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RESEARCH ARTICLE

Cytotoxic and genotoxic evaluation of copper oxychloride through *Allium* test and molecular docking studies

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Abstract

Copper oxychloride gained great importance due to its broad-spectrum antifungal action to combat various fungal diseases of plants. However, excess quantity of cupric fungicides on plants causes enzymatic changes and toxic effects. Thus, the current study was aimed to investigate the cytotoxicity and genotoxicity of copper oxychloride on *Allium cepa* root cells. The root growth, mitotic index (MI), chromosomal aberrations (CAs), and DNA damage were assessed through root growth inhibition, *A. cepa* ana-telophase, and alkaline comet assays. Furthermore, molecular docking was performed to evaluate binding affinities of two copper oxychloride polymorphs (atacamite and paratacamite) on DNA. In root growth inhibition test, onion root length was statistically significantly decreased by changing the copper oxychloride concentration from lower (2.64±0.11 cm) to higher (0.92±0.12 cm). Concentration- and time-dependent decrease in MI was observed whereas increase in CAs such as disturbed ana-telophase, chromosome laggards, stickiness, anaphase bridges, and DNA damage were caused by the copper oxychloride on *A. cepa* root cells. Molecular docking results revealed that the two main polymorphs of copper oxychloride (atacamite and paratacamite) bind selectively to G and C nucleotides on the B-DNA structure. It is concluded that the atacamite- and paratacamite-induced DNA damage may be through minor groove recognition and intercalation. Findings of the current study revealed the cytotoxic and genotoxic effects of copper oxychloride on *A. cepa* root cells. However, further studies should be carried out at the molecular level to reveal the cyto-genotoxic mechanism of action of copper oxychloride in detail.

Keywords Atacamite · Chromosomal aberrations · Mitotic index · DNA damage · Paratacamite · Molecular docking

Introduction

Fungicides are used to combat different fungi that invade the plants. Fungicides are classified as organic, inorganic, systematic, and unsystematic fungicides on the basis of their chemical composition and mode of application (Fisun and Rasgele 2009). Composition of fungicides is important as various metals ions present in these agents assist to eliminate the fungal diseases. The mechanism of action through which fungicide act is the blockade of mitosis via ketone, amines, and phenol used, while some substances in fungicides like carbamates and copper sulfate act on the fungal metabolism. Commonly used broad spectrum fungicides are copper sulfates and copper oxychloride having copper as the main metal ion (Yuzbasioglu 2003). Copper is an important metal and have physiological functions, but large quantity of copper is lethal and can interfere with enzymatic activity (Laurén and McDonald 1987; Osman et al. 2011). Copper oxychloride toxicity occurs through oxidative stress. It accumulates in tissues and cause cell damage due to formation of reactive

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oxygen species (ROS) by Fenton reaction (Sevcikova et al. 2016). The fungicide copper oxychloride is mainly used in various agricultural fields like tomato, potato, tea, nuts, grapes, banana, tobacco, spices, and citrus to combat fungal diseases. Other common names of this synthetic are Blitox, coprantol, coprex, and coppesean. The empirical formula is $H_6Cl_2Cu_4O_6$. Copper oxychloride is used to resist bacterial ailment, leaf spot, early and late blight, brown deterioration, bacterial cancer, leaf twist, downy mildew, and powdery mold infections. It is a bluish-green powder and its fungitoxicity depends on the solubilization and release of ionic copper (Brooks 1992). It is a low soluble copper in different formulation as wettable powder, colloidal liquid, or ready-to-use dust. Extensive studies indicated its effectiveness against plant pathogenic fungi such as *Rhizoctonia solani*, *R. bataticola*, *Botrytis cinerea*, *Fusarium semitectum*, *F. culmorum*, *F. moniliforme*, *F. solani*, *F. oxysporum*, *Stemphylium radicinum*, *Hirschmanniella oryzae*, *Otinia sclerotiorum*, and *Colletotrichum gloeosporioides* (Kućmierz et al. 1989; Bhaskar and Ahmad 1991; Thakae and Patil 1995; Gharieb et al. 2004). It controls harmful fungi which is inhabited on seeds including *Helminthosporium* sp., *Rhizopus nigricans*, *Chaetomium* sp., *Aspergillus niger*, *A. flavus*, *and Stachybotrys atra* (Narayanappa and Sohi 1985). It may be applied alone or in combination with other fungicides, seed treatment, foliar application, and soil drenching pre- or post-emergence. Copper oxychloride administration led to the inhibition of growth and a reduction in auxin, gibberellin, and cytokinin levels in *A. flavus*, *A. terreus*, and *Penicillium palitans* (El-Mehalawy 1997).

