

Evaluation of Genotoxic and Cytotoxic Activities of Stem Extract of *Telfairia Occidentalis*

Ukeme E. Andrew¹, Favour Mbanefo², Chinyelu C. Osigwe^{2*}, Jude E. Okokon¹, Ugonma F. Uwaeme², Imoh I. Johnny³

¹Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria; ²Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria; ³Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

*Correspondence: drchinyeluosigwe@gmail.com; +234-8037169260

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Abstract

The vegetable, *Telfairia occidentalis* Hook (Cucurbitaceae), is useful in the preparation of meals and treatment of various ailments and diseases in ethnomedicine. Evaluation of the stem extract for genotoxic and cytotoxic activities using *Allium cepa* test was carried out. *Allium cepa* roots were exposed to various concentrations of the stem extract (2.5, 5.0, and 10 mg/mL) and macroscopic and microscopic examinations were carried out to ascertain the effects of the stem extract on the root meristem cells. Water and methotrexate (0.1 mg/ml) were used as negative and positive controls respectively. Significant ($p < 0.05$) concentration-dependent inhibition of root growth was observed relative to the negative control group. Cytotoxic effects were observed on cell division in *A. cepa* in all tested concentrations of the stem extract. Chromosomal aberrations and micronuclei (MNC) formations were significantly ($p < 0.05$) induced in *A. cepa* root tip cells relative to the control group. Cell death, ghost cells, cells membrane damage, and binucleated cells were observed also to be induced by the extract. These findings demonstrate that *Telfairia occidentalis* stem extract can exert cytotoxic and genotoxic effects on *A. cepa*.

Keywords: *Telfairia occidentalis*; *Allium cepa*, Apoptosis; Cytotoxic, Genotoxic

Introduction

Some green plants have phytoconstituents that are antimutagenic and others naturally toxic [1], while some are either cytotoxic and/or genotoxic [2]. A number of studies have revealed that some plants employed as food or medicine locally possess mutagenic potential as well as cytotoxic and genotoxic properties *in vitro* and *in vivo* [2-4]. Some plants, useful in nutrition and medicine, that contain compounds with mutagenic and/or carcinogenic properties [5,6], have been linked with high incidence of cancer in humans [7-9]. This has instigated serious interest and concern about the toxic potentials that may arise from frequent and long-term consumption of such plants especially as vegetables. In southern region of Nigeria, domestic or wild vegetables including *Telfairia occidentalis*, are employed in preparing varieties of dishes. This calls for investigation into the toxic potentials of the different parts of these vegetables to provide useful information for better and safer exploitation of the plants.

Telfairia occidentalis Hook (Cucurbitaceae), commonly called fluted pumpkin, is consumed as food in Nigeria [2]. It is popular all over Nigeria, especially in the Niger-Delta region and the Eastern Nigeria. Meals of many kinds are prepared from the stem, seeds and leaves of *T. occidentalis* [10]. The seeds, leaves and stem of *T. occidentalis* are reported to be used traditionally in the treatment and management of many disease conditions [11, 12]. Antiplasmodial activities of the seed, leaves, stem and roots of *T. occidentalis* have been previously documented [11-13] as well as the antinociceptive activity of the stem extract [6]. Other scientific reports on *T. occidentalis* included but not limited to antioxidant activity of the stem extract [15], alpha amylase and alpha glucosidase inhibition by the stem extract in rats [15]. The leaf and seed extracts of *T. occidentalis* reportedly exert genotoxic and cytotoxic potentials [2] as well as anti-inflammatory activity [16]. Fatty acids especially polyunsaturated fatty acids have been identified in the various fractions of the stem extract as well as secondary metabolites such as alkaloid, terpenes, saponin, flavonoid and tannin in the crude extract [15]. Evaluation of stem extract of *T. occidentalis* for genotoxic and cytotoxic activities using *Allium cepa* test was carried out in this investigation.

