020) and that capacity is linked to anti-proliferative and cytotoxic mechanisms in some cases (Yanez~ et al. 2004). CIT inhibits yeast growth at high concentrations (100 ppm), activating stress response genes such as AADs, FLR1, OYE3, GRE2 and MET17 that are responsible for the glutathione synthesis.

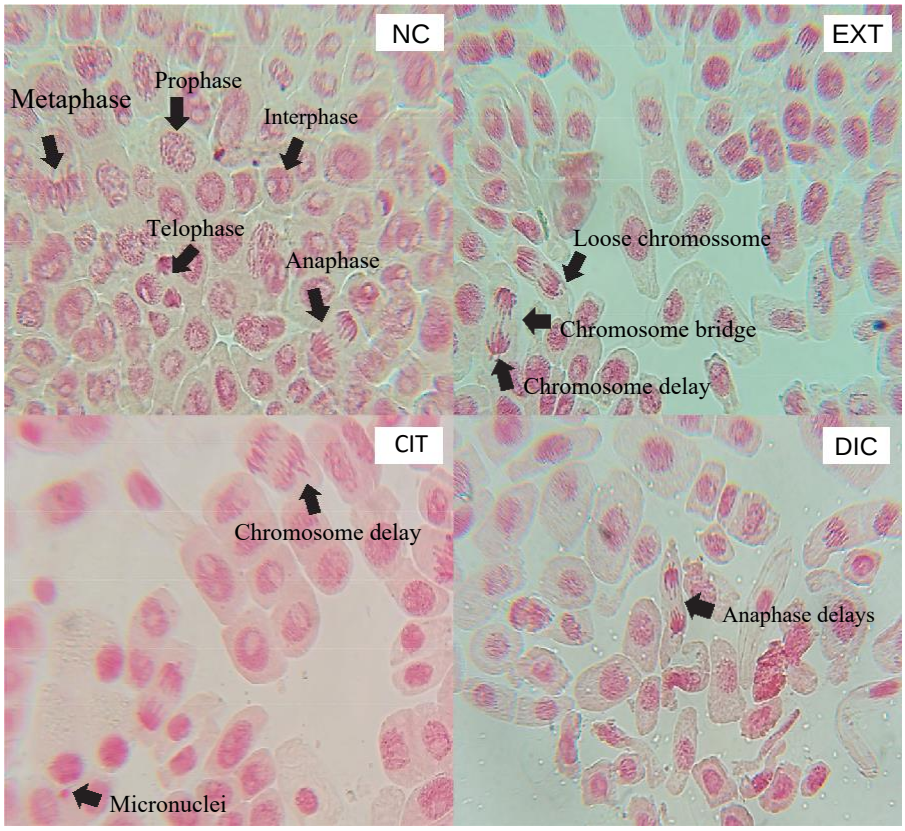

Figure 2. Cell division and chromosomal aberration in A.cepa meristematic cells.

Table 4. Pretreatment of S. cerevisiae strains with AEPc, CIT and DIC-A.

