The detrimental effects and mutagenical potential of Schiff base sulfadiazine derivative engineered particles on *Allium cepa* (L)

Essam Ahmed Abdel-Azeem

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt
essam_1616@hotmail.com

Abstract: The objective of this work carried to evaluate the effects of two doses (50 and 100 mg) of Schiff base sulfadiazine derivative (SDD) engineered particles (EPs), at time intervals 3, 6, and 12 (hrs), on the contents of the genetic material (DNA & RNA), soluble proteins as well as cell division and chromosomal behaviour of root meristems of *Allium cepa* (onion bulbs) as a tested material. Scan Electron Microscope (SEM) studies revealed that ovoid-to rectangular-shaped SDD were present as clusters with an average particle size of 696 ± 17.5 nm. The obtained data showed that the contents of nucleic acids and total soluble proteins in root meristems of *Allium cepa* were found to be decreased by increasing both the concentration and time of exposure. Regarding the effect of SDD on the mitotic index (MI) and chromosome instability of onion root tips, the results showed significant reductions in MI accompanied by high significantly increased in the total percent of chromosomal abnormalities. Different types of chromosomal aberrations were induced following the exposure to the engineered particles of SDD such as disturbed chromosomes, chromosomal stickiness, laggards, chromosomal fragmentation and diagonal (disorientation of spindle fibers). Our investigation suggests that plant cells as an important marker of the ecosystems need to be included when evaluating the overall toxicological impact of the engineered particles in the environment.
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Keywords: Schiff base sulfadiazine derivative (SDD); *Allium cepa* root meristems; DNA; RNA; soluble proteins; mitosis; chromosomal aberrations

1. Introduction

Our understanding of how engineered particles (EPs) may effect on human, non-human biota and natural ecosystems are yet thoroughly assessed. To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds is a matter of urgency. The Schiff bases constitute one of the most active class of the compounds possessing diversified biological activity such as anticancer[1], antibacterial[2], antifungal[3], analgesic and anti-inflammatory[4].

Schiff bases are used as starting material for the synthesis of various bioactive hetrocyclic compounds. One of the important role of Schiff base is an intermediate in the biologically important transamination reaction[5]. The Schiff bases showed greater activity than their metal complexes; the metal complexes exhibited differential effects on the bacterial strains dependent on the molecular structure of the compound, the solvent used and the bacterial strains[6,7]. Zinc sulfadiazine products were more effective against Gram positive and Gram negative bacteria as well as fungi. These compounds displayed a potential value in wound healing. The toxicity of zinc sulfadiazine derivative was much lower than that of silver sulfadiazine[8].

Miller et al.[9] stated that silver-contained sulfadiazine compounds have applied as standard topical therapy for patients with partial-thickness burns. They concluded that use of silver sulfadiazine(SSD) decrease mortality, prevent infection, lowered cytotoxicity and improve wound healing in human.

Chen et al.[10] concluded that engineered sulfadiazine compounds in combination with silver metal ions in forming of cream can be used on second degree burn wound and can decrease the risk of wound infection and accelerate wound healing. *In vitro* release studies, it has been stated that the releasing of engineered silver sulfadiazine ESSD nano-suspension of average size (367.85nm) was faster as compared to nano-gel owing to the influence of the gel matrix on SSD releasing[11]. The same authors reported that the inhibitory efficiency of silver sulfadiazine nano-suspension was a good against *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*. *In vivo* studies revealed that a nano-gel containing 0.5% SSD was more effective in wound healing compared to 0.5% and 1.0% marketed cream[12]. The microbicidal effects involve both altering the function of the cell membrane and linking to the cell's DNA, disrupting cell reproduction[13].

Recently, silver sulfadiazine (SSD) contained nano-fiber scaffolds lead to enhanced regeneration of burn-wounds owing to its releasing was able to prevent the growth of a wide array bacteria and accelerate the wound healing minimizing the risk of infection[14]. A

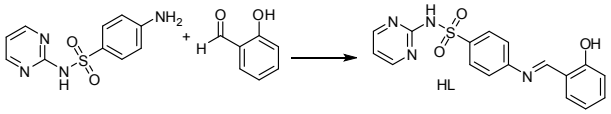
successful preparation of engineered nanoparticles of Schiff base was carried out by many authors, an antimicrobial activity of these modified particles was inspected also[15]. Thus, this investigation aims to evaluate the expected effects of the Schiff base sulfadiazine derivative engineered particles (SDDEPs) on nucleic acids, total soluble proteins, mitotic division and chromosomal behaviour in *Allium cepa* root tip cells which has been used for clastogenicity studies[16] and as recommended by the International Programme on Chemical Safety (IPCS,WHO) and the United Nations Environmental Programme (UNEP) as a suitable standard assay for the chemical screening and genotoxicity of environmental substances[17].