Materials and Methods

Plant materials

Matured stems of *Telfairia occidentalis* were freshly collected from farms in Uyo LGA, Akwa Ibom State, Nigeria. Identification and authentication of the plant materials was done by a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria. A voucher specimen (FPH 20a) was preserved at the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

Extraction

The collected *Telfairia occidentalis* fresh stem were washed in running tap water, reduced to smaller pieces and shade-dried for two weeks. The dried stems were powdered with electric grinder and the powder (2 kg) soaked in 50% ethanol (5 L) at room temperature for 3 days with intermittent stirring, after which the soaked material was filtered and the resultant liquid filtrate was concentrated to dryness using a rotary evaporator (BuchiLab Switzerland) at 40 °C. The weighed extract was stored in a refrigerator at -4 °C, until used for the proposed experiments.

Allium cepa test

Allium cepa (onion) bulbs (small in size), were bought from Itam market, Itu LGA, Akwa Ibom State, Nigeria. The bulbs were thereafter prepared for the study by cutting off dried old roots using a small knife at the bottom base of the bulbs without destroying the root primordia [3, 17]. The extract (20 g) was dissolved in distilled water (200 mL) and diluted to make varying concentrations of the extract (2.5, 5.0 and 10.0 mg/mL respectively) from the stock solution. The different extract test concentrations (2.5 mg/mL, 5 mg/mL, and 10 mg/mL) were filled in 50 mL beakers (5 per test concentration) and arranged serially as shown in figures 1(A-E). On top of each beaker in the different tested concentrations, one onion bulb was placed with the root primordial touching the surface of the liquid. Tap water and methotrexate (0.1 mg/mL) were respectively used as negative and positive controls. After 24 hours, the test samples were changed in the controls and all test concentrations. The experiment was for a period of 72 hours. Thereafter, the roots numbers were counted in all the beakers used in the respective tested concentrations and mean root number per concentration was calculated. The mean root length per concentration was also calculated after measuring the roots' length with a metre rule. The root tips (10 mm) of some roots per bulb were cut off and respectively fixed in 3:1 (v/v) ethanol: glacial acetic acid and 1N HCl in respective sample bottles and stored in a refrigerator [3, 17].