Groups	SODWT	Sod1D	Sod2D	Sod1DSod2D	Cat1D	Sod1DCat1D
NC	0.50 ± 0.58	0.50 ± 0.61	0.37 ± 0.39	0.41 ± 0.45	0.52 ± 049	0.50 ± 0.55
PC	11.25 ± 0.9	15.00 ± 2.16	15.00 ± 0.81	20.25 ± 1.70	20.00 ± 2.06	22.00 ± 1.82
AEPc (mg/mL)						
0.5	3.25 ± 0.50 ,	4.50 ± 0.58 ,	4.25 ± 0.96 ,	3.5 ± 0.58 ,	4.25 ± 0.50 ,	6.75 ± 0.96 ,
1.0	2.00 ± 0.00	7.25 ± 0.96 ,	7.75 ± 0.96 ,	7.25 ± 1.50 ,	7.00 ± 0.82 ,	3.00 ± 0.81 ,
1.5	5.50 ± 0.58 ,	6.25 ± 0.96 ,	7.75 ± 0.50 ,	6.5 ± 0.58 ,	8.25 ± 0.50 ,	6.00 ± 0.58 ,
2.0	6.75 ± 0.50 ,	8.0 ± 1.15 ,	7.25 ± 0.96 ,	7.25 ± 0.96 ,	5.25 ± 0.50 ,	6.00 ± 0.82 ,
CIT (mg/mL)						
0.5	8.75 ± 0.50 ,	7.50 ± 0.58 ,	8.75 ± 0.58 ,	6.00 ± 0.82 ,	6.25 ± 0.96 ,	3.80 ± 1.00 ,
1.0	6.75 ± 0.50 ,	7.25 ± 0.96 ,	9.00 ± 0.82 ,	6.00 ± 0.00 ,	5.75 ± 0.96 ,	6.50 ± 0.57 ,
1.5	10.25 ± 0.96	8.50 ± 0.58 ,	8.75 ± 0.50 ,	7.25 ± 0.96 ,	7.75 ± 0.96 ,	7.00 ± 0.82 ,
2.0	9.75 ± 1.25 ,	7.00 ± 1.15 ,	9.75 ± 0.96 ,	8.25 ± 0.96 ,	8.75 ± 0.96 ,	9.50 ± 0.58 ,
DIC-A (mg/mL)						
0.5	2.00 ± 0.00	4.50 ± 0.58 ,	7.00 ± 0.82 ,	7.25 ± 0.96 ,	4.50 ± 0.58 ,	3.50 ± 0.50 ,
1.0	8.00 ± 0.82 ,	6.00 ± 0.82 ,	6.25 ± 0.50 ,	7.75 ± 0.96 ,	7.25 ± 1.00 ,	8.25 ± 0.96 ,
1.5	6.50 ± 0.58 ,	8.50 ± 0.58 ,	8.25 ± 0.50 ,	8.00 ± 0.82 ,	6.75 ± 0.96 ,	7.25 ± 0.50 ,
2.0	6.70 ± 0.50 ,	7.25 ± 0.50 ,	8.0 ± 1.15 ,	8.25 ± 0.50 ,	9.25 ± 0.96 ,	7.50 ± 0.50 ,

AEPc: Acetonitrile extract of Penicillium citrinum; CIT: Citrinin; DIC-A: Dicitrinin A; NC: (vehicle); PC: Hydrogen peroxide (stressor). Values are mean ± SD of inhib-ition halos measured in mm; ANOVA, two-way, Bonferroni post-test. p < .01 compared to the NC; p < .01 compared to the PC.

Conversely, CIT was not found to induce DNA repair gene expression (Iwahashi et al. 2007), although it may allow activation of certain defensive genes, promoting adaptation and survival (Santos et al. 2012). In another study, using yeast cells, CIT (1600 lM) induced strong gene expression of 68 genes related to oxidative stress, suggesting a confirmation of the toxicity triggered by CIT is fundamentally based on its ability to generate ROS (Vanacloig-Pedros et al. 2016). In this context, CIT is capable of triggering oxidative stress responses through induction of natural genes and transcrip-tion factors (e.g., Skn7 and Yap1) (Pascual-Ahuir et al. 2014).

AEPc, CIT and DIC-A, in the three lowest concentrations and all strains, participated in the modulation of H_2O_2-induced damage, but did not eliminate oxidative damage when compared to the NC group. However, CIT at the concentration of 2.0 lg/mL did not modulate H_2O_2-induced damage, as observed in all tested strains (Table 6).

In this study, AEPc, CIT and DIC-A exerted a concentration dependent toxic and cytotoxic effects on A. salina and A. cepa test systems. In A. salina the test substances showed toxic effects by increasing the percentage mortality with the increasing of test concentration. In A. cepa test system, the substances reduced the MI value more than 50% in comparison to NC group, which indicates the cytotoxic characteristics of them. Moreover, AEPc, CIT and DIC-A, at all tested concentrations significantly increased the CA parameters, where