A. cepa test is the most commonly used assay for the cytotoxic and genotoxic assessment of toxic agents due to its easiness in handling, cost effectiveness, greater sensitivity, and its potential to produce meaningful results (Fiskesjö 1985; Caritá and Marin-Morales 2008; Gupta et al. 2018; Liman et al. 2015; Rahman et al. 2017). It is a biomarker of genotoxicity (Ghosh et al. 2011; Liman 2013; Kumar et al. 2015; Rajeshwari et al. 2016a; Becaro et al. 2017; Kaygisiz and Ciğerci 2017). The comet assay, also known as single cell gel electrophoresis, is widely used to assess DNA damage of various mutagens due to its sensitivity, adaptability, and consistency, and its application is relatively inexpensive (Demir et al. 2014; Pakrashi et al. 2014; Ghosh et al. 2015; De et al. 2016; Ciğerci et al. 2015; Cvjetko et al. 2017; Mangalampalli et al. 2018;). Scarcity of data is present on the cytogenetic properties of copper oxychloride through *A. cepa* cells, and no study investigating its binding mode and affinity to the DNA molecule has been observed. The importance of in silico structure-activity models in the assessment of the genotoxic prospective of small molecules is steadily increasing. The reliability of the investigation of in silico receptor-ligand interaction is because of its great compatibility, little rate of false negative/positive results, and fast results with reasonable amount of data (Dördü et al. 2020; Istifli et al. 2020). The data obtained from in vitro genotoxicity and cytotoxicity assessments can be analyzed and correlated with the structural characteristics of small molecules. In addition, the genotoxic effects of small molecules at the atomic level can also be visualized and confirmed using computational methods (Pandey et al. 2009; Snyder et al. 2013). In this context, whether the copper oxychloride has the structural characteristic that supports non-covalent bonding with the DNA molecule has been tested using molecular docking analysis via AutoDock Vina. Moreover, investigations on the MI, CAs, and DNA structural damage induced by fungicide copper oxychloride in *A. cepa* root tips were also carried out in the current study.

Materials and methods

Chemicals

Copper oxychloride was purchased from Supelco Solutions Ltd., USA (Cat. No. PS 292). Other chemicals such as low melting agarose, glacial acetic acid, magnesium chloride, Trizma hydrochloride, sodium and potassium chloride, normal melting agarose, EDTA, Triton X-100, potassium disulfide, and ethidium bromide were procured from Sigma-Aldrich and were of analytical grade.

A. cepa root growth inhibition test

A. cepa bulbs (diameter 24–31mm) were purchased from the local market to perform the root growth inhibition and *Allium* ana-telophase test. *A. cepa* root growth inhibition test was performed as described by Fiskesjö (1985) with modifications as suggested by Küçük and Liman (2018). Onion bulbs were cleaned, and then outer shells of onions were peeled off. Then onion roots were exposed to the series of different concentrations (0.25, 0.5, 1, 2, 3, and 4 mg/L) in test tubes for 96 h to perform root growth inhibition test. Distilled water was established as control. The complete set of experiments was performed in dark and at room temperature. The average root lengths of onion bulbs were measured by taking ten roots from each bulb (total of 50 roots from the 5 onion bulbs) to find half maximal effective concentration (EC_{50}) after 96 h. Since *Allium cepa* cell cycle is 24 h, application process was carried out at 24, 48,72, and 96 h.

Allium ana-telophase test

The *Allium* ana-telophase test was conducted with minor modifications as explained by Rank and Nielsen (1994). On the basis of root inhibition test, three concentrations of copper oxychloride (0.25 (½xEC_{50}), 0.5 (EC_{50}), and 1(2xEC_{50}) mg/L) were further employed to onion cells for 24 h, 48 h, 72h,

and 96 h for *Allium* ana-telophase test. Distilled water was used as negative control, and for positive control, methyl methanesulfonate (MMS, 10 mg/L) was utilized. At the end of the respective exposure time, root tips were cut and kept in fixative solution of ethanol and glacial acetic acid (3:1 v/v) at 4°C and then preserved in alcohol (70%). After this step, fixed tips of roots were hydrolyzed in 2 mL of 1N HCl for 10 min at 60 °C. At room temperature, the root tips were cleaned with distilled water and stained using Feulgen technique for 25 min. Stained tips were crushed and covered with coverslips, and cells were counted under trinocular light microscope (Nikon Eclipse Ci-L, Japan). A total of 5000–5250 cells (1000–1050 cells from each slide per bulb) and total 500 ana-telophase cells (100 ana-telophase cells from each slide per bulb) were counted to find the MI and CAs frequencies using the following formulas:

$$MI = \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ cells} \times 100$$

$$\text{Phase index} = \frac{\text{Particular index}}{\text{Total number of dividing cells}} \times 100$$

$$\text{CAs} = \frac{\text{Total aberrant cells}}{100\text{ana}-\text{telophase cells}} \times 100$$

Alkaline comet assay

Alkaline comet assay was performed to assess the level of DNA damage induced by copper oxychloride on the onion cells by using same concentrations as described above according to the protocol of Tice et al. (2000) with minor changes as proposed by Küçük and Recep (2018). Root tips were crushed in ice-cold nucleus isolation buffer (0.2 M Tris, 4 mM $MgCl_2$–$6H_2O$, 0.5% w/v Triton X-100 at pH 7.5) and sieved further to separate the cells. Onion root cells were centrifuged for 7 min at 1200 rpm. Then, 50 μL of nucleus suspension were combined with 50 μL of low melting agarose (1.5%) and spread on slides that were already coated with normal melting point agarose (1%). In turn, agarose was solidified on ice for 5 min. Slides were kept in alkaline buffer (300 mM NaOH, 1mM EDTA, pH > 13) in dark at 4°C for 20 min, and then samples were run in electrophoresis chamber at (25 V, 300 mA) for 20 min at 4 °C. Finally, cells were stained with (20 μg/mL) ethidium bromide and scored as described by Liman et al. (2020). Arbitrary unit (AU) was used to express the DNA damage and scored from 0 to 4, depending upon the extent of DNA damage. Scores were arranged as 0 for no damage, 1 for insignificant damage, 2 for modest damage, 3 for severe destruction, and 4 for complete impairment (Supp. Fig 1). A total of 50 randomly selected comets from each slide (totally 150 comets) were scored through fluorescence microscope (TAM-F, Turkey). Experiments were run in triplicate. The following formula of AU was used to find DNA damage.

$$Arbitrary\ \text{unit} = \sum_{i=0}^{4} Ni \times i$$

where i is the degree of DNA damage in terms of score (0, 1, 2, 3, 4) and Ni is number of cells.