2. Materials and Methods

2.1. Preparation of Schiff base sulfadiazine derivative (SDD) engineered particles

Schiff base sulfadiazine derivative (SDD) engineered particles were supplied from Department of Chemistry, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt and that synthesized by adding (1.2219g, 10 mmol) of Salicylaldehyde, in 50ml absolute ethanol drop-wise with stirring to Sulfadiazine (2.50g, 10 mmol) in 50ml absolute ethanol. The mixture was heated to reflux for 6 hours, during which the color of the solution changes to Yellow. The formed yellow solid product (Fig. 1) was left to coagulate, then filtered off and recrystallized from absolute ethanol. The yield was 2.457g (69.6%), its melting point was 259^{o}C[18]. Schiff base resulting from reaction between salicylaldehde and sulfadiazine, has been investigated under Scan Electron Microscope(SEM) and tested in the present work (Fig. 2) and (scheme 1).

Sulfadiazine + Salicylaldehyde → Schiff base



Scheme 1:Synthesis of Schiff bases derived from Sulfadiazine.

Figure 1. The formed yellow solid product of modified Schiff base particles.

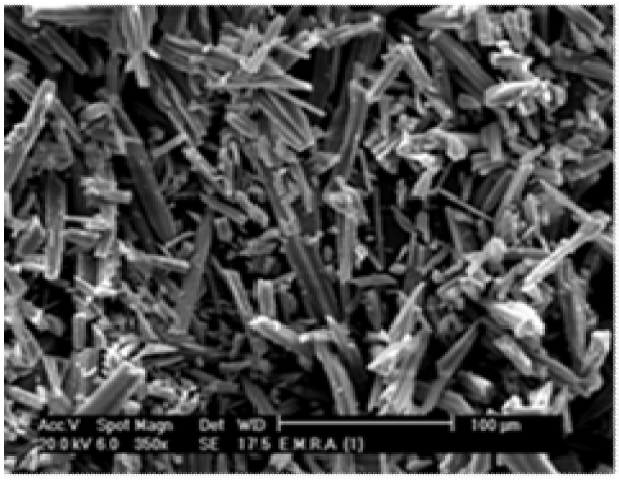


Figure 2. Scan Electron Microscope (SEM) of sulfadiazine derivative engineered particles (696nm ± 17.5) as ovoid to rectangular-shaped clusters.

2.2. Bulbs of *Allium cepa* as a tested plant

Bulbs of *Allium cepa* L. (var. Giza 6) were obtained from the National Institute of Agriculture, Giza, Cairo, Egypt. Five clean and healthy bulbs of *Allium cepa* L. (2n=16) were taken for each treatment as well as the control. The outer brown dry scales were removed and then the bulbs were germinated in de-ionized water at room temperature range (20-25^{o}C). When the roots reached a length of about 1.5 cm, they were treated with 10ml of (50 and 100 mg SDD/100ml distilled water) for the time interval of 3, 6, and 12 hrs. For control experiments, distilled water was used for the same time periods.

2.3. Molecular analysis

2.3.1. Nucleic acids determination

Extraction of nucleic acids (DNA & RNA) was carried out according to method of Schneider technique (1945)[21] where 0.5 gm powdered samples were homogenized in 10% perchloric acid and extracted in a gradient pH series of perchloric acid. Estimation of total DNA was done using Diphenylamine reaction according to method of Burton[22] (1956) and the optical density was measured at 595 nm. whereas total RNA was determined using Orcinol reaction according to method of Schneider (1957)[23] and the optical density at 660 nm.

2.3.2.Protein determination

Estimation of total soluble proteins was determined according to Bradford[24], where 0.1 gm of fresh root tissues was powdered in liquid nitrogen, extracted by 1ml 80% ethanol, precipitated for 15 min at 4000 rpm and dissolved in 1ml phosphate buffer (pH 7.0). Optical density was read at 595 nm.

2.4.Cytological studies

The treated root tips of length about 2 cm were detached then fixed in Carnoy's fixative [acetic acid : ethanol (1:3 v/v)] for 24 hrs, then hydrolyzed in 1 N HCL at 60ºC for 6-8 min. After hydrolysis, the roots were washed with de-ionized water several times then stained using Feulgen stain squash technique. After complete staining 50-60 min, the dark stained meristems were then transferred to clean slides and squashed in one drop of acetic acid (45%). At least, five temporary slides were prepared for each treatment as well as the control. Semi-permanent slide was prepared by putting one drop of 1:1 (Glycerol : 45% Acetic acid) around glass covering slide then keeping in refrigerator until nearly examination. The mitotic index (MI) was calculated for each treatment and related control as the ratio of the number of dividing cells to the total number of examined cells multiplied by 100[19]. Number and types of chromosomal aberrations were recorded in all treatments and the control. Total percent of chromosomal abnormalities was calculated as the number of total aberrant cells to the number of dividing cells (normal and abnormal) multiplied by 100[20]. Microphotographs were taken from selected semi-permanent slides.