Groups	SODWT	Sod1D	Sod2D	Sod1DSod2D	Cat1D	Sod1DCat1D
NC	0.50 ± 0.57	0.50 ± 0.57	0.50 ± 0.57	0.50± 0.57	0.50 ± 0.57	0.50 ± 0.57
PC	11.25 ± 0.95	15.00 ± 2.16	15.00 ± 0.81	17.25± 1.7	15.00 ± 2.06	16.00 ± 1.82
AEPc (mg/mL)						
0.5	0.75 ± 0.95	1.75 ± 0.50	1.00 ± 1.15	1.75± 0.50	2.25 ± 1.70	0.25 ± 0.50
1.0	1.25 ± 0.95	3.00 ± 0.82	2.25 ± 0.50	6.25± 0.50 ,	4.75 ± 0.95 ,	0.25 ± 0.50
1.5	2.50 ± 1.29 ,	5.75 ± 0.96 ,	2.25 ± 0.96	7.25± 0.96 ,	6.25 ± 0.96 ,	0.25 ± 0.50
2.0	2.75 ± 0.96 ,	4.5 ± 1.29 ,	1.25 ± 0.96	8.50± 0.58 ,	4.25 ± 0.96 ,	1.75 ± 1.26
CIT (mg/mL)						
0.5	3.75 ± 1.26 ,	8.75 ± 1.50 ,	6.25 ± 1.26 ,	6.00± 1.15 ,	6.75 ± 1.50 ,	5.25 ± 0.96 ,
1.0	5.75 ± 0.96 ,	9.00 ± 0.82 ,	8.00 ± 0.82 ,	7.75± 1.26 ,	6.00 ± 0.00 ,	6.75 ± 0.50 ,
1.5	6.00 ± 1.15 ,	8.75 ± 0.96 ,	7.25 ± 0.96 ,	6.75± 0.96 ,	7.25 ± 0.96 ,	7.00 ± 0.82 ,
2.0	8.25 ± 1.26	9.25 ± 1.26	8.00 ± 0.82 ,	8.00± 1.29	9.00 ± 0.58 ,	8.00 ± 1.41
DIC-A (mg/mL)						
0.5	5.50 ± 0.58 ,	5.75 ± 0.96 ,	5.00 ± 1.41 ,	7.25± 0.96 ,	5.50 ± 0.58 ,	3.75 ± 0.96 ,
1.0	6.75 ± 096 ,	9.00 ± 0.82 ,	6.00 ± 0.82 ,	8.29± 0.96 ,	8.00 ± 0.82 ,	4.25 ± 0.50 ,
1.5	6.25 ± 1.26 ,	9.25 ± 0.96 ,	7.75 ± 0.96 ,	7.00± 0.82 ,	7.50 ± 1.29 ,	5.75 ± 0.96 ,
2.0	9.00 ± 1.29	9.00 ± 1.41	9.25 ± 0.96	8.25± 1.26	8.00 ± 1.41	9.00 ± 1.70

AEPc: Acetonitrile extract of Penicillium citrinum; CIT: Citrinin; DIC-A: Dicitrinin A; NC: (vehicle); PC: Hydrogen peroxide (stressor). Values are mean ± SD of inhib-ition halos measured in mm; ANOVA, two-way, Bonferroni post-test. p < .0001 compared to the NC; p < .0001 compared to the PC.

Table 6. Effects of AEPc, CIT, DIC-A and control groups on damages induced by H_2O_2 in S. cerevisiae strains.