Molecular docking

Copper oxychloride has four polymorphic crystal systems: atacamite (orthorhombic), paratacamite (rhombohedral), clinoatacamite (monoclinic), and botallackite (monoclinic). Atacamite and paratacamite are the two most common polymorphs of $Cu_2(OH)_3Cl$, and both have two possible octahedral sites for Cu atoms (Boita et al. 2014). In case of atacamite, the Cu atom is surrounded by four OH^- and two Cl atoms in one of the sites, while in the other site, one of the Cl atoms is changed by OH^- (Fig. 1a). In case of paratacamite, first site has Cu surrounded by four OH^- and two Cl atoms; on the other hand, the other site contains Cu atom bounded by six OH^- (Fig. 1b). These two polymorphs of copper oxychloride were used in molecular docking calculations in this study.

Molecular docking was performed using AutoDock Vina (version 1.1.2) to calculate binding affinities of two major

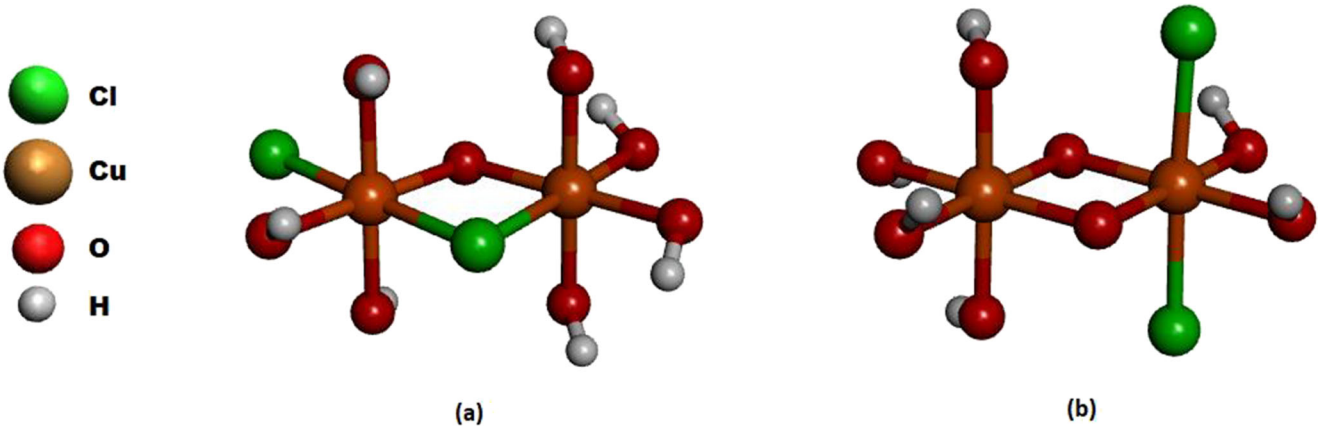

Fig. 1 Possible octahedral Cu sites in $Cu_2(OH)_3Cl$ polymorphs: **a** atacamite and **b** paratacamite

polymorphs of copper oxychloride (atacamite and paratacamite) to the possible binding sites on crystal arrangement of a synthetic B-DNA dodecamer (PDB ID: 1BNA; resolution: 1.90Å) downloaded from Protein Data Bank (PDB). The crystal structures of atacamite and paratacamite were drawn in ChemOffice version 19.1. Since the atomic parameters and charge of the Cu element is not listed by default in AutoDock software, the atomic charges of the two ligands were initially calculated on the Atomic Charge Calculator website (https://webchem.ncbr.muni.cz/Platform/ChargeCalculator) and downloaded as mol2 files. Then, the ligands were geometrically optimized with the Avogadro software using the united-atom force field (UFF) (Rappé et al. 1992), saved in pdbqt format using Open Babel GUI, and the parameters of the Cu atom were registered in the AD4_parameters.dat file.

In this study, the crystal structure of B-DNA dodecamer was designated as the target (receptor) molecule. AutoDockTools-1.5.6 was used in target preparation and two ligand molecules and parameters before initiating docking study by AutoDock Vina (Sanner 1999). In molecular docking studies with B-DNA dodecamer, polar hydrogen atoms in receptor and ligand molecules were retained; however, non-polar hydrogens were merged. Kollman charges were assigned to the receptor molecule (B-DNA dodecamer), while Gasteiger charges were assigned for the ligands. During the docking calculations, rotatable bonds of the ligands were allowed for free rotation. The size of grid box 52×52×52 Å points with 0.375 Å grid spacing was set for B-DNA and ligands. Grid box size were set in such a way that ligands could easily interact with the DNA molecule.

After two separate docking runs (10 dockings for each ligand) of the ligands against B-DNA receptor, all potential binding modes (conformations) of the ligands were clustered through AutoDock Vina and were ranked based on binding free energy (ΔG^{o}; kcal/mol) of the ligand conformation which showed the lowest binding free energy against the B-DNA molecule. The best docking conformation of the ligands gained by AutoDock Vina among different poses against the receptor structure was visualized and analyzed using Discovery Studio Visualizer v16.