2.5. Statistical analysis of data

Means and standard deviations (±) were derived from five repeated samples of each of the experimental group. For the determination of the significance of the difference between the mean, t-test was carried out only, for cytological observations, within the two levels (0.05 and 0.1) of significance.

2.6. Microphotographs

Microphotographs of chromosomal instability were taken from selected semi-permanent slides using Digital Camera Solution Disk (Toup Camera of 2 Mega Pixel, Ver.3.2).

3. Results and Discussion

3.1. Molecular analysis

The results of the present investigation clearly revealed that exposure of root meristems of *Allium cepa* to high dose (100 mg) of SDD led to reduction in both nucleic acids and soluble proteins contents as compared with their respective control, as can be seen in table (1) and Fig. (3). This reduction was dose and time dependent. Regarding DNA, the recorded maximum contents was 1.36 mg/gm D.W. after 3 hrs of exposure to the lowest dose (50 mg) of engineered particles of SDD whereas the minimum value (0.33 mg/gm D.W.) was recorded after 12 hours exposure to the highest dose. The effects of the tested engineered particles on RNA contents were similar to that of DNA where the decreases, also, were dose and time dependent (Table 1 and Fig. 4).

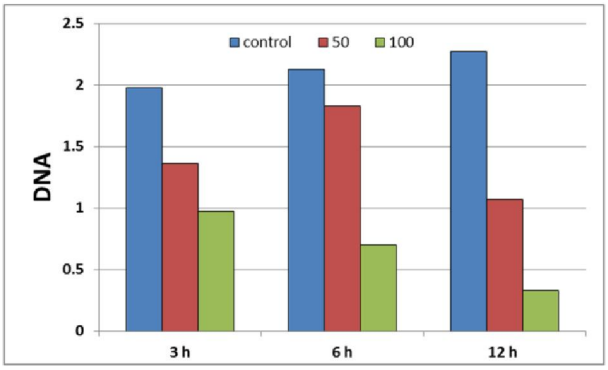


Figure 3. Effect of SDD on DNA contents (mg/g D.W.) of *Allium cepa* root tips.

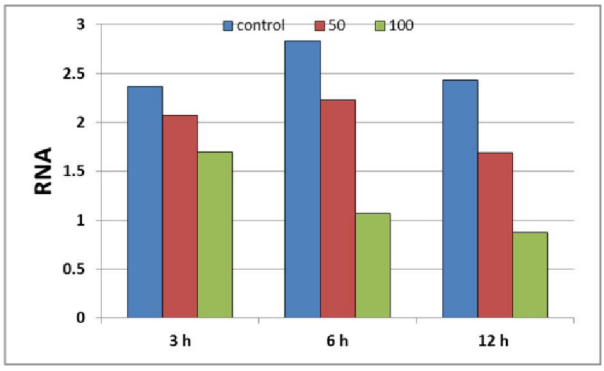


Figure 4. Effect of SDD on RNA contents (mg/g D.W.) of *Allium cepa* root tips.

As regard to the effect of SDDs on the contents of total soluble proteins, we documented greater reductions in all treatments. This depression in total soluble protein content was also dose and time dependent. The maximum value was 25.66 mg/gm F.W. after 3 hours of exposure to the lowest dose (50mg) while the minimum content (9.31 mg/gm F.W.) was recorded after 12 hrs exposure to the highest dose of SDD (Table 1 and Fig. 5). This investigation shows the ability of engineered sulfadiazine derivative compound (SDD) to induce a progressive decrease in nucleic acids as well as the total soluble protein contents. In this respect, It has been reported that silver engineered particles (SEPs) have the ability to damage the genetic material, since the engineered nanoparticles ENPs are able to cross cell membranes and reach the cellular nucleus causing DNA damage[25].

AshaRani et al.[26] showed that the exposure of human cell to silver engineered particles caused damage to DNA which was in a dose-dependent manner. Transmission Electron Microscope (TEM) analysis indicated the presence of Ag NPs inside the mitochondria and nucleus ,implicating their direct involvement in the mitochondrial toxicity and DNA

damage[26]. Silver engineered nanoparticles (SENPs) are believed to alter the membrane structure by attaching to the sulfur containing proteins of the cell membrane thereby damaging the cell membrane as well as the DNA of the bacterial cell[27]. Some investigators demonstrated that silver engineered nanoparticles produce reactive oxygen species (ROS) which can interfere with cellular metabolism causing damage DNA and proteins[28]. A number of researchers have shown that silver nanoparticles can destroy the ability of DNA to replicate or can damage DNA and death of the cells[29,30].