Groups	SODWT	Sod1D	Sod2D	Sod1DSod2D	Cat1D	Sod1DCat1D
NC	0.50 ± 0.58	0.50 ± 0.58	0.50 ± 0.58	0.50 ± 0.58	0.50 ± 0.58	0.50 ± 0.58
PC	11.25 ± 0.95	15.00 ± 2.16	15.00 ± 0.81	16.25 ± 1.7	17.00 ± 2.06	15.00 ± 1.82
AEPc (mg/mL)						
0.5	5.75 ± 0.96 ,	6.00 ± 0.82 ,	6.0 ± 1.15 ,	5.50 ± 0.58 ,	4.00 ±0 .82 ,	4.50 ± 0.58 ,
1.0	6.25 ± 0.5	8.00 ± 1.15 ,	7.25 ± 0.96 ,	6.00 ± 1.15 ,	5.00 ± 0.0 ,	8.25 ± 0.96 ,
1.5	8.50 ± 0.58 ,	8.75 ± 1.5 ,	7.75 ± 0.96 ,	8.00 ± 1.63 ,	6.75 ± 0.58 ,	5.75 ± 0.5
2.0	9.50 ± 0.58	12.50 ± 1.29	11.50 ± 1.29	9.00 ± 0.82 ,	9.00 ± 1.29	8.50 ± 0.5
CIT (mg/mL)						
0.5	6.50 ± 1.0	7.00 ± 0.82 ,	7.50 ± 1.29 ,	6.50 ± 1.0 ,	7.00 ± 0.0 ,	4.50 ± 0.58 ,
1.0	5.00 ± 0.82 ,	7.75 ± 0.96 ,	7.25 ± 0.96 ,	6.75 ± 0.96 ,	7.50 ± 0.58 ,	7.75 ± 0.50 ,
1.5	7.00 ± 1.63 ,	9.50 ± 2.38 ,	7.75 ± 1.89 ,	6.50 ± 1.0 ,	7.25 ± 2.22 ,	5.50 ± 0.58 ,
2.0	10.25 ± 0.96	13.25 ± 0.96	12.00 ± 0.58	10.00 ± 0.5	11.00 ± 0.96 ,	8.00 ± 0.82 ,
DIC-A (mg/mL)						
0.5	4.25 ± 0.5	7.00 ± 0.82 ,	5.0 ± 0.82 ,	5.50 ± 1.0 ,	4.75 ± 0.96 ,	2.25 ± 0.96 ,
1.0	6.00 ± 1.63 ,	7.75 ± 0.5 ,	5.50 ± 0.58 ,	5.50 ± 0.58 ,	5.75 ± 0.96 ,	5.00 ± 0.82 ,
1.5	6.50 ± 1.29 ,	6.50 ± 0.58 ,	7.75 ± 0.5 ,	6.75 ± 0.5 ,	8.00 ± 0.82 ,	4.75 ± 0.96 ,
2.0	8.00 ± 0.82	10.00 ± 0.82	11.00 ± 0.5	9.00 ± 0.82	9.25 ± 0.96	9.00 ± 0.58

AEPc: Acetonitrile extract of Penicillium citrinum; CIT: Citrinin; DIC-A: Dicitrinin A; NC: (vehicle); PC: Hydrogen peroxide (stressor). Values are mean ± SD of inhib-ition halos measured in mm; ANOVA, two-way, Bonferroni post-test. p < .0001 compared to the NC; p < .0001 compared to the PC.

AEPc and DIC-A were seen to increase the number of MN for-mation at the two highest test concentrations. In mutagenic and non-mutagenic S. cerevisae strains, AEPc, CIT and DIC-A modulated the H_2O_2-induced oxidative damage in compari-son to the NC group. AEPc at 2 lg/mL and CIT at the two highest concentrations did not affect the H_2O_2-induced DNA damage in the test strains.

4. Conclusion

AEPc, CIT and DIC-A showed toxicity in A. salina. In A. cepat-est system, the compounds also showed an inhibitory effect on cell division phases. The test substances also induced mutagenicity, especially at higher concentrations. Pre-, co-and post-treatments of AEPc, CIT and DIC-A significantly modulated H_2O_2-induced oxidative damage in S. cerevisiae strains. The substances also showed a DNA damage repair capacity in S. cerevisiae test strains. Further investigations are necessary to understand the exact mechanisms regarding the toxic, cytotoxic and mutagenic effects of these substances.

Acknowledgement

We are owed to the Federal University of Piauı (UFPI) and NTF, Brazil for hosting and funding this project.

References

Aleo, M.D., Wyatt, R.D., and Schnellmann, R.G., 1991. The role of altered mitochondrial function in citrinin-induced toxicity to rat renal prox-imal tubule suspensions. Toxicology and Applied Pharmacology, 109 (3), 455–463.

Andrade, T.J.A.S., et al., 2011. Antioxidant properties and chemical com-position of technical Cashew Nut Shell Liquid (tCNSL). Food Chem, 126 (3), 1044–1048.

Arai, M., and Hibino, T., 1983. Tumorigenicity of citrinin in male F344 rats. Cancer Letters, 17 (3), 281–287.