Results and discussion

In root growth inhibition test, the EC_{50} of copper oxychloride was found 0.5 mg/L (50.76%), and onion root lengths were statistically reduced from lower concentration of copper oxychloride to higher concentrations in a dose-dependent manner (r= −0.928, Fig. 2). The root growth was inhibited gradually starting from lower concentration: at 0.25 mg/L (2.64±0.11 cm, 74.42%), at 0.5 mg/L (1.8±0.11 cm, 50.76%), at 1 mg/L (1.51±0.08 cm, 42.59%), at 2 mg/L (1.36±0.1 cm, 38.37%), at 3 mg/L (1.13±0.07 cm, 31.94%),

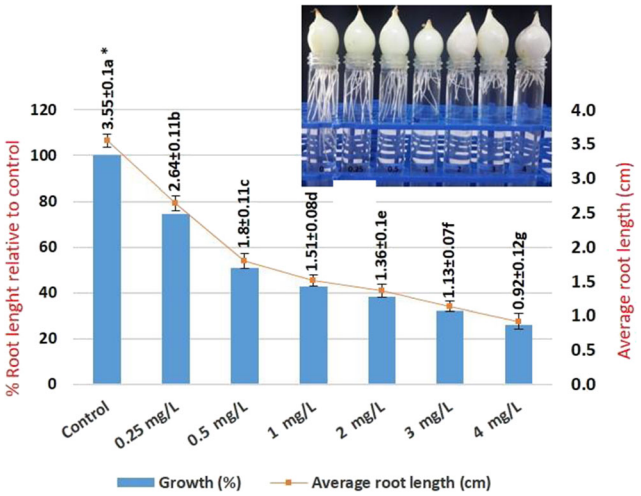

Fig. 2 Allium root growth inhibition test at different copper oxychloride concentrations after 96 h. *Different letters are significantly different at $p \leq 0.05$

and at 4 mg/L (0.92±0.12 cm, 25.92%), respectively. There are different factors which could lead to the suppression of root growth like inhibition of meristematic activity of apex (Webster and Macleod 1996), halting of cell cycle (Fusconi et al. 2006), or the inactivation of enzymes of cell division (Silveira et al. 2017). Inhibition of root growth could be due to the decrease in the mitotic activity of allium cepa cells of root tips in the current study. Copper oxychloride have shown different levels of EC_{50} in different organisms: it was found 2.83 mg/L for mice, 333 mg Cu/kg bw/day for the *Colinus virginianus*, for *Oncorhynchus mykiss*, it was > 43 mg/L, and for *Eisenia foetida* 489.6 kg/bw at 14 days (EFSA 2008).

Concentration- (for 24 h r= −0.973, for 48 h r=−0.900, and for 72 h r= −0.768 at p=0.01, except for 96 h) and time-dependent (for 0.25 mg/L r= −0.933, for 0.5 mg/L r= −0.938 at p=0.01, and for 1 mg/L r= −0.458 at p=0.05) decrease in the MI was observed by the copper oxychloride (Table 1). Copper oxychloride reduced the prophase index (except at 0.5 mg/L and at 48 h); however, it increased the telophase index (except at 0.25 mg/L and at 24 h) compared to the control group. Blitox and Raxil fungicides having copper oxychloride as an active ingredient had also shown to decrease the MI along with an increase in concentrations and treatment time in onion root cells (Fisun and Rasgele 2009; Paul et al. 2013). Decrease in MI induced by the copper oxychloride could be due to inhibition of cell cycle based either on the blockage of DNA synthesis or the entry of the cell cycle into G2 phase (El-Ghamery et al. 2000; Sudhakar et al. 2001). Furthermore, decreased mitotic activity or changes in duration of mitotic stages by the toxic chemicals (Hidalgo et al. 1989; Saxena et al. 2005) or anti-mitotic effects could be due to inhibition of DNA polymerase enzyme along with the related network of other cell cycle proteins (Hidalgo et al. 1989; Türkoğlu 2015). Therefore, it can be presumed that MI of onion root cells is reduced by copper oxychloride due to its cytotoxic action.

Table 1 Mitotic index and mitotic phase of *A. cepa* root meristematic cells exposure to copper oxychloride