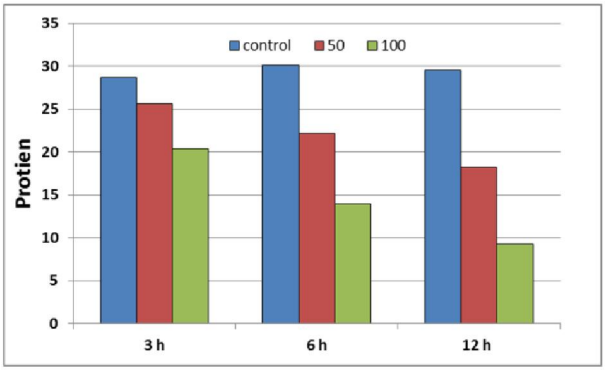


Figure 5. Effect of SDD on soluble protein contents (mg/g F.W.) of *Allium cepa* root meristems.

3.2. Cytological observations

3.2.1. Mitotic Index (MI)

The obtained results recorded in table (2) and represented in Fig. (6) showed drastic decrease in mitotic index of root tip cells of onion bulbs as compared to the control (untreated). The depression in the rate of mitotic cell division was also dose and time dependent. In general, these observed decreases in (MI) were highly significant after exposure to the highest dose of the tested engineered particles. The minimum percent of mitotic index (4.10%) was recorded after 12 hours exposure to the highest concentration as compared to the untreated onion root tips (14.0%). Our results were consistent with those observed by Pulate et al.[31], Kumari et al.[32] and Patlolla et al.[33]. The reduction in MI clearly indicates the mitodepressive and cytotoxic effects of engineered nanoparticles on meristems of *Allium cepa*. It has been suggested that the cytotoxicity level can be determined by the decreased rate of MI[34]. It has been showed that, *in vitro*, cytotoxicity, genotoxicity and inflammation were related to the nanometer-size particles[35], or to the concentration of dose exposure[32]. The reduction of mitotic index may be attributed to the detrimental effect of engineered particles on the microtubules[36], or mitochondria[26]. The mitodepression of cell cycle may be due to a slower progressive of cells from S (DNA synthesis) phase and second gap phase (G2) period to M (mitotic cell cycle) phase[33,37]. This might have been achieved by the inhibition of DNA synthesis at S-phase [38], and this is consistent with our investigation which concluded that both variation in time intervals and dose concentration may have an inhibitory effect on mitotic cell cycle. Additionally, mitodepressive and cytotoxic effect induced by engineered silver nanoparticles (ESNPs) is dependent on surface coating and examined cell type[39].

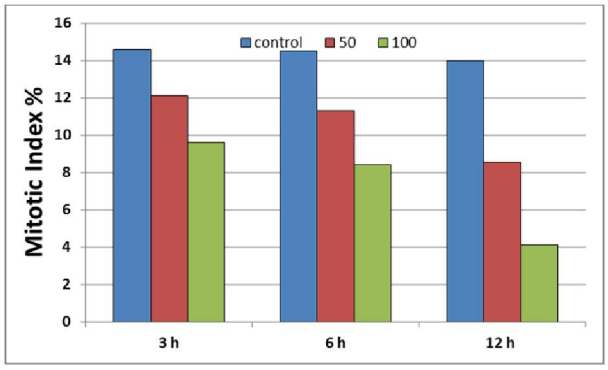


Figure 6. Mitotic index (MI) percentage following exposure of *Allium cepa* root tips to SDD for different time intervals

3.2.2. Frequency of mitotic phases

The results in table (2) and Figs. (7-9), clearly showed an obvious arrest of the cells at metaphase stage on response of both prophase and anatelophases. Root tip cells of *Allium cepa* exposed to the highest dose of sulfadiazine derivative engineered particles(SDD EPs) for 12 hours showed maximum value of arresting metaphases (49.82%) whereas the minimum proportion was 33.31% after 3 hours exposure to the lowest dose (50mg) as compared to the control (31.18%). A similar results of arresting metaphases during mitotic division was reported by some investigators[32,40,41]. Arresting cells at metaphases could be due to either disassembled of the spindle[42] or to inactivation of M-phase Promoting Factor (MPF) complex which is known to stimulates a number of processes essential for nuclear and cell division[41]. It has also been reported that dissociation of MPF complex to cyclin and cdc2 subunits[43] or inhibition of the genes that controlled its activation[44] may resulted in disfunctional of nuclear localization signal (NLS)[45].

Many published reports stated that metaphase arrest can be achieved either to tightly binding of the drug to microtubules[46] or to spindle disrupting producing a strong signal that greatly prolongs metaphase stage[47].

Table 1. DNA, RNA contents (mg/gm D.W) and total soluble protein contents (mg/gm F.W) after exposure to SDD of two doses (50 &100 mg) for the time interval of 3, 6, 12 hours.