Arcanjo, D.D.R., et al., 2012. Bioactivity evaluation against Artemia saline leach of medicinal plants used in Brazilian Northeastern folk medicine. Brazilian Journal of Biology, 72 (3), 505–509.

Arora, D.S., and Chandra, P., 2011. In vitro antioxidant potential of some soil fungi: screening of functional compounds and their purification from Penicillium citrinum. Applied biochemistry and Biotechnology, 165 (2), 639–651.

Bennett, J.W., and Klich, M., 2003. Mycotoxins. Clinical Microbiology Reviews, 16 (3), 497–516.

Blasko, A., et al., 2013. Citrinin-induced fluidization of the plasma mem-brane of the fission yeast Schizosaccharomyces pombe. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 59, 636–642.

Blunt, J.W., et al., 2015. Marine natural products. Natural Product Reports, 32 (2), 116–211.

Blunt, J.W., et al., 2008. Marine natural products. Natural Product Reports, 25 (1), 35–94.

Blunt, J.W., et al., 2009. Marine natural products. Natural Product Reports, 26 (2), 170–244.

Bouslimi, A., et al., 2008. Cytotoxicity and oxidative damage in kidney cells exposed to the mycotoxins ochratoxin a and citrinin: individual and combined effects. Toxicology Mechanisms and Methods, 18 (4), 341–349.

Chagas, G.M., et al., 1995. Mechanism of citrinin-induced dysfunction of mitochondria. III. Effects on renal cortical and liver mitochondrial swel-ling. Journal of Applied Toxicology, 15 (2), 91–95.

Chan, W.H., 2007. Citrinin induces apoptosis via a mitochondria-depend-ent pathway and inhibition of survival signals in embryonic stem cells, and causes developmental injury in blastocysts. The Biochemical Journal, 404 (2), 317–326.

Chan, W.H., 2008. Effects of citrinin on maturation of mouse oocytes, fer-tilization, and fetal development in vitro and in vivo. Toxicology Letters, 180 (1), 28–32.

Chan, W.H., Wu, H.J., and Shiao, N.H., 2007. Apoptotic signaling in meth-ylglyoxal-treated human osteoblasts involves oxidative stress, c-Jun N-terminal kinase, caspase-3, and p21-activated kinase 2. Journal of Cellular Biochemistry, 100 (4), 1056–1069.

Chang, C.H., et al., 2009. Activation of ERK and JNK signaling pathways by mycotoxin citrinin in human cells. Toxicology and Applied Pharmacology, 237 (3), 281–287.

Chang, C.H., et al., 2011. Mycotoxin citrinin induced cell cycle G2/M arrest and numerical chromosomal aberration associated with disruption of microtubule formation in human cells. Toxicological Sciences: An Official Journal of the Society of Toxicology, 119 (1), 84–92.

Clark, B.R., et al., 2006. Citrinin revisited: from monomers to dimers and beyond. Organic & Biomolecular Chemistry, 4 (8), 1520–1528.

Csepregi, R., et al., 2020. Cytotoxic, antimicrobial, antioxidant properties and effects on cell migration of phenolic compounds of selected tran-sylvanian medicinal plants. Antioxidants Basel, 9 (2), 166.

Da Lozzo, E.J., Oliveira, M.B.M., and Carnieri, E.G.S., 1998. Citrinin-induced mitochondrial permeability transition. Journal of Biochemical and Molecular Toxicology, 12 (5), 291–297.

De La Torre-Ruiz, M.A., Pujol, N., and Sundaran, V., 2015. Coping with oxi-dative stress. The yeast model. Current Drug Targets, 16 (1), 2–12.

De Oliveira, I.M., et al., 2014. Dicholesteroyldiselenide: cytotoxicity, genotoxicity and mutagenicity in the yeast Saccharomyces cerevisiae and in Chinese hamster lung fobroblasts. Mutation Research, 763, 1–11.

Donmez€-Altuntas, H., et al., 2007. Effects of the mycotoxin citrinin on micronucleus formation in a cytokinesis-block genotoxicity assay in cultured human lymphocytes. Journal of Applied Toxicology, 27 (4), 337–341.