Concentration (mg/L)	CCN	MI±SD*	Mitotic phases (%)±SD*			
			Prophase	Metaphase	Anaphase	Telophase
Control-24 h	5217	70.32±0.95a	89.62±0.67a	1.69±0.26 ac	1.5±0.32a	7.2±0.77a
MMS-10	5242	58.13±1.52b	83.76±0.76b	3.23±0.71b	4.04±0.48b	8.97±0.84b
0.25	5104	62.76±0.98c	88.33±0.61c	2.16±0.34cd	1.34±0.37a	8.18±0.9ab
0.5	5241	57.77±0.89b	88.6±0.88c	1.19±0.36a	1.46±0.23a	8.76±0.59b
1	5189	49.95±1.53d	82.35±0.32d	2.5±0.64d	2.5±0.64c	12.58±0.92c
Control-48 h	5311	71.13±1.05a	87.08±0.79a	2.22±0.61a	4.18±0.6a	6.52±0.52a
MMS-10	5170	54.88±1.24b	83.02±0.69b	2.53±0.46a	2.86±0.22b	11.59±0.46b
0.25	5121	53.61±0.99bc	85.75±0.36c	2.67±0.3a	1.72±0.51c	9.86±0.67c
0.5	5151	52.3±0.69c	87.2±0.89a	1.41±0.17b	1.11±0.21d	10.28±0.85c
1	5183	48.07±1.14d	83.74±0.98b	2.34±0.38a	2.61±0.44b	11.32±0.6b
Control-72 h	5222	69.15±1.15a	86.61±0.89a	2.55±0.28ab	2.81±0.49a	8.03±0.54a
MMS-10	5055	53.35±1.15b	83.7±0.88b	2.18±0.48a	2.56±0.38a	11.57±0.68b
0.25	5133	52.17±1b	83.49±0.99c	2.89±0.41b	2.28±0.37ab	11.34±0.89b
0.5	5123	50.19±1.38c	84.52±0.89bc	2.06±0.23a	1.98±0.33b	11.44±0.96b
1	5094	49.04±1.09c	84.91±0.93bc	2.56±0.4ab	2.4±0.41ab	10.13±0.76c
Control-96 h	5163	70.04±0.31a	86.39±0.94a	2.52±0.33a	2.52±0.54a	8.57±0.38a
MMS-10	5092	51.55±0.87b	84.31±0.57b	2.13±0.32ab	2.62±0.49a	10.93±0.44b
0.25	5125	48.36±0.36c	84.05±0.62b	1.92±0.38b	1.84±0.23b	12.19±0.72c
0.5	5056	48.18±0.79c	82.79±0.74c	2.59±0.26a	2.39±0.44ab	12.23±0.66c
1	5089	47.79±0.79c	84.58±0.72b	2.22±0.32ab	2.06±0.33ab	11.14±0.67b

*Different letters for each treatment time are statistically significant ($p \leq 0.05$). *CCN* counting cell numbers, *SD* standard deviation

In this study, copper oxychloride induced a significant increase in the CAs in a concentration- (r= 0.641 at p=0.05 for 24 h, r= 0.83 for 48 h, r=0.89 for 72 h, and r=0.889 for 96 h at p=0.01) and time-dependent manner (r= 0.834 for 0.25 mg/L, r=0.931 for 0.5 mg/L, and r=0.821 for 1 mg/L at p=0.01). The lowest frequency of total CAs (7.8±0.84) was observed at the

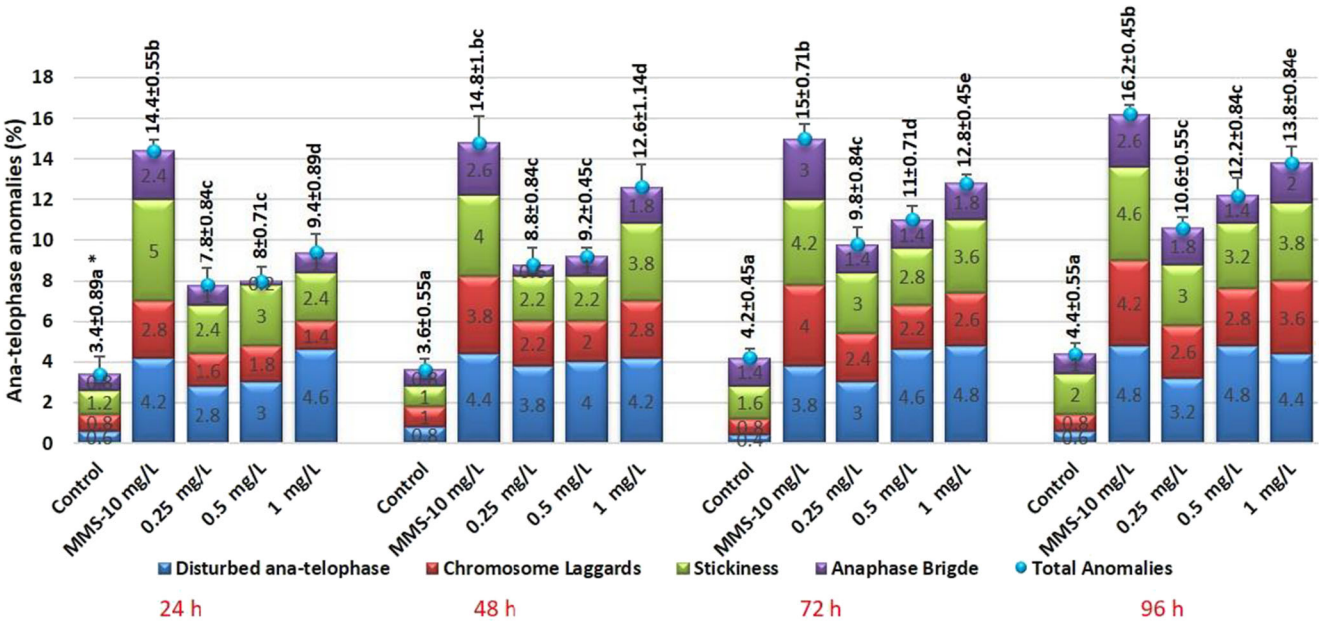

Fig. 3 Copper oxychloride induced CAs in ana-telophase in *A. cepa* root cells. *Different letters for each treatment time are significantly different at $p \leq 0.05$