Time (hrs)	Conceration Dose (mg)	DNA (mg/gm D.W.)	RNA (mg/gm D.W.)	Total soluble protein (mg/gm F.W.)
3	Control	1.98±0.27	2.37±0.60	28.73±0.66
	50	1.36±0.78	2.07±1.18	25.66±1.12
	100	0.97±0.34	1.70±0.85	20.38±0.46
6	Control	2.13±0.47	2.83±0.54	30.12±0.81
	50	1.33±0.43	2.23±o.88	22.17±0.71
	100	0.70±0.66	1.07±0.45	13.97±0.45
12	Control	2.27±1.02	2.43±0.74	29.54±0.67
	50	1.07±0.67	1.69±0.93	18.23±0.93
	100	0.33±0.83	0.87±1.0	9.31±1.12

Mean value (±) standard deviation

Table 2. Mitotic index and percentage of mitotic phases in *Allium cepa* meristems after exposure to SDD of two doses (50 & 100 mg) for time intervals of 3, 6 and 12 hours.

Time (hrs)	Concentration Dose (mg)	Total examined Cells	Total Dividing cells	Mitotic Index (%) ±SD	Mitotic Phases		
					Prophase %	Metaphase %	Ana-Telophase %
3	Control	5057	737	14.6±0.66	36.32	31.18	32.50
	50	5049	612	12.12±0.69*	33.31	33.13	33.56
	100	4979	478	9.60±0.47**	30.12	37.16	32.72
6	Control	4938	718	14.5±0.84	34.60	30.76	34.64
	50	5067	573	11.3±0.49*	34.40	36.16	29.44
	100	5000	421	8.42±1.08**	31.17	41.33	27.5
12	Control	5126	719	14.0±0.93	36.26	28.22	35.58
	50	4871	416	8.54±0.57**	30.88	40.17	28.95
	100	5066	208	4.10±0.64**	26.15	49.82	24.03

*Significant to control at 0.05 level
**Highly significant compared to control at 0.01 level

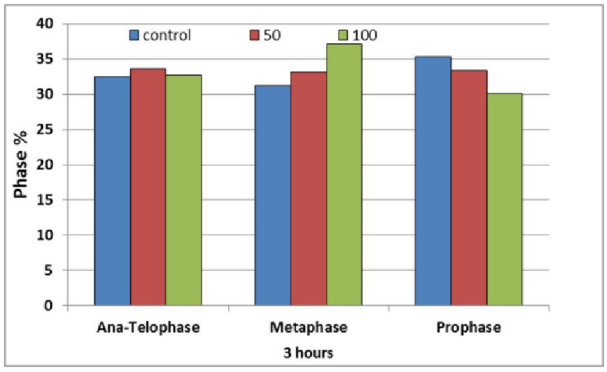


Figure 7. Frequency of mitotic phases induce in *Allium cepa* root meristems treated with different doses of SDD for 3 hours.

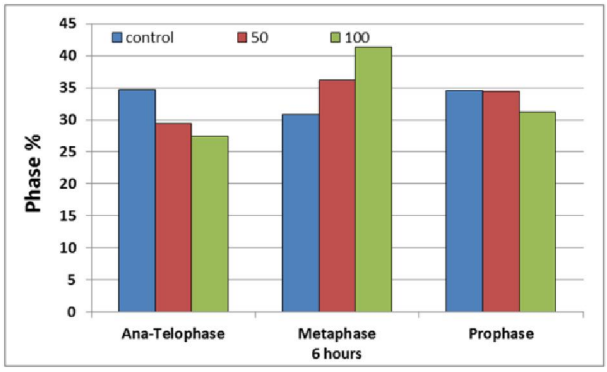


Figure 8. Frequency of mitotic phases induced in *Allium cepa* root tips treated with different doses of SDD for 6 hours.

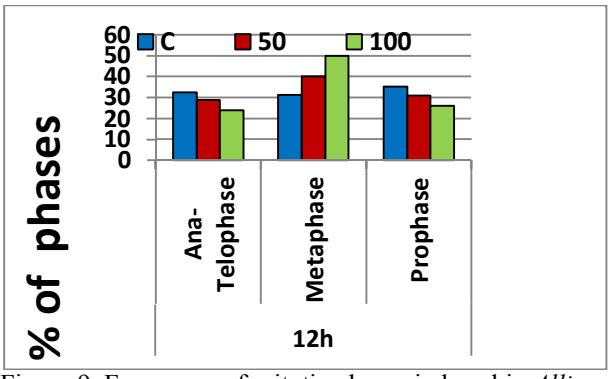


Figure 9. Frequency of mitotic phases induced in *Allium cepa* root tips treated with different doses of SDD for 12 hours.

