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Original article

Genotoxicity and cytotoxicity potential of organoselenium compounds in human leukocytes *in vitro*

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ABSTRACT

In the current work, cytotoxicity and genotoxicity of different organoselenium compounds were examined using Trypan blue exclusion and alkaline comet assays with silver staining respectively. Leukocytes were subjected to a 3-hour incubation with organoselenium compounds at concentrations of 1, 5, 10, 25, 50, and 75 μM, or with the control vehicle (DMSO), at a temperature of 37 °C. The viability of the cells was evaluated using the Trypan blue exclusion method, while DNA damage was analyzed through the alkaline comet assay with silver staining. The exposure of leukocytes to different organoselenium compounds including i.e. (Z)-N-(pyridin-2-ylmethylene)-1-(2-((2-(1-((E)-pyridin-2-ylmethyleneamino)ethyl)phenyl)diselanyl)phenyl)ethanamine (C1), 2,2′(1Z,1′E)-(1,1′-(2,2′-diselanediylbis(2,1-phenylene))bis(ethane-1,1-diyl)) bis(azan-1-yl-1-ylidene)bis -methan-1-yl-1-ylidene) diphenol (C2), and dinaphthyl diselenide $(NapSe)_2$, At concentrations ranging from 1 to 5 μM, no significant DNA damage was observed, as indicated by the absence of a noteworthy increase in the Damage Index (DI).

Our results suggest that the organoselenium selenium compounds tested were not genotoxic and cytotoxic to human leukocytes in vitro at lower concentration. This study offers further insights into the genotoxicity profile of these organochalcogens in human leukocytes. Their genotoxicity and cytotoxicity effects at higher concentration are probably mediated through reactive oxygen species generation and their ability to catalyze thiol oxidation.

1. Introduction

Organoselenium compounds have been found to possess a range of pharmacological properties, including antioxidant, anti-inflammatory, antinociceptive, neuroprotective, hepatoprotective, and anticonvulsant effects (Ibrahim et al., 2015, 2014; Orian and Toppo, 2014; Stefanello et al., 2013). These beneficial effects have generally been at-tributed to the presence of selenium (Se), an essential micronutrient for biological functions. Selenium plays a crucial role in various physiological processes and is an essential component of living cells. Within biological systems, selenium takes the form of selenoproteins such as glutathione peroxidase, thioredoxin reductase, and selenoprotein P (Arnér and Holmgren, 2000; Kryukov et al., 2002; Saito and Takahashi, 2002). Over the past couple of decades, there has been a growing interest in the field of organoselenium chemistry and biochemistry. This interest has mainly stemmed from the utilization of diverse organoselenium compounds with roles as antioxidants, inhibitors of enzymes, agents that protect against neurodegeneration, anticancer agents, substances that combat infections, as well as inducers of cytokines and modulators of the immune response(Nogueira et al., 2004; Ibrahim et al., 2014a,b, 2012a,b; Mugesh et al., 2001). Conversely, organoselenium compounds have the potential to induce a range of adverse effects (Nogueira and Rocha,

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2010). For example, the expanding utilization of organic compounds containing selenium, which serve as significant intermediates and valuable agents in organic synthesis (Zeni et al., 2001), Could increase the chances of people being exposed to risks in their workplace (Marino et al., 2002; Zeni et al., 2006).

It has been reported that organoselenium compounds may be genotoxic or carcinogenic at high doses (Zhang et al., 2023; Huang et al., 2023), probably mediated through reactive oxygen species generation and its ability to catalyze thiol oxidation (Spallholz et al., 2001). Therefore, studies on the genotoxicity and cytotoxicity as well as the doses of organoselenium compounds that exhibit beneficial effect is critical to explore.

The comet assay, also known as single-cell gel electrophoresis, has gained significant fame as an effective, straightforward, rapid, and dependable technique for assessing DNA damage (Zhu et al., 2021; Liu et al., 2023). This assay identifies effects such as single-strand breaks in DNA, incomplete excision repair sites, and alkali labile sites. These effects are determined by analyzing the extent of DNA migration from immobilized cell nuclei, which are subjected to electrophoresis (Knöbel et al., 2007; Zeng et al., 2020).