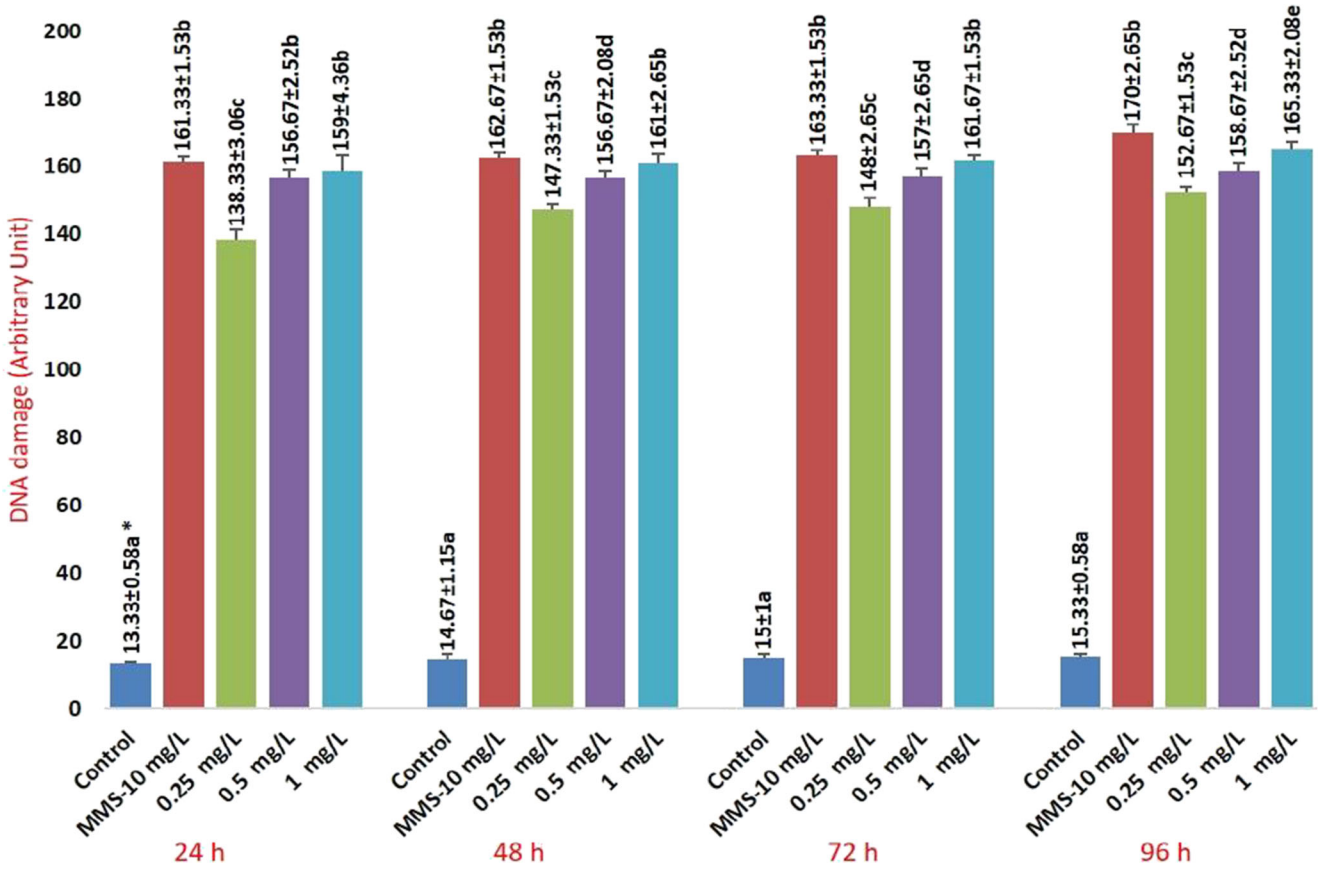

Fig. 4 Copper oxychloride induced DNA damage in *A. cepa* roots.*Different letters for each treatment time are significantly different at $p \leq 0.05$

lowest concentration (0.25 mg/L) in 24 h; however, the highest frequency of total CAs (13.8±0.84) was found at the highest concentration (1 mg/L) in 96 h (Fig. 3). The most common CAs were disturbed ana-telophase, chromosome laggards, stickiness, and anaphase bridges (Supp. Fig. 1).

Previously, fungicides were shown to induce chromosomal aberrations such as c-mitosis, adhesiveness, multipolarity, laggards, picnosis, star-anaphase-telophase, bridges, and cell division breakups in *A. cepa* root cells and other plants (Behera et al. 1982; Armbruster et al. 1991; Bayram et al. 2016; Paul et al. 2013). Accumulation of fungicides in onion root tip may cause inacted spindle development, chromosomal non-histone protein distortions, and structural gene mutations (Mann 1977; Yuzbasioglu 2003; Stanić 2008). Chromosomal adhesiveness is described as the cohesion of chromosomes during stages of cell cycle. Genomic and environmental elements are the main reason of clumping (Badr 1983; Caetano-Pereira et al. 1998; Panneerselvam et al. 2012). It is also hypothesized that adhesive chromosomes are produced due to imperfect functioning of specific non-histone proteins which are involved in chromosomal organization, required for segregation of the chromatids. The defective functioning of these proteins occurred due to mutagens or mutations in genes coding structural proteins (Pagliarini 2000; Türkoğlu 2007).