3.2.3. Types and total percentage of mitotic chromosomal aberrations

Regarding the effect of SDD engineered particles on the total percentage of chromosomal abnormalities, the presented results in table (3) and Fig. (10) clearly showed that root-tip cells of *Allium cepa* are susceptible to exposure to the highest dose (100mg) for the different time intervals. The maximum value (49.04%) was recorded after 12 hours exposure to the tested drug while the minimum proportion (15.85%) was observed following exposure to the lowest dose for 3 hours. The obtained results clearly revealed that all treatments were concentration and time dependent. The obtained data were statistically high significant as compared to their relative controls which showed no aberrant cells following exposures to the different treatments. Our results generally were in similar with those observed by many investigators[29-31,38].

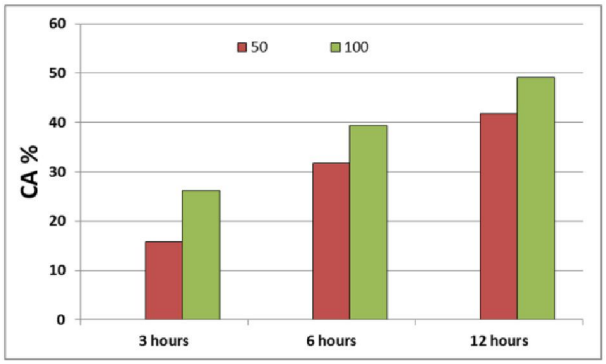


Figure 10. Total percentages of mitotic chromosomal abnormalities (CA) induced in root meristems of *Allium cepa* following exposure to the SDD for different time intervals.

Sulfadiazine derivative(SDD) engineered particles treated cells exhibited different types of chromosomal aberrations (CA). Types and frequencies of mitotic abnormalities are given in table (3) and Figs. (11-18). These findings are in agreement with those observed after treatments of *Allium cepa* and *Vicia faba* root tip cells with engineered silver suspension nanoparticles [29-31]. It could be noticed that the majority of aberrant cells were observed at metaphase as well as ana-telophase stages. Arresting chromosomes at metaphase stage resulting in failure of properly chromosome segregation to anaphase stage. This may be a consequence of defeats in its regulatory mechanisms leading to induction of mitotic instability[48].

The major clastogenic abnormalities were chromosomal disturbance at prophase (Fig. 15), metaphase (Fig. 11), anaphase (Figs. 13 & 18) and chromosomal stickiness (Figs. 14, 15 & 17). The frequencies of the first type (disturbances) was decreased as the dose and the time of treatment increased where it reached the maximum value (40.14%) after 3 hrs treatment with the lowest dose while the frequencies of the other type (sticky chromosomes) was increased especially after treatment with the higher dose for long periods where extremely high proportion (49.55%) was recorded. Lagging chromosome (Fig. 17), breaks (Fig. 16) and bridges (Figs. 13, 16 & 18) were also observed with minimum proportions, following all treatments whereas cells with micro-nuclei (Mn) were only shown after exposure to the highest tested dose for 12 hrs.

The Induction of disturbed chromosomes at ana-telophases primarily reflects its effect on mitotic spindles, altering the orientation of chromosomes during cell cycle resulting in diagonal (Fig. 12). Impairment of mitotic spindle function is probably due to the interaction of engineered particles (EPs) with tubulin –SH group[49].

Stickiness of chromosomes is one of the frequent observations in all treatments with the colloidal (SDD) engineered particles. This major clastogenic aberrations was induced mainly by intermixing of chromatin fibers which leads to subchromatid connection bridges between chromosomes[50]. The induction of chromosomal fragments (breaks) by SDD engineered particles indicates the clastogenic potential of the tested drug, which may lead to a loss of genetic material indicating a mutagenicity of the inducer[51]. Bridges (single, double or multi-bridges) also were noticed in treated root meristems of *Allium cepa*. Chromosomal bridges may be the results of reunion of broken chromosomes ends[52] or may be due to chromosomal stickiness and subsequent failure of free anaphase separation or can be attributed to unequal translocation and/or inversion of chromosome segments[53].

Micronuclei were also observed with very low frequency after only exposure to the higher dose of

SDD for 12 hours. Similar observations were reported in *Allium cepa* root tips exposed to ZnO engineered particles[54] and *Vicia faba* meristems treated with engineered silver nano-particle[33]. Micronuclei is the result of acentric fragments or laggards being excluded from the nucleus proper during mitotic cell division[55] and may lead to a loss of genetic material[56]. Observation of diagonal ana-telophase (Fig. 12) was another type of CA induced by SDD at 12 hours indicate inactivation of ATPase by the interaction of endosulfan on spindle protein[57].

Table 3. Percentages and different types chromosomal abnormalities induced in *Allium cepa* root tips treated with the SDD for different durations of 3, 6 and 12 hours.