As a result, the detection of Deoxyribonucleic acid damage in leukocytes can serve as an indicator of oxidative stress within the organism (Hu et al., 2022; Knöbel et al., 2007). On the basis of the aforementioned information, the current investigation was conducted to explore the possible genotoxicity and cytotoxicity of three organoselenium compounds in healthy human leucocytes using single cell gel electrophoresis assay (comet assay) and trypan blue respectively, as endpoint of toxicity.

2. Materials and methods

2.1. Materials

The compounds used in this work are displayed in Fig. 1. (Z)-N-(pyridin-2-ylmethylene)-1-(2-((2-(1-((E)-pyridin-2-ylmethyleneamino) ethyl)phenyl)diselanyl)phenyl)ethanamine (C1), and 2,2′-(1Z,1′E)-(1,1′-(2,2′-diselanediylbis(2,1-phenylene))bis(ethane-1and 1-diyl))bis(azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)diphenol (C2) were synthesized according to literature methods (Braga et al., 2005; Liu et al., 2005), with slight modifications. The dinaphthyl diselenide $(NapSe)_2$ was synthesized according to the method described by Kozlov and Suvorova (1961). Examination of the ^{1}HNMR and ^{13}CNMR spectra revealed that the obtained compound (with a purity of 99.9 %) displayed analytical and spectroscopic characteristics that perfectly matched its assigned structure. The compounds were dissolved in DMSO. All other chemicals were purchased from standard suppliers.

2.2. Sample preparation

Blood samples treated with heparin were collected from healthy volunteers at the Hospital of the Federal University of Santa Maria (UFSM) in Santa Maria. The donors' average age was 30 ± 12 years. The research protocol underwent a comprehensive evaluation and obtained authorization from the UFSM Committee Guidelines (0089-0-243-000.07). For the separation of samples, a method involving differential erythrocyte sedimentation with dextran was employed. Specifically, 8 mL of blood sample was mixed with two ml of dextran (100–200 kDa, 5 % w/v in Phosphate buffer Saline), followed by gentle inversion. This mixture was then kept at room temperature for forty-five minutes. Subsequently, the supernatant, devoid of erythrocytes, was transferred to another tube, then centrifuged (490*g*) for 8 min, and the supernatant was discarded.

The resulting pellet underwent a process of isotonic erythrocyte lysis with NH_4Cl. To achieve this, 1 ml of cold lysis solution (NH_4Cl, 150 mM; $NaHCO_3$, 10 mM; disodium EDTA, 1 mM, pH 7.4) was added to the pellet, followed by inversion and a 5-minute incubation at room temperature. After this, the tube was subjected to centrifugation (490*g*) for

