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Antigenotoxic potential of gel extract of *Aloe vera* against Sodium azide genotoxicity in *Allium cepa* cells

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ABSTRACT

Nowadays, the increasing rate of human exposure to various kinds of environmental mutagens has necessitated the search for natural antimutagens /antigenotoxic agents in natural products. In this study, *Aloe vera* gel extract was tested for its possible antigenotoxicity following the *Allium cepa* assay. Ten onions (*Allium cepa*) per dose were grown for 48 and 72 hours on gel extract of *A. vera* at 6.25%, 12.5%, 25.0%, 50.0% and 100.0% in combination with sodium azide (0.05mg/ml) solution for microscopic and macroscopic evaluations, respectively. Distilled water and sodium azide were the negative and positive controls, respectively. The cell division in the root tips, and root growth in the exposed *A. cepa* were inhibited in a dose dependent manner by the mixture of *A. vera* and sodium azide. However, the mixture of absolute (100.0%) dose and sodium azide completely arrested cell division and induced lower root length than that recorded for sodium azide alone. The genotoxicity of sodium azide was inversely reduced by the doses of *A. vera* except at 100.0%. These results show that gel extract of *A. vera* possesses strong antigenotoxic /antimutagenic potency at lower dose range of 6.25% to 25.0% in *A. cepa* cells, however, its higher doses above 50.0% to 100.0% could be severely toxic when being considered for suppression of environmental mutagens' mutagenicity or genotoxicity. This suggests that gel extract of *A. vera* contains phytochemical(s) that can be useful in the development of anticancer drug.

KEYWORDS: Aloe vera; *Allium cepa*; Antimutagenicity; Mutagenicity; Sodium azide

INTRODUCTION

Protection of the genetic system in cellular organisms is an indispensable task to ensure normal biochemical and physiological functions. Antigenotoxicity is one of the ways to prevent assaults onto the genetic material of a living organism at both unicellular and multicellular levels. Chemical substances that prevent / inhibit alterations in the organizational structure of genetic element could have an application in the development of anticancer agents because they can prevent the very crucial initiation stage of cancer development. Natural products and compounds known with medicinal properties are receiving much attentions of scientific investigators today in an attempt to discover their possible new pharmacological properties and also validate their existing medicinal claims. Naturally occurring antigenotoxic agents found in major foods or food supplements are beneficial as they delay or inhibit cancer formation (Skrzypczak et al., 2015). Antimutagenic compounds prevent the genetic system of cells from damages through drugs, foods and their metabolites, as well as radiations (Akeem et al., 2011). Deaths from cancers have been projected to reach 11.4 million by the year 2030 (Loh et al., 2009). Environmental pollution and unhealthy lifestyle through nutrition and physical activities have been suggested to come with significant number of genotoxic factors related to development of cancer, diabetes and obesity. *Aloe arborescens* Miller and *Aloe barbadenesis* Miller also known as *Aloe vera* (Linn.) have been reported for their beneficial medicinal values including antitumoral, antidiabetic and immune boosting activities (Berti et al., 2016; Guo & Mei, 2016).

Aloe vera originated from the warm, dry climates of Africa and belongs to the family Liliaeceae. It is readily adaptable and grows worldwide (Taiwo et al., 2005). The gel extracts of this plant as moisturizing cosmetics are topically applied on the skin by man to heal wound, and is consumed in health foods and beverages as laxative. It is also used to cure fever, burns, gastroenteritis, diabetes, inflammation, sexual diseases, infertility problems, cancer, and problems of the immune system. There are reports of antigenotoxic effects of gel extract of *A. vera* against benzo[a] pyrene - induced DNA damages, as an indirect acting mutagen that can cause adducts formation. Also, potentiality of *A. vera* gel extract in suppressing mutagenicity of ethyl methane sulphonate, a direct acting mutagen, following the Drosophila sex-linked recessive lethal test (SLRL) in 3-day old adults was

investigated (Snežana, 2007; Shamim et al., 2013; Słoczyńska et al., 2014). The immune stimulating effect of the plant was suggested as one of its mechanisms of suppressing cancer development. The whole leaf of *Aloe vera* contains 98.5% water and the remaining solid materials (leaf extract, gel and latex) contain more than 200 chemicals substances, of which over 75 of them are biologically active compounds such as vitamins, minerals, enzymes, polysaccharides, amino acids, phenolics and organic acids, anthraquinones, lignins and phytosterol which confer the aforementioned medicinal properties on the plant (Shamim et al., 2013; Guo & Mei, 2016). Genotoxicity and antigenotoxicity of extract of *A. vera* were conducted using chromosome aberrations test in the bone marrow cells of rats, sister chromatids exchange, micronucleus and chromosomal aberrations in human lymphocytes, and the Ames Salmonella/microsome test system (Anil et al., 2015). However, none of these reported tests adopted the *Allium cepa* root mitosis assay as it is being used to evaluate potency of gel extract of *A. vera* in suppressing genotoxicity of sodium azide, a direct acting mutagen, in this present study.