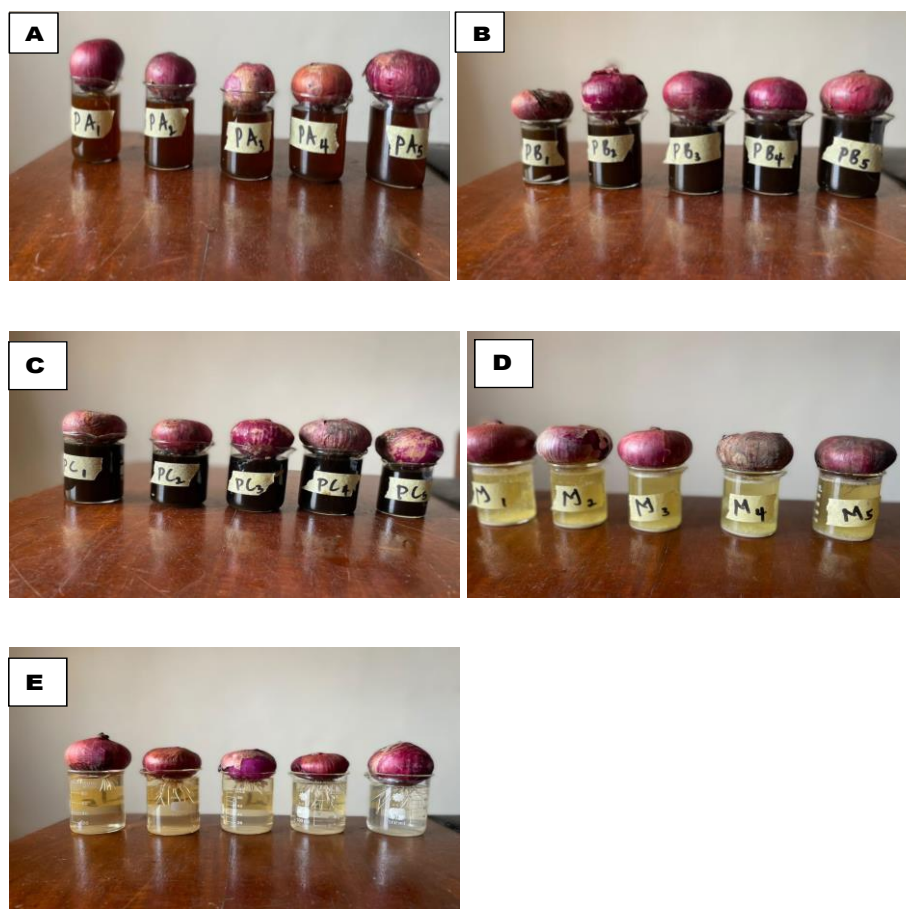


Figure 1: Treatment group treated with (A) 2.5 mg/mL (B) 5.0 mg/mL (C) 10 mg/mL of *Telfairia occidentalis* extract, (D) Methotrexate 0.1mg/mL, (E) Control group treated with tap water.

Microscopy

Each of the root tips was heated at 50°C for 6 minutes in a test tube containing 1N HCl in order to fix and macerate them. They were, thereafter, placed with a forceps on microscopic slides on a white piece of tile and their terminal tips cut off. Orcein stain (2%) was added and with a stirring spatula, mixed with the rootlets. This was covered with a cover slip and squashed by gently applying pressure on the covered treated root tips with the thumb. Colourless finger nail polish was used to seal each slide which was examined under a Light Microscope at a magnification of x40. Photomicrographs of chromosomal aberrations were taken in each slide. The mitotic index and total number of chromosomal aberration were calculated based on the number of abnormal cells per total cells counted in each concentration of the sample [2, 18]. The mitotic inhibition was determined using the following formula:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\% \text{Aberrant cells} = \frac{\text{Number of Aberrant cells}}{\text{Total number of cells}} \times 100$$

$$\% \text{root growth of control} = \frac{\text{Overall mean root length of test solution}}{\text{Overall mean root length of control}} \times 100$$

The parameters used in evaluating cytotoxicity and genotoxicity included: (i) the mitotic index (MI) (ii) chromatin aberrations (stickiness, bridges, breaks and polar deviation) and micronuclei (MNC) were scored per 500 cells [2, 18].

Statistical Analysis

The results gotten from this experiment were statistically analysed using Instat Graphpad software, (San Diego, USA). One –way ANOVA followed by Tukey-Kramer multiple comparison test were employed. Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

Results

Physicochemical Characterization

Table 1 shows the effects of *T. occidentalis* stem extract on the physicochemical indices (mean root number and root length). The results demonstrate that all tested concentrations of *T. occidentalis* stem extract exhibited significant inhibition of root- growth relative to the negative and positive controls. This inhibition of growth in root numbers was shown to be concentration dependent. Increase in concentrations of the stem extract of *T. occidentalis* caused corresponding root number and root length inhibitions. The mean root length in negative (tap water-treated) and positive control (methotrexate-treated) groups were 5.48 ± 0.27 and 0.28 ± 0.16 cm respectively. However, mean root length in 10 mg/mL tested group of the stem extract was 0.32 ± 0.05 . These observed decreases in root lengths of groups exposed to the stem were significant ($p < 0.05$) relative to that of the negative control (Table 1). The root length means of extract-exposed groups were shortened with increasing concentrations. The root morphology of the negative control treatment group appeared to be normal, while 2.5 mg/mL test group of the stem extract was slightly yellowish in appearance. The morphology of roots from 5 and 10 mg/mL treatment groups of the extract appeared brownish (Table 1).

Cytogenetic Analysis

The effects of *T. occidentalis* stem extract on cytogenetic indices of *Allium cepa* roots are presented in Table 2. Analysis of cytogenetic parameters demonstrated that *T. occidentalis* stem extract exhibited significantly ($p < 0.05$) concentration-dependent reductions in the mitotic index relative to that of negative control. Mitotic index of 26.60 ± 6.29 was recorded for 10 mg/mL of stem extract of *T. occidentalis* and negative control group had mitotic index of 64.80 ± 8.65 (Table 2).