Chromosomal laggards (Supp. Fig. 2a) and disturbed ana-telophase (Supp. Fig 2b) could be due to cessation of chromosomal movements towards opposite poles or due to aberrated microtubule formation (Evseeva et al. 2005; Kumari et al. 2009; Rajeshwari et al. 2016b). Stickiness (Supp. Fig. 2c) may be caused by the formation of cross-linking proteins with proteins or with DNA-DNA itself (Amin 2002; Barbério et al. 2011). Clastogenic effects of fungicides may lead to the

Table 2 Molecular docking results for atacamite and paratacamite, the two major polymorphs of copper oxychloride

Compound (ligand)	DNA (receptor)	ΔG_{best}	Hydrogen bond distances (receptor-ligand)
Atacamite	B-DNA	−5.4	C3 (2.52 Å), G22 (2.07 Å)
Paratacamite	B-DNA	−5.4	G2 (2.62 Å)–C23 (2.06 Å) (complementary bases) C3 (2.07 Å), G4 (3.00 Å)

ΔG_{best}: most favorable binding free energy (kcal/mol)

formation of anaphase bridges (Supp. Fig. 2d) due to intermingling or breaking of chromosomes, asymmetrical exchanges of chromatids and dicentric chromosomes, or due to variations in replicating enzymes (Dutta et al. 2018; El-Ghamery et al. 2000; Luo et al. 2004). Cytogenetic and molecular tests demonstrated that copper oxychloride induced dose-dependent CAs in human lymphocytes. Increase in polymorphic bands, chromosomal breakage, and chromatid

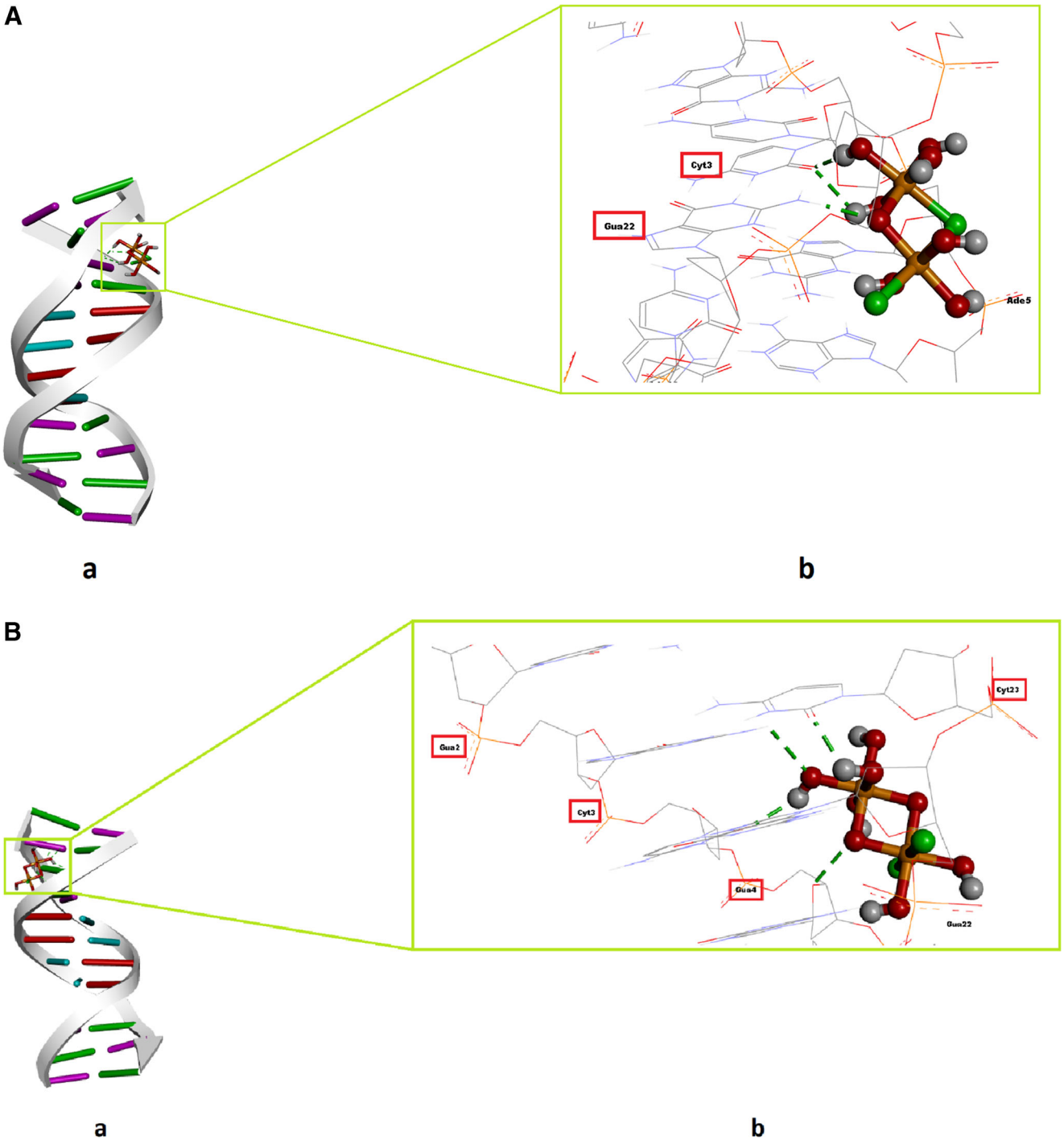

Fig. 5 **A** Top-ranked conformation of the interaction between the constituent (atacamite) of copper oxychloride and B-DNA structure. *a* DNA—atacamite complex; *b* 3D ligand interaction diagram of the top-ranked docking pose. DNA nucleotides that interact with atacamite are outlined in red boxes (*b*). Green dashed lines on the right image represent hydrogen bonds. **B** Top-ranked conformation of the interaction between the constituent (paratacamite) of copper oxychloride and B-DNA structure, *a*. DNA – paratacamite complex, *b*. 3D ligand interaction diagram of the top-ranked docking pose. DNA nucleotides that interact with paratacamite are outlined in red boxes (*b*). Green dashed lines on the right image represent hydrogen bonds