MATERIALS AND METHODS

Preparation of Gel Extract

Aloe vera plant weighing 5 kg was extracted by squeezing out gel extract from the leaves and this was preserved in the refrigerator at 4°C for antigenotoxic evaluation.

Allium cepa Assay

Onions were bought from Wazo market in Ogbomoso, Oyo state, Nigeria. They were sun dried for 1 week in order to facilitate their growth when planted for the antigenotoxicity evaluation. The outer scales of the dried onions were removed and the primordial root ring was carefully trimmed to remove dried old roots (Akinboro et al., 2017; Akwu et al., 2019). The absolute Aloe vera gel extract (100.0%) was diluted with distilled water to prepare 6.25%, 12.5%, 25.0%, 50.0% doses. The positive and negative controls were sodium azide solution prepared at 0.05 mg/ml and distilled water, respectively. Ten onions per dose were placed in the solution of sodium azide at 0.05 mg/ml (a direct mutagen) inside a 100 ml capacity glass beaker and placed in a cupboard for 24 hours. Sprouted onions after 24 hours of growth in the sodium azide solution were transferred into the doses of *Aloe vera* gel extract so as to continue root growth for another 24 hours (Akinboro et al., 2011; Akinboro et al., 2016; Madić et al., 2019). After 48 hours, root tips from four onions from each dose were harvested with a scissors, fixed in Canoy's fixative (3:1 ethanol acetic acid, v/v) and kept at 4°C for slides preparation. The fixed root tips were hydrolyzed in 1NHCL for 10 minutes in a water-bath heated at 60°C. The hydrolysed root tips were rinsed three times with distilled water. Two root tips were macerated on a glass slide and 2 drops of acetoorcein stain were added to the root tips smear on the slide and this was allowed to stand for 15 minutes. Further preparations of the slides were done as previously described (Akinboro & Bakare, 2007; Çavuşoğlu et al., 2016; Priyanka et al., 2019. Five slides were observed for the stages of mitosis and chromosomal aberrations induced by each dose of *A. vera* gel extract in combination with sodium azide (Akinboro et al., 2012; Akinboro et al., 2014; Akinboro et al., 2016). The remaining six onions were suspended in freshly prepared *Aloe vera* gel extract for another 24 hour root growth. After 72 hours of root growth of onions, the root lengths of these onions were measured using a ruler in order to examine the effect of the mixture of *Aloe vera* gel extract and sodium azide on onions root growth.

Statistical Analysis

Obtained data were summarized to mean, standard deviation and percentage using descriptive statistics in the SPSS software (version 21.0). Means were separated with Duncan's multiple range test where significant difference was set at $p \leq 0.05$.

RESULTS

Cytotoxicity of the mixture of gel extract of *Aloe vera* and sodium azide in *Allium cepa* root tip cells was dose dependent (Table 1). Sodium azide (0.05 mg/ml), the positive control induced the least mitotic index value of 1.16%, while the distilled water (negative control) recorded highest mitotic index value of 9.05%. *Aloe vera* gel extract dose of 6.25%, 12.5%, 25.0%, 50.0%, 100.0% in combination with sodium azide (0.05 mg/ml) produced mitotic index values 6.26%, 4.76%, 3.45%, 3.10% and 0%, respectively. The phase index was observed to have highest frequency of prophase in all the doses of *Aloe vera* gel extract and controls. Other dividing stages of mitosis were also observed at lower frequencies at all the selected doses except 100.0% that induced complete arrest of cell division. Toxicity of sodium azide to cell division in the root tips of onions was reduced by 56.35% at 6.25% dose, while 12.5%, 25.0% and 50.0% doses were able to reduce the mutagen's cytotoxicity by 39.78%, 25.30% and 21.44%,

Table 1: Effects of mixture of gel extract of *Aloe vera* and sodium azide on mitosis in *Allium cepa* cells

Dose (%)	Prophase	Metaphase	Anaphase	Telophase	No of dividing cells	Mitotic index (%)	Reduction of NaN_3-cytotoxicity (%)
Negative control	161	98	66	60	380	9.05	-
NaN3(+ve control)	18	12	11	7	49	1.16	-
6.25	104	63	49	48	263	6.26	56.35
12.5	77	40	34	48	200	4.76	39.78
25.0	61	34	26	28	145	3.45	25.30
50.0	57	30	22	21	130	3.10	21.44
100.0	0	0	0	0	0	0	N.C

N.C.: not calculable

respectively. However, it was not possible to calculate the degree of reduction of sodium azide - induced cytotoxicity by the *Aloe vera* gel extract at 100.0% because there was no dividing cells at this