Table 1: Cytotoxicity of *Telfairia occidentalis* stem extract on growing roots of onion (*Allium cepa*)

Treatment group	Concentration of extract (mg/mL)	Average root Number $\pm$ S.D	Average root length $\pm$ S.D (cm)
Negative control	Tap water	36.21 ± 5.14	5.48 ± 0.27
Methotrexate	0.1	10.55 ± 5.28^a	0.28 ± 0.16^a
<i>Telfairia occidentalis</i>	2.5	10.80 ± 3.96^a	2.38 ± 0.07^a
	5.0	22.20 ± 12.59	1.08 ± 0.17^a
	10.0	17.0 ± 7.10^a	0.32 ± 0.05^a

Values are expressed as mean $\pm$ SEM (n=5). Significant at $p < 0.05$ when compared to negative control

Table 2: Dividing and total cells counted under microscopic observations and mitotic values in control and treatment concentrations

Treatment group	Concentration of extract (mg/mL)	Total Number of cells	Dividing cells	M.I (%)± S.E
Negative control	Tap water	500	324	64.80±8.65
Methotrexate	0.1	500	18	3.60±0.25*
<i>Telfairia occidentalis</i>	2.5	500	281	56.20±7.29
	5.0	500	164	32.80±4.39*
	10.0	500	133	26.60±6.29*

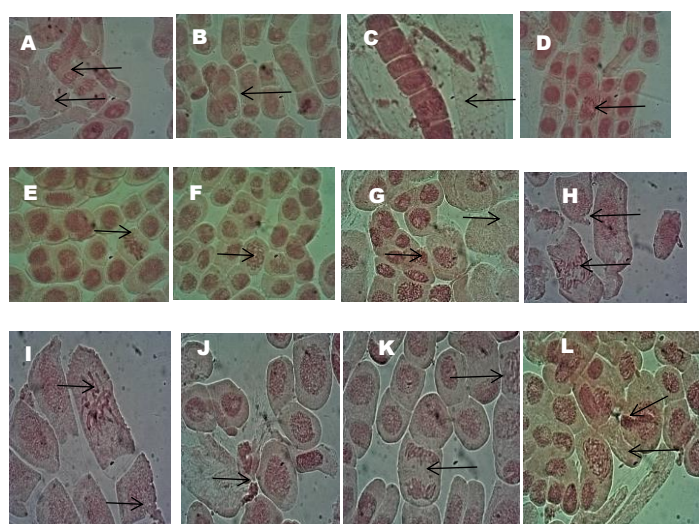
Values are expressed as mean ±SEM (n=5). *Significant at $p < 0.05$ when compared to negative control. M.I- mitotic index, SE- standard error

Cytogenetic abnormalities exhibited by *Telfairia occidentalis* stem extract are presented in Table 3. Chromosomal and cytological abnormalities were recorded groups exposed to tap water (negative control), methotrexate (positive control), and *T. occidentalis* stem extract as shown in Table 3. Chromosomal abnormalities analysis in groups exposed to the stem extract showed apoptotic bodies, membrane damaged cells and stickiness (Table 3; Figure 2A), binucleated cells (Figure 2B), as well as laggard and bridge chromosomes (Figures 2C, 2E and 2 I). Polar deviations (wrong directions of chromosome movement) (Figure 2D), fragments or clastogenic breaks (Table 3; Figure 2 H) and sticky metaphase/telophase (Figures 2(F and L)) were also observed in the stem extract exposed groups. These were significant ($p < 0.05$) relative to negative control group. These aberrations were also found to be concentration-dependent and statistically significant ($p < 0.05$) relative to the negative control (Table 3; Figure 2). However, methotrexate-treated group (positive control) had the highest number of aberrant cells (Table 3). The stem extract further demonstrated genotoxic potential by inducing micronuclei formation in the root tip meristem cells of *A. cepa*, which was not concentration-dependent. Also, cells with nuclear and membrane damage (Figures 2 (H, J and L)), binucleated cells (Figure 2 (B)), ghost cells 2 (G)) and apoptotic cells (Figure 2 (A)) were found in various frequencies in the different extract-treated groups.

Table 3: Chromosomal and mitotic aberrations in the root meristematic cells of *Allium cepa* after treatment of stem extract of *Telfairia occidentalis*.