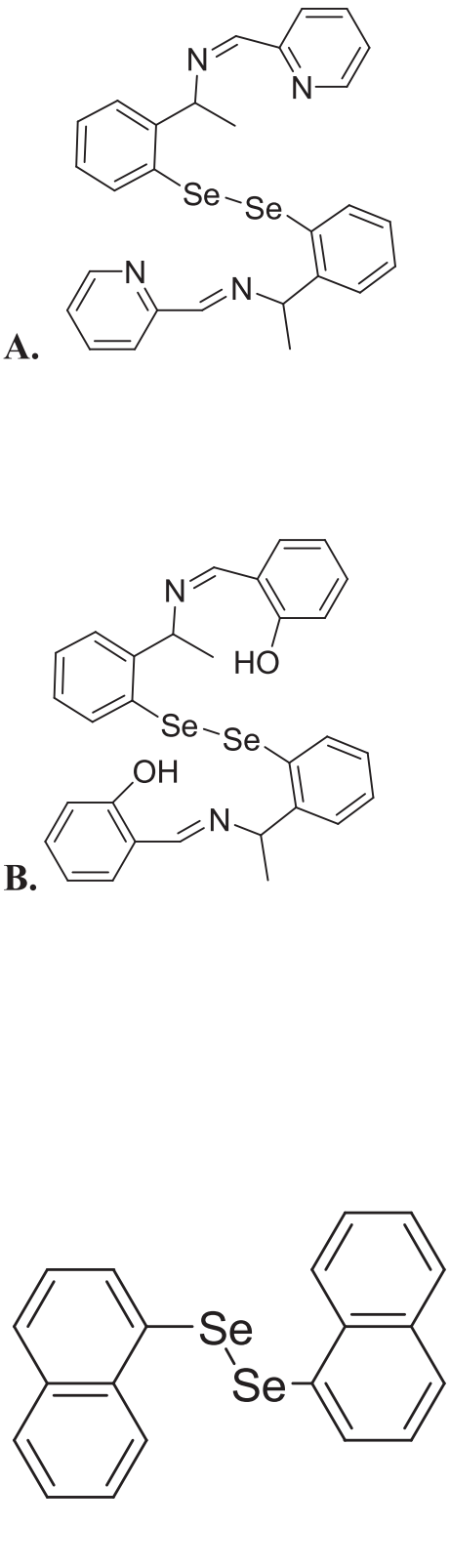

Fig. 1. (A) Chemical structure of compound A, i.e. (Z)-N-(pyridin-2-ylmethylene)-1-(2-((2-(1-((E)-pyridin-2-ylmethyleneamino)ethyl)phenyl)diselanyl) phenyl)ethanamine (B) B. i.e. 2,2′-(1Z,1′E)-(1,1′-(2,2′-diselanediylbis(2,1-phenylene))bis(ethane-1,1-diyl))bis(azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)diphenol diphenyl diselenide i.e. $(PhSe)_2$ and (C) dinapthyl diselenide i.e. $(NapSe)_2$.

2 min, the supernatant was removed by decanting, and the resulting pellet underwent two washes with 1 ml of the lysis solution.

Following the second wash, the pellet was suspended in 2 mL of Hank's buffer solution saline (HBSS)/heparin. The HBSS solution was prepared with the following constituents: KCl 5.4 mM, Na_2HPO_4 0.3 mM, KH_2PO_4 0.4 mM, $NaHCO_3$ 4.2 mM, $CaCl_2$ 1.3 mM, $MgCl_2$ 0.5 mM, $MgSO_4$ 0.6 mM, NaCl 137 mM, d-glucose 10 mM, and Tris–HCl 10 mM, adjusted to pH 7.4. The suspension was adjusted to a concentration of 2 × 106 leukocytes/mL using a buffer salt solution, HBSS.

2.3. Compounds exposure

Leukocytes were subjected to a range of different concentrations (5–75 μM) of C1, C2 and $(NapSe)_2$ or an equivalent volume of DMSO for a duration of 3 h at 37 °C (pre-incubation). The concentrations used were leveraging findings from prior research within our research group demonstrating that mostly the toxicity start from above 5 μM which could be its narrow margin between antioxidant activity and potential toxicity (Tian et al., 2022).

2.4. Cell viability analysis

The techniques were carried out in accordance with the procedure outlined by Mischell and Shiingi (1980), with minor adjustments. Following exposure to either DMSO or various concentrations of the organoselenium compounds, a treated cell suspension of 50 μL was added with 20 μL of HBBS and 10 μl of a 0.4 % Trypan blue solution. To assess cell viability, the suspension was examined under a microscope (at 400× magnification) using a hemocytometer. The calculation of cell viability involved determining the ratio of living cells (those unstained by Trypan blue) to the total cell count, afterward, multiplied by a factor of 100.