Du, L., et al., 2010. Novel carbon-bridged citrinin dimers from a volcano ash-derived fungus Penicillium citrinum and their cytotoxic and cell cycle arrest activities. Journal of Tetrahedron, 66 (47), 9286–9290.

EFSA, Panel on Contaminants in the Food Chain (CONTAM). 2012. Scientific opinion on the risks for public and animal health related to the presence of citrinin in food and feed. EFSA Journal, 10, 2605.

Eleftheriou, E.P., Adamakis, I.D.S., and Melissa, P., 2012. Effects of hexavalent chromium on microtubule organization, ER distribution and callose deposition in root tip cells of Allium cepa L. Protoplasma, 249 (2), 401–416.

El-Neketi, M., et al., 2013. Alkaloids and polyketides from Penicillium citri-num, na endophyte isolated from the Moroccan Plan Ceratoniasliqua. Journal of Natural Products, 76 (6), 1099–1104.

Farrugia, G., and Balzan, R., 2012. Oxidative stress and programmed cell deadth in yeast. Frontiers in Oncology, 2, 1–21.

Fernandes, T.C.C., Mazzeo, D.E.C., and Marin-Morales, M.A., 2007. Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to trifluralin herbicide. Pesticide Biochemistry and Physiology, 88 (3), 252–259.

Fiskesjo,€ G., 1985. The Allium test as a standard in environmental moni-toring. Hereditas, 102 (1), 99–112.

Flajs, D., and Peracia, M., 2009. Toxicological properties of citrinin. Arhiv za higijenu rada i toksikologiju, 60 (4), 457–464.

Folkmann, J.K., et al., 2009. Oxidatively damaged DNA in rats exposed by oral gavage to C60 fullerenes and single-walled carbon nanotubes. Environmental Health Perspectives, 117 (5), 703–708.

Follmann,€ W., Behm, C., and Degen, G.H., 2014. Toxicity of the mycotoxin citrinin and its metabolite dihydrocitrinone and of mixtures of citrinin and ochratoxin A in Vitro. Archives of Toxicology, 88 (5), 1097–1107.

Gajardo, G.M., and Beardmore, J.A., 2012. The brine shrimp Artemia: adapted to critical life conditions. Frontiers in Physiology, 3, 1–8.

Gayathri, L., et al., 2015. Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: in vitro study in HepG2 cell. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 83, 151–163.

Gerwick, W.H., and Fenner, A.M., 2013. Drug discovery from marine microbes. Microbial Ecology, 65 (4), 800–806.

Gubiani, J.R., et al., 2014. Biologically active eremophilane-type sesquiterpenes from Camarops sp., an endophytic fungus isolated from Alibertia macrophylla. Journal of Natural Products, 77 (3), 668–672.

Hu, Y., et al., 2017. Pencitrin and pencitrinol, two new citrinin derivatives from an endophytic fungus Penicillium citrinum salicorn 46. Phytochemistry Letters, 22, 229–234.

Islam, M.T., et al., 2017. Evaluation of toxic, cytotoxic and genotoxic effects of phytol and its nanoemulsion. Chemosphere, 177, 93–101.

Iwahashi, H., et al., 2007. Evaluation of toxicity of the mycotoxin citrinin using yeast ORF DNA microarray and oligo DNA microarray. BMC Genomics, 8 (1), 95.

Jeswal, P., 1996. Citrinin-induced chromosomal abnormalities in the bone marrow cells of Mus musculus. Cytobios, 86 (344), 29–33.

Jordan, M.A., and Wilson, L., 2004. Microtubules as a target for anticancer drugs. Nature Reviews Cancer, 4 (4), 253–265.

Kashif, M., et al., 2015. Cytotoxic and antioxidant properties of phenolic compounds from Tagetes patula flower. Pharmaceutical Biology, 53, 672–681.

Keller, N.P., 2019. Fungal secondary metabolism: regulation, function and drug discovery. Nature Reviews Microbiology, 17 (3), 167–180.

Knasmuller,€ S., et al., 2004. Structurally related mycotoxins ochratoxin A, ochratoxin B, and citrinin differ in their genotoxic activities and in their mode of action in human-derived liver (HepG2) cells: implica-tions for risk assessment . Nutrition and Cancer, 50 (2), 190–197.