Treatment group	Concentration of extract (mg/mL)	Chromosome breaks (%)±S.E	Stickiness(%) ±S.E	Polar deviation (%)±S.E	Aberrant cells (%)±S.E	MNC (%)± S.E
Negative control	Tap water	-	0.18±0.02	0.14±0.06	2.12±0.14	-
Methotrexate	0.10	2.23±1.06 ^a	19.36±4.29 ^a	15.10±3.16 ^a	48.68±5.88 ^a	2.84±0.38 ^a
<i>Telfairia</i>	2.5	1.12±1.16 ^a	2.44±0.16 ^a	1.45±0.22 ^a	15.18±3.26 ^a	5.32±1.85 ^a
<i>occidentalis</i> stem	5.0	1.68±1.04 ^a	5.83±2.11 ^a	5.28±0.59 ^a	29.35±2.34 ^a	1.18±0.78 ^a
extract	10.0	3.33±1.22 ^a	9.66±2.48	5.44±0.32 ^a	38.15±8.18	3.04±0.88 ^a

Values are expressed as mean ±SEM (n=5). ^aSignificant at $p < 0.05$ when compared to negative control. MNC –micronuclei

**Figure 2: Photomicrograph showing the mitotic and chromosomal aberrations of *Allium cepa* root meristem cells after leaf and seed extracts of *Telfairia occidentalis* treatment under light microscope X40 magnification**

(A) arrows indicate Stickiness and apoptotic bodies (B) binucleated cells (C) Bridge and laggard chromosomes (D) Polar deviation (E) Laggard chromosomes (F) Sticky chromosomes (G) Ghost cells and binucleated cells (H) nuclear damage and fragmentation (I) Bridge (J) Membrane and nuclear damage (K) Sticky telophase (L) Sticky chromosomes and nuclear damage

Discussion

This study investigated the toxic effects of *Telfairia occidentalis* stem extract on root growth and morphology of *A. cepa*. Root growth inhibition, which was observed in all extract concentrations tested (2.5–10.0 mg/mL) were statistically significant relative to control group. The cyto- and genotoxicity effects were evaluated by assessing the mitotic index and various chromosome abnormalities. Methotrexate (0.1 mg/mL) treated group exhibited a significantly decreased mitotic index (MI) relative to control. Also, the *Telfairia occidentalis* stem extract exhibited significant inhibition of root length with mitotic indices of 56.20%, 32.80% and 26.60% for 2.5, 5.0 and 10.0 mg/mL respectively. These were significant relative to control. The root growth inhibition correlated with decrease of Mitotic Index. Serious toxic effect on the organism are depicted when the mitotic index value falls below 22% when compared to negative control [19], while a value $\geq$ 50% usually indicates sublethal effects [20] and is called cytotoxic limit value [21]. Mitotic index gives information on the percentage of cells that are in the M-phase of the cell cycle and its inhibition could be considered as cellular death or a delay in the cell proliferation kinetics [22]. Prevention of synthesis of DNA or suppression of G2 phase activities in the cell cycle could be responsible for lowered mitotic activity, thus inhibiting cell division [23]. These mitotic inhibitory activities of plant extracts have previously been reported [24–26]. The stem extract's ability to alter cell cycle and cause chromosomal abnormalities due to the DNA synthesis depressive activities of its phytoconstituents could have resulted in the observed significantly lowered mitotic indices in *A. cepa* roots treated with the stem extract, suggesting inhibitory and mito-depressive potentials of the stem extract on root growth and cell division of *A. cepa*. The stem extract may be exerting its cytotoxic effect by preventing DNA synthesis and reducing the number of dividing cells in the root tips of *A. cepa*. The stem extract further demonstrated its toxic potentials by inducing sticky metaphase (where the chromosomes stick together and lose their normal appearance) [27], as observed in all the extract concentrations and suggested to result from interaction of some chemical compounds with DNA and/or protein, thereby forming complexes with phosphate groups in DNA and affecting DNA condensation as well as formation of inter- and intra-chromatid cross links [28]. Alteration of chromosomal structures due to fragmentation or exchange of chromosomal material were observed, some of which could be lethal to cause genetical changes, either somatic or inherited [29]. Formation of bridges in anaphase/telophase could be responsible for the observed chromosomal fragmentation in this investigation especially in the stem extract treated groups [30]. The stem extract was found to induce polar deviation in all the treated groups, with a significant increase in frequencies of total chromosome aberrations indicating clastogenic activity. Formation of MNC in *A. cepa* root cells at 2.5–10 mg/mL concentrations of the stem extract was observed in the study. The lowest concentration (2.5 mg/mL) of the stem extract was observed to have induced a higher number of MNC, while significantly lowered MNC frequency was produced at the highest concentration of the stem extract (10 mg/mL), resulting probably from high cytotoxicity. The micronuclei frequency which indicates cytogenetic potentials of a tested chemical, results from chromosomal abnormalities that prevent the integration of the chromosomes into the daughter nuclei during telophase of the dividing cells and can lead to cellular death as a result of primary genes deletion [31,32]. Previous studies have suggested MNC-induced effect of various plant extracts such as *Hippocratea africana* [33], *Setaria megaphylla* [34], *Heinsia crinata*, *Lasianthera africana* and *Justicia insularis* [3], *Solanum anomalum* fruit [35], *Croton zambesicus* [36].