2.5. Comet assay

Exposing peripheral blood leukocytes to a 3-hour incubation with various concentrations of the organic chalcogens. (1, 5, 10, 25, 50, and 75 μM). Following this incubation, the leukocytes (15 μL) were mixed with low-melting point agarose (0.75 % w/v, 90 μL) and positioned on a microscope slide that had been previously coated with normal melting point agarose (1.0 % w/v). Once placed, a coverslip was added, and the slides were promptly cooled on ice for 5 min. After the agarose had set, the coverslips were taken off, and the slides were placed into a lysis solution. This solution comprised 2.5 M NaCl, 100 mM EDTA, and 10 mM Tris, with a pH range of 10.0 to 10.5. Furthermore, the lysis solution included 1 % Triton X-100 and 10 % DMSO. The slides stayed frozen in this solution at 4 °C, shielded from light, for about 14 h. Afterward, they were transferred to a freshly prepared alkaline buffer (containing 300 mM NaOH and 1 mM EDTA, pH > 13.5) for a 20-minute period to enable DNA unwinding.

Electrophoresis was conducted in the same buffer for 20 min at 300 mA and 25 V. Each phase was performed via indirect yellow light to curtail possible light-induced effects.

After the electrophoresis step, the slides were treated with a 400 mM Tris solution for neutralization (pH 7.5), subsequently followed by three washes with distilled water. The slides were then left to air dry overnight at room temperature. Once they were dry, they were briefly soaked in distilled water for 3 min. Then, they were fixed for 10 min in a solution containing 15 % trichloroacetic acid, 5 % zinc sulfate, and 5 % glycerol. After rinsing with water and allowing them to dry for 5 h, the slides were soaked in water again for 3 min. Later, they were stained in a dark environment using a solution made of sodium carbonate, ammonium nitrate, silver nitrate, tungsto silicic acid, and formaldehyde.During staining, the slides were continuously agitated at 37 °C for a duration of 25 min. After staining, the slides were submerged in a stop solution (1 % acetic acid), rinsed once again, and promptly labeled for subsequent analysis.

2.6. Damage evaluation

A total of one hundred cells were randomly selected from each sample and evaluated visually, categorizing them into five classes based on the intensity of the tail (ranging from 0 for undamaged to 4 for maximum damage). As such, the severity of damage for each sample could vary from 0 (indicating complete lack of damage − 100 cells × 0) to 4 (representing maximum damage − 100 cells × 4). The computation of the damage index (DI) took into consideration both the extent of migration and the quantity of DNA within the tail.

To assess the overall damage incurred by the compounds and to make a comparison against negative controls (DMSO), the DI was determined using the following formula:

$$DI = n1 + 2n2 + 3n3 + 4n4$$

where n1 signifies the count of cells exhibiting damage level 1, n2 corresponds to cells with damage level 2, n3 stands for cells with damage level 3, and n4 denotes cells with damage level 4. The evaluation of the slides was conducted in a blinded manner, involving a minimum of two distinct individuals.

3. Statistical analysis

It comprises the analysis of variance (ANOVA), supplemented by Duncan's test as applicable. The analysis of the comet assay encompassed repeated measures ANOVA/MANOVA, while the damage index was scrutinized through one-way ANOVA. Cell viability data underwent analysis via one-way analysis of variance (ANOVA), followed by Duncan's tests for post hoc comparison. The data were represented as mean ± SEM, originating from four separate experiments. Significance was determined at levels of $p < 0.05$ and $p < 0.01$.

4. Results

Cellular viability was assessed by Trypan blue exclusion as an index of cytotoxicity. The control vehicle DMSO did not lead to a noteworthy decrease in cell viability.H_2O_2 (4 mM) used as positive control, significant decreased cellular viability when compared to DMSO control ($p < 0.05$). Exposure of human leucocytes to C1 (10–75 μM), C2 (5–75 μM) and $(NapSe)_2$ (10–75 μM) for 3 h caused a significant decrease in cell viability when compared to DMSO control ($p < 0.05$) (Table 1). However, this effect was not concentration dependent.

4.1. DNA damage by organoselenium compounds in blood leukocytes

As presented in In Table 2, it was observed that subjecting leukocytes to H_2O_2 (4 mM) led to a noteworthy rise in DNA migration compared to the control ($p < 0.05$). In a similar manner, the exposure of leukocytes to C1 (ranging from 5 to 100 μM), C2 (ranging from 10 to 100 μM), and $(NapSe)_2$ (ranging from 5 to 100 μM) brought about a considerable elevation in the DNA Damage Index (DI) in comparison to the control ($p < 0.05$). However, this effect did not show a direct correlation with concentration. It's important to emphasize that the genotoxic impact of these organoselenium compounds was linked to a high occurrence of cells displaying DNA damage levels ranging from 3 to 4 (Table 2).