Kogika, M.M., Hagiwara, M.K., and Mirandola, R.M., 1993. Experimental cit-rinin nephrotoxicosis in dogs: renal function evaluation. Veterinary and Human Toxicology, 35 (2), 136–140.

Kumar, M., et al., 2014. Apoptosis and lipid peroxidation in ochratoxin A-and citrinin-induced nephrotoxicity in rabbits. Toxicology and Industrial Health, 30 (1), 90–98.

Kumar, R., et al., 2011. Citrinin-generated reactive oxygen species cause cell cycle arrest leading to apoptosis via the intrinsic mitochondrial pathway in mouse skin. Toxicological Sciences: An Official Journal of the Society of Toxicology, 122 (2), 557–566.

Kuroda, K., et al., 2013. Cell cycle progression, but not genotoxic activity, mainly contributes to citrinin-induced renal carcinogenesis. Toxicology, 311 (3), 216–224.

Leme, D.M., and Marin-Morales, M.A., 2009. Allium cepa test in environmental monitoring: a review on its application. Mutation Research, 682 (1), 71–81.

Lesgards, J.F., et al., 2014. Anticancer activities of essential oils constitu-ents and synergy with conventional therapies: a review. Phytotherapy Research: PTR, 28 (10), 1423–1446.

Liu, B.H., et al., 2003. Evaluationof genotoxic risk and oxidative DNA dam-age in mammalian cells exposed tomycotoxins, patulin and citrinin. Toxicology and Applied Pharmacology, 191 (3), 255–263.

Liu, S., et al., 2017. Marine-derived Penicillium species as producers of cytotoxic metabolites. Marine Drugs, 15 (10), 329.

Mazzeo, D.E.C., Fernandes, T.C.C., and Marin-Morales, M.A., 2011. Cellular damages in the Allium cepa test system, caused by BTEX mixture prior and after biodegradation process. Chemosphere, 85 (1), 13–18.

Mesi, B.N., and Koplicu, D., 2013. Cytotoxic and genotoxic potency screening of two pesticides on Allium cepa L. Procedia Technology, 8, 19–26.

Meyer, B.N., et al., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica, 45 (5), 31–34.

Nakajima, Y., et al., 2016. Low doses of the mycotoxin citrinin protect cortical neurons against glutamate-induced excitotoxicity. The Journal of Toxicological Sciences, 41 (2), 311–319.

Nong, X.H., et al., 2013. Polyketides from a marine-derived fungus Xylariaceae sp. Marine Drugs, 11 (5), 1718–1727.

Nunes, B.S., et al., 2006. Use of the genus Artemia in ecotoxicity testing. Environmental pollution (Barking, Essex: 1987), 144 (2), 453–462.

Nunes, X.P., et al., 2008. Constituintes quımicos, avaliac¸˜ao das atividades citotoxica e antioxidante de Mimosa paraibana Barneby (Mimosaceae). Revista Brasileira de Farmacognosia, 18, 718–723.

Pascual-Ahuir, A., Vanacloig-Pedros, E., and Proft, M., 2014. Toxicity mech-anisms of the food contaminant citrinin: application of a quantitative yeast model. Nutrients, 6 (5), 2077–2087.

Perveen, S., and Al-Taweel, A. M., 2017. Phenolic compounds from the natural sources and their cytotoxicity. In: M., Soto-Hernandez, M., Palma-Tenango, M., Del Rosario Garcia-Mateos, eds. Phenolic compounds natural sources, importance and applications. London, UK: Intech Open, pp. 61–88.

Sangian, H., et al., 2013. Antiplasmodial activity of ethanolic extracts of some selected medicinal plants from the northwest of Iran. Parasitology Research, 112 (11), 3697–3701.

Santos, C.L., Pourrut, B., and Oliveira, J.M.F., 2015. The use of comet assay in plant toxicology: recent advances. Frontiers in Genetics, 6, 1–18.

Santos, S.C., et al., 2012. Yeast toxicogenomics: genome-wide responses to chemical stresses with impact in environmental health. Pharmacology and Biotechnology. Frontiers in Genetics, 3, 11–17.