In all the tested extract groups, cells with damaged membrane were found in various stem extract concentrations treated groups (2.5, 5.0 and 10.0 mg/mL), which is indicative of the cytotoxic potentials of the stem extract. Binucleated cells were found to be present in all the tested concentrations. This may be due to inhibition of microtubules which are involved in cytokinesis or cell plate formation, thereby preventing this process. Dead cell with visible outline but without stainable nucleus and cytoplasmic structures or ghost cell [37], were observed in various frequencies in all concentrations of the stem extract but higher in the 10 mg/mL treated group. Some phytochemicals in the stem extract could have caused serious damages to the nucleus and cytoplasmic structures leading to death of cells. Various frequencies of dead cells or apoptotic cells were observed in this study which could have been due to damaged DNA damage and cell death and/or apoptosis. This was prominent at high concentrations of the extract perhaps as a results of high concentrations of toxic substances such as toxin, heavy metals, chemicals and others including stress.

The present findings indicate that the stem extract of *T. occidentalis* demonstrated genotoxic and cytotoxic potentials by causing various forms of chromosomal abnormalities and cell death in *A. cepa* root tips.

Previous researches have revealed some phytochemical constituents of the stem extract such as alkaloid, flavonoid, tannins, terpenes, saponin, and cardiac glycosides [15,39], and their presence in the stem extract among other constituents may have contributed significantly to the effects observed in this investigation.

In addition, the identified terpenoids, high phenolic and flavonoid contents of the stem extract as reported previously by Enin et al [15] may have also accounted to the observed activities in this investigation. Quercetin, a flavonoid and others flavonoids have been shown in various studies to exhibit mutagenic and genotoxic potentials [39] and these phenolic compounds reportedly present in large quantity in the leaves of this vegetable, and likely in the stem, are suggestively responsible for the observed activities. These findings corroborate earlier reports [2, 16] on the cytotoxic and genotoxic activities of the seed and leaf extracts of *Telfairia occidentalis*. The observed cytotoxic and genotoxic potentials of the stem extract can be attributed to the activities of the phytoconstituents especially flavonoids.

Conclusion

This study revealed that *Telfairia occidentalis* stem extract exhibits cytotoxic and genotoxic effects in all tested concentrations on the root number, root length and root morphology of the *Allium cepa* meristems after exposure. The degree of chromosomal aberrations (based on increasing extract concentration), the inhibition of cellular mitotic processes, and the general abnormalities observed in all root bulbs treated with test samples further indicate cytotoxic potentials of the stem extract of *T. occidentalis*.

Conflict of interests

The authors have not declared any conflict of interests.

Authors' contributions

JEO, UEA, FM - Research concept and design; JEO, FM, IJJ Data analysis and interpretation; JEO,UUF, CCO, FM Writing the article; JEO,CCO,UEA and UUF read and approved the final manuscript.

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Disclaimer (Artificial Intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript

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