Time (hrs)	Concentration Dose (mg)	Total Dividing cells	Total Aberrant cells	Total Percent of aberrant cells	Types and percentages of abnormalities						
					Disturb	Sticky	Lagging	Breaks	Diagonal	Bridge	Micronuclei
3	Control	737	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	50	612	97	15.85±0.75**	40.14	28.17	20.55	0.0	0.0	11.14	0.0
	100	478	125	26.15±0.11**	37. 77	32.43	22.85	0.0	0.0	9.95	0.0
6	Control	718	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	50	573	182	31.76±0.39**	28.12	39. 67	25.85	0.0	0.0	6.36	0.0
	100	421	166	39.42±0.65**	23. 88	40 .12	17 .36	6.98	6. 56	5.10	0.0
12	Control	719	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	50	416	174	41,83±0.96**	22.65	41.94	19 .34	7.10	5.97	3.00	0.0
	100	208	102	49.04±1.02	18.54	49.40	5.32	12.55	8.42	5.32	0.45

** Highly significant compared to control at 0.01 level

4. Conclusion

This work concluded that Schiff base derivative (SDD) engineered particles (EPs) Have a multiple mechanism of action that dose and time dependent. A possible mechanism of action of Schiff base modified particles is their ability to cross both the cellular and nuclear membranes, altering their structures and induced ROS which can interfere with the cellular metabolism resulting in DNA and proteins damage. The cytological observations showed an obvious reduction in mitotic cell division after exposure of root meristems to the highest dose of SDD for 12 hrs. The reduction in the rate of cell division may be achieved to the effect of the tested drug on DNA replication resulting in arresting in the mitotic cell cycle and blocking in chromosomal segregation as well as inducing different types of chromosomal anomalies. However, some investigators suggested that sulfadiazine (SD) was effectively when loaded with Ag in nano-composite structures for wound healing**[58]**, showed no cytotoxicity and enhanced the drug efficiency of the engineered particles**[59]**, as well as significantly increased the rate of wound healing than silver sulfadiazine without engineered nano-form**[60]**. Finally, a further investigation is needed to determine how to safety design, use, and dispose of SDD without creating a new risk to humans and the environment. Plants, which was considered as important component of the ecosystems, need to be included when evaluating the overall toxicological impact of the engineered nanoparticles(ENPs) in the environment.

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Corresponding Author:
Dr. Essam Ahmed Abdel-Azeem
Botany and Microbiology Department
Faculty of science
Al-Azhar University, Cairo, Egypt
E-mail: essam_1616@hotmail.com

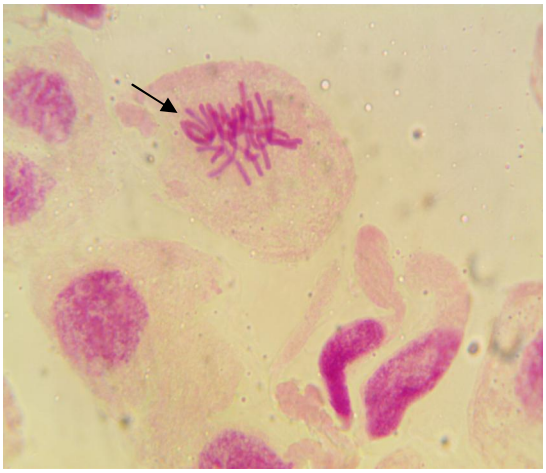

Figure 11. Disturbed metaphase.

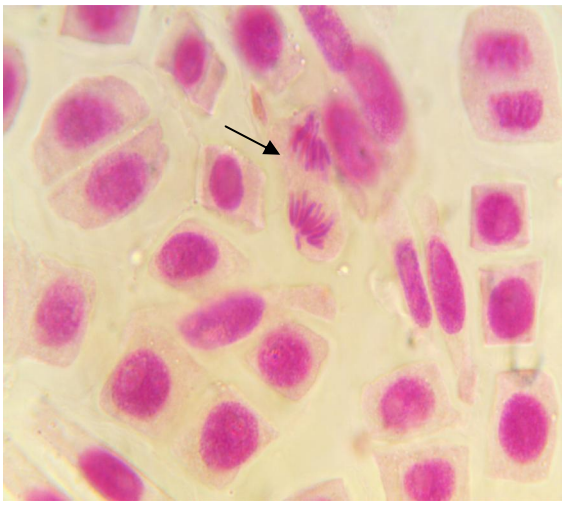

Figure 12. Diagonal at anaphase.