5. Discussion

Over the past decade, there has been a growing fascination with numerous organoselenium compounds, driven in part by their potential as chemopreventive agents and their promising role in cancer prevention. Some of these compounds even find application in the synthesis of pharmacologically active drugs (Mugesh et al., 2001). Similar to certain

Table 1
Effect of organoselenium compounds on the cell viability in human leukocytes in vitro.

Treatment	Concetration (μM)	Cell viability (%)
DMSO (control)	0	92.66 ± 1.85
Compound 1	1	93.33 ± 0.88
	5	84.33 ± 0.33
	10	77.33 ± 3.75**
	25	34.33 ± 7.79**
	50	20.66 ± 0.33**
	75	20.66 ± 0.88**
Compound 2	1	92.66 ± 1.20
	5	71.00 ± 4.61**
	10	80.66 ± 4.33*
	25	82.00 ± 2.30*
	50	75.66 ± 2.02**
	75	60.33 ± 4.33**
Compound 3	1	87.66 ± 3.33
	5	88.33 ± 0.88
	10	68.66 ± 4.70**
	25	17.00 ± 3.46**
	50	9.00 ± 1.73**
	75	8.33 ± 2.60**

Table 1. Effect of organoselenium compounds on the cell viability in human leukocytes in vitro.Cell viability was determined after 3 h of incubation and calculated as the number of living cells divided by the total number of cells multiplied by 100.
* Denoted $p < 0.05$ as compared to control (DMSO) value.
** Denoted $p < 0.01$ as compared to control (DMSO) value.

other trace elements, selenium follows a bimodal pattern, where its beneficial attributes are realized within a specific range of daily intake, below which its essential functions are compromised, and above which it becomes toxic (Nogueira et al., 2004; Ibrahim et al., 2014a,b, 2012a,b; Mugesh et al., 2001).

When assessing a synthetic compound effectiveness as a drug, finding the right balance between its therapeutic benefits and potential toxic effects is crucial.

In this regard among the tested compounds $(NapSe)_2$, synthesized in our laboratory as a potent antioxidant and have hepato, renal and neuro protective activities (Ibrahim et al, 2012a,b), and,C1, C2, have neuroprotective activities (Lopes et al., 2012).

While prior research has presented compelling evidence for the neuroprotective properties of $(NapSe)_2$, its potential application in clinical models demands thorough investigation, including an indepth assessment of its toxicity and potential adverse effects. Numerous investigations have underscored that DNA damage or genotoxicity holds paramount significance in initiating, apoptosis, mitotic death, and cellular transformations that contribute to carcinogenesis (Riley, 1994). The forms of damage contributing to genotoxic effects encompass oxidative modifications to DNA bases and sugars leading to mutations, single and double strand breaks, and more intricate alterations like deletions, translocations, and fusions (Kunwar et al., 2010). Thus, a pivotal criterion that must be thoroughly evaluated before classifying a compound as toxic or non-toxic revolves around its genotoxic potential.

Presently, the comet assay has found widespread application in quantifying DNA strand breaks, identifying targeted oxidative damage to DNA bases within genotoxic investigations, and assessing DNA repair capabilities. Multiple investigations have demonstrated the comet assay's sensitivity, efficiency, and extensive utilization for detecting the mutagenic and genotoxic attributes of chemicals and xenobiotics, both in laboratory settings and real world scenarios (Li et al., 2021; Tian et al., 2022).

Therefore in present study we evaluated the genotoxic andcytotoxicityeffectsof (Z)-N-(pyridin-2-ylmethylene)-1-(2-((2-(1-((E)-pyridin-2-ylmethyleneamino)ethyl)phenyl)diselanyl)phenyl)ethanamine (C1), 2,2′-(1Z,1′E)-(1,1′-(2,2′-diselanediylbis(2,1-phenylene))bis(ethane-1,1-diyl))bis(azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)diphenol (C2) and dinaphthyldiselenide, $(NapSe)_2$) an attempt to screen their toxicity.