frequency were observed in all concentrations of copper oxychloride, and genomic template constancy and cell proliferation were reduced (Bayram et al. 2016). Blitox also has demonstrated dose-dependent clastogenic and aneugenic effects on the *A. cepa* cells by inducing C-mitosis, adhesiveness, multipolarity, laggards, picnosis, star-anaphase-telophase, and bridge types of chromosomal aberrations (Paul et al. 2013).

In this study, copper oxychloride also induced concentration- (r=0.927 at for 24 h, r=0.936 for 48 h, r=0.932 for 72 h, and r=0.95 for 96 h at p=0.01) and time-dependent (r=0.885 at p= 0.01 for 0.25 mg/L and r= 0.592 for 1 mg/L at p= 0.05) increases in DNA damage in onion root tip cells of *A. cepa* (Fig. 4). Copper oxychloride caused recessive mutation on the X-chromosome of *Drosophila melanogaster* in I and II broods which further led to the genotoxic effects in spermatids and spermatozoids within 72 h after its application (Stanić 2008). Dose-dependent DNA damage was also exerted by the copper oxychloride in human lymphocytes (Bayram et al. 2016). Copper oxychloride had shown mutagenic effects on white mice (Pirtskhelani et al. 2008) and testicular atrophy in *Gallus domesticus* fed with higher dosage of this agent (Shivanandappa et al. 1983). The cytotoxic and genotoxic effects of copper oxychloride could be due to higher oxidative stress, stimulated selective mitotic activity, and distressed spreading of cell (Bakkali et al. 2008; Kocaman et al. 2014) which resulted in DNA damage and inhibition of cell division (Vock et al. 1998; Kirkland and Müller 2000).

To rationalize the genotoxic mode of action of copper oxychloride at the molecular level, we have investigated the binding affinities of atacamite and paratacamite on crystal structure of synthetic B-DNA dodecamer (PDB code: 1BNA) utilizing a 3D computational analysis using AutoDock Vina. The top-ranked ligand conformations based on molecular docking studies were visualized using BIOVIA Discovery Studio Visualizer v16. All nucleotides involved and hydrogen bonds formed in the interactions of atacamite and paratacamite with B-DNA were depicted (Figs. 5a and b; Table 2). Atacamite showed its best binding affinity into the minor groove of B-DNA mediated by nucleotides C3 and G22. The binding mode of atacamite with the receptor was predominantly consisted of conventional H-bonds (C3 and G22) (Fig. 5, Table 2). It was determined that the binding free energy (ΔG^{o}) between atacamite and B-DNA was moderately strong (ΔG_{best} = −5.4). Likewise, paratacamite demonstrated its best binding affinity into the minor groove of B-DNA mediated by nucleotides G2, C23, C3, and G4. Interestingly, paratacamite also displayed an intercalative mode of action by entering between G2-C23 complementary nucleotides via H-bonds in this region of B-DNA. The binding mode of paratacamite with the receptor was predominantly consisted of conventional H-bonds (G2, C23, C3, and G4) (Fig. 5, Table 2). Furthermore, the binding free energy (ΔG^{o}) between paratacamite and B-DNA was determined to be moderately strong (ΔG_{best} = −5.4). Based on the results of molecular docking studies, it can be proposed that the main components of copper oxychloride, atacamite and paratacamite, induced DNA damage through minor groove recognition and intercalation. According to the docking results, it was observed that the non-covalent forces that stabilize these two interaction types are mainly formed by strong hydrogen bonds. In addition, it has been determined that atacamite and paratacamite bind selectively to G and C nucleotides in B-DNA structure. DNA minor groove binders can intercalate into GC-rich regions of DNA and can act as a DNA topoisomerase-I poison (Miskovic et al. 2013). Inhibition of topoisomerases causes the DNA replication to stall, induces formation of DNA single and double strand breaks, and finally leads to disruption of genomic stability (Gaulden 1987; Mitscher 2005; Pandey et al. 2009). This hypothesis is consistent with the high frequency of chromosomal aberrations and DNA damage observed in our study.

Conclusion

It was revealed that copper oxychloride had the cytotoxic and genotoxic effects on *A. cepa* root tip cells. Moreover, molecular docking studies further explored the mechanism that polymorphs of copper oxychloride, atacamite and paratacamite, induced DNA damage by DNA minor groove recognition and intercalation into GC-rich regions of DNA. However, further research should be carried out to explore the cyto-genotoxic mechanisms of copper oxychloride in detail.

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Availability of data and materials All additional data and material will be available on demand.

Author contribution R.L. conceived the idea and supervised the research. E.S.I., C.S., and I.H.C. performed the experiments. R.L. and M.M.A. did the data analysis and wrote the manuscript.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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