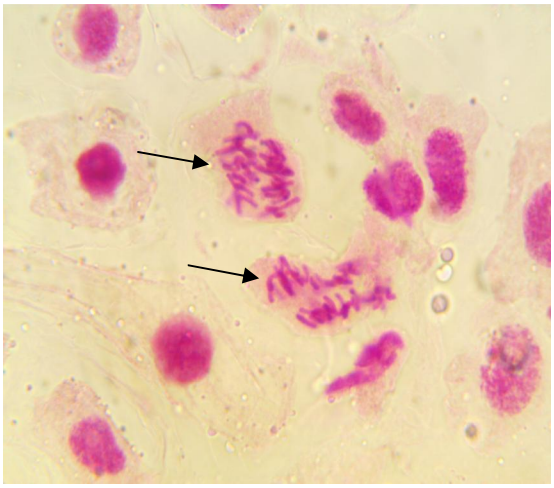

Figure 13. Disturbed anaphases with bridges

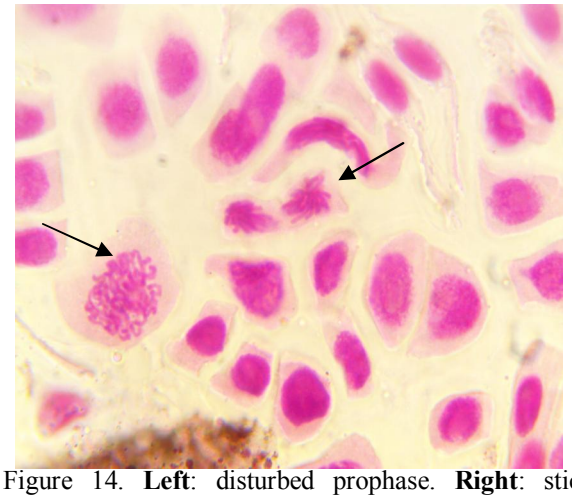

Figure 14. **Left**: disturbed prophase. **Right**: sticky metaphase.

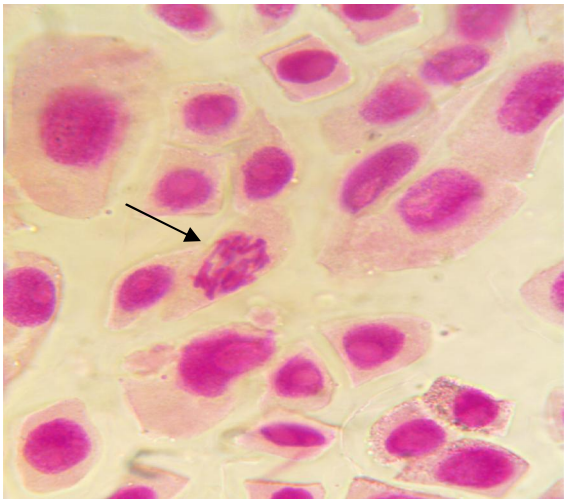

Figure 15. Sticky metaphase showing Disoriented chromosomes.

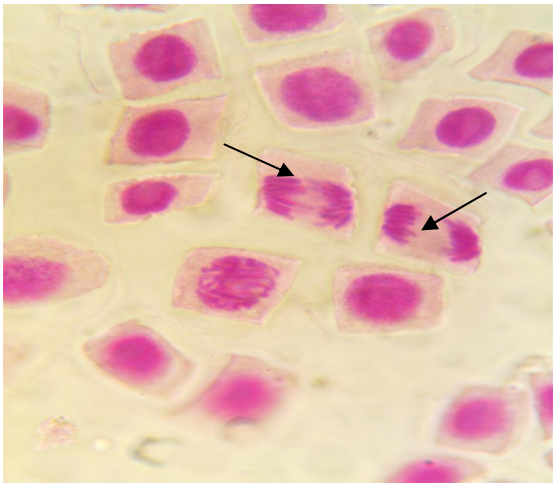

Figure 16. **Left**: chromosome bridge. **Right**: chromosome fragment

Figure 17. Sticky anaphase with lagging chromosome.

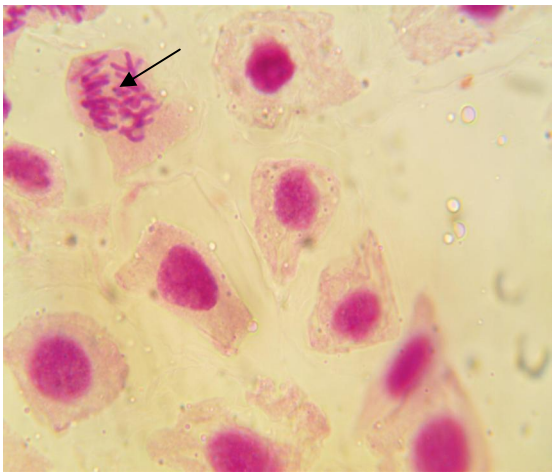

Figure 18. Disturbed anaphase with bridge.

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