Our results demonstrated that the use of (Z)-N-(pyridin-2-ylmethylene)-1-(2-((2-(1-((E)-pyridin-2-ylmethyleneamino)ethyl)phenyl)diselanyl)phenyl)ethanamine (C1), 2,2′-(1Z,1′E)-(1,1′-(2,2′-diselanediylbis(2,1-phenylene))bis(ethane-1,1-diyl))bis(azan-1-yl-1-ylidene)bis

Table 2
DNA damage in human leukocytes exposed in vitro to different concentrations of organoselenium compounds.

	Concentration (μM)	DNA damage levels					DI
		0	1	2	3	4	
Control		65.33 ± 8.19	25.66 ± 8.56	3.66 ± 0.88	2.66 ± 0.66	2.66 ± 0.66	51.66 ± 8.37
C1	1	49.33 ± 0.88	17.00 ± 1.73	7.33 ± 2.60	4.66 ± 1.45	16.66 ± 3.92	112.33 ± 16.47
	5	39.00 ± 4.61	21.00 ± 5.19	11.66 ± 0.88	5.33 ± 0.33	23.00 ± 1.15	152.33 ± 1.45*
	10	11.00 ± 6.35	22.00 ± 11.54	12.33 ± 0.88	17.33 ± 5.48	36.33 ± 13.29	244.00 ± 56.40**
	25	0.0 ± 0.0	0.0 ± 0.0	2.00 ± 1.15	0.0 ± 0.0	98.00 ± 1.15	369.33 ± 28.68**
	50	3.25 ± 1.49	2.5 ± 1.19	12.75 ± 6.79	26.5 ± 1.32	55.00 ± 9.24	327.50 ± 20.35**
	75	4.00 ± 2.73	0.75 ± 0.75	5.25 ± 2.13	22.00 ± 3.80	68.00 ± 5.75	349.25 ± 13.60**
	100	5.25 ± 2.68	1.5 ± 0.86	1.75 ± 0.75	11.25 ± 2.56	80.25 ± 4.32	359.75 ± 12.65**
C2	1	60.00 ± 6.92	21.00 ± 2.30	6.00 ± 0.00	5.33 ± 1.45	7.66 ± 3.17	79.66 ± 19.34
	5	55.00 ± 9.81	14.00 ± 5.77	10.33 ± 3.17	7.00 ± 1.15	13.66 ± 0.33	103.66 ± 15.76
	10	10.00 ± 2.30	26.33 ± 0.33	19.00 ± 1.15	15.00 ± 2.88	29.66 ± 1.45	221.33 ± 3.92**
	25	4.00 ± 1.73	5.33 ± 0.33	10.66 ± 2.60	9.33 ± 3.75	70.66 ± 8.37	337.33 ± 16.74**
	50	6.25 ± 1.93	0.75 ± 0.75	1.5 ± 0.64	4.75 ± 2.13	86.75 ± 2.46	365 ± 7.93**
	75	1.75 ± 1.75	0.25 ± 0.25	1.25 ± 0.62	4.5 ± 1.65	92.25 ± 2.89	380 ± 8.98**
	100	3.25 ± 2.92	1 ± 0.57	1.25 ± 0.94	3.5 ± 1.84	91 ± 3.48	376.75 ± 10.11**
C3	1	45.33 ± 6.64	31.33 ± 7.21	3.00 ± 1.73	5.00 ± 1.15	15.33 ± 3.48	119.00 ± 6.50
	5	49.33 ± 7.79	19.66 ± 7.53	4.66 ± 0.33	9.33 ± 1.45	17.00 ± 6.42	125.00 ± 22.47*
	10	3.66 ± 0.33	22.33 ± 0.33	6.66 ± 0.33	12.66 ± 0.66	54.66 ± 0.33	292.33 ± 0.33**
	25	0.66 ± 0.33	0.33 ± 0.33	3.33 ± 2.02	8.00 ± 1.73	87.66 ± 3.75	381.66 ± 5.48**
	50	5.25 ± 3.32	4.00 ± 3.36	10.75 ± 4.6	22.25 ± 4.75	58.50 ± 6.34	326.25 ± 19.54**
	75	6.25 ± 2.17	6.25 ± 3.06	12.75 ± 3.42	15.50 ± 1.84	59.25 ± 7.23	315.25 ± 18.85**
	100	8.25 ± 3.54	5.00 ± 2.54	10.00 ± 3.24	16.75 ± 1.93	60.00 ± 5.19	315.25 ± 16.33**