Shaala, N.M.A., et al., 2015. Lethal concentration 50 (LC_{50}) and effects of diuron on morphology of brine shrimp Artemia salina (Branchiopoda: Anostraca). Nauplii Proceeding Environmental Science, 30, 279–284.

Shi, Y.C., and Pan, T.M., 2012. Red mold, diabetes, and oxidative stress: a review. Applied Microbiology and Biotechnology, 94 (1), 45–47.

Skoneczna, A., Kaniak, A., and Skoneczny, M., 2015. Genetic instability in budding and fission yeast-sources and mechanisms. FEMS Microbiology Reviews, 39 (6), 917–967.

Tacar, O., Sriamornsak, P., and Dass, C.R., 2013. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery sys-tems. Journal of Pharmacy and Pharmacology, 65 (2), 157–170.

Thacker, H.L., Carlton, W.W., and Sansing, G.A., 1977. Citrinin mycotoxico-sis in the Guinea-Pig. Food and Cosmetics Toxicology, 15 (6), 553–561.

Vanacloig-Pedros, E., Proft, M., and Pascual-Ahuir, A., 2016. Different tox-icity mechanisms for citrinin and ochratoxin A reveled by transcrip-tomic analysis in yeast. Toxins, 8 (10), 273–292.

Visagie, C.M., et al., 2014. Identification and nomenclature of the genus Penicillium. Studies in Mycology, 78, 343–371.

Wakana, D., et al., 2006. New citrinin derivatives isolated from Penicillium citrinum. Journal of Natural Medicines, 60 (4), 279–284.

Wali, A.F., et al., 2019. Natural products against cancer: review on phyto-chemicals from marine sources in preventing cancer. Saudi Pharmaceutical Journal: SPJ: The Official Publication of the Saudi Pharmaceutical Society, 27 (6), 767–777.

Wang, M.L., et al., 2013. Four new Citrinin derivatives from a marine-derived Penicillium sp. Fungal strain. Molecules (Basel, Switzerland), 18 (5), 5723–5735.

Wen, C., Guo, W., and Chen, X., 2014. Purification and identidication of a novel antifungal protein srcreted by Penicillium citrinum from the Southwest Indian Ocean. Journal of Microbiology and Biotechnology, 24 (10), 1337–1345.

Williams, D.E., and Andersen, R.J., 2020. Biologically active marine natural products and their molecular targets discovered using a chemical genetics approach. Natural Product Reports. doi:10.1039/c9np00054b

Xiao, D., et al., 2003. Induction of apoptosis by the garlic-derived com-pound S-allylmercaptocysteine (SAMC) is associated with microtubule depolymerization and c-Jun NH(2)-terminal kinase 1 activation. Cancer Research, 63 (20), 6825–6837.

Xu, B.J., et al., 2006. Review on the qualitative and quantitative analysis of the mycotoxin citrinin. Food Control, 17 (4), 271–285.

Yanez,~ J., et al., 2004. Cytotoxicity and antiproliferative activities of sev-eral phenolic compounds against three melanocytes cell lines: rela-tionship between structure and activity. Nutrition and Cancer, 49 (2), 191–199.

Yao, G., et al., 2011. Citrinin derivatives from the soil filamentous fungus Penicillium sp. Journal of the Brazilian Chemical Society, 22 (6), 1125–1129.

Yu, F., et al., 2015. Pseudolaric acid B circumvents multidrug resistance phenotype in human gastric cancer SGC7901/ADR cells by downregu-lating Cox-2 and P-gp expression. Cell Biochemistry and Biophysics, 71 (1), 119–126.

Yu, F.Y., et al., 2006. Citrinin induces apoptosis in HL-60 cells via activa-tion of the mitochondrial pathway. Toxicology Letters, 161 (2), 143–151.

Zhang, J., Zhang, F., and Niu, R., 2015. Functions of Shp2 in cancer. Journal of Cellular and Molecular Medicine, 19 (9), 2075–2083.

Zheng, Y.K., et al., 2016. Diversity, distribution and biotechnological potential of endophytic fungi. Annals of Microbiology, 66 (2), 529–542.