(methan-1-yl-1-ylidene)diphenol (C2) and dinaphthyldiselenide. $(NapSe)_2$) at relatively high concentrations can be considered as possible factors causing genetic and cellular damage in human leukocytes, as indicated in (Tables 1 and 2). Exposure of human leukocytes to C1 (10–75 μM), C2 (5–75 μM) and $(NapSe)_2$ (10–75 μM) for 3 h induced a significant decrease in cell viability, and that the C2 at lower concentration (5 μM) seems to be more cytotoxic compared to other two organoselenium (Table 1). Similarly, the alteration in cell viability was associated with DNA damage. The compounds C1 (5–100 μM), C2 (10–100 μM), and $(NapSe)_2$ (5–100 μM) caused significant escalate in DNA damage index. In contrast, to that observed in the cell viability, the compounds C1 and $(NapSe)_2$ were genotoxic at lowest concentration tested (5 μM Different concentration of organoselenium were used based on previous studies from our research group demonstrating that mostly the toxicity start from above 5 μM which could be its narrow margin between antioxidant activity and potential toxicity (Tian et al., 2022; Xiao et al., 2023).

As previously discussed, selenium compounds are characterized by a dual nature, showcasing divergent behavior contingent on the concentration employed. At lower concentrations, selenium yields advantageous outcomes, whereas elevated levels can give rise to toxicity (Nogueira et al., 2004; Ibrahim et al., 2014a,b; Mugesh et al., 2001). In this study, even the lowest dose tested (5 μM) of the selenium organic forms were potent genotoxic and cytotoxic agents to the cells, suggesting that their beneficial effects might probably observed at concentrations lower than 5 μM. These findings align with earlier studies, indicating the genotoxic effects of organoselenium compounds on human leukocytes (Santos et al., 2009).

Indeed, a prior investigation demonstrated that diphenyl diselenide, an organoselenium compound, exhibited genotoxic effects on bacteria, yeast, and certain tumor cell lines. However, these effects were observed at concentrations significantly higher than those found within the typical pharmacological dosage range(Nogueira et al., 2004) Consistently, several authors have reported that organoselenium compounds might directly inhibit DNA synthesis or protein repair. It has been proposed that the mechanism underlying these toxic effects of selenium stems from its strong tendency to substitute sulfur in proteins containing SH groups, regardless of specificity.

6. Conclusions

To summarize, the findings presented in this study suggest that higher concentrations of $(NapSe)_2$ are associated with both genotoxic and cytotoxic effects, whereas at the same dose, $(NapSe)_2$ displays protective properties. These effects seem to be linked to the pro-oxidant activity demonstrated by organoselenium compounds. These results align with existing research concerning the toxicological and pharmacological impacts of organochalcogens across various pathological models. Additionally, this data indicates that $(NapSe)_2$ may exhibit protective effects upon *in vivo* administration in mice, potentially owing to its antioxidant characteristics. Similarly, compounds C1 and C2 demonstrate neuroprotective effects at very low concentrations.Hence, additional research will be required to clarify the mechanisms behind the actions of C1, C2, and $(NapSe)_2$.

Author contributions

Each author participated sufficiently in taking public responsibility for appropriate portions of the content. Study conception and design: J. B.R and M.I, D.F., conceived the idea and designed experiments and performed the experiments. M.K. and M.I.K; wrote the manuscript analyzed the data; A.F.A., M.A., A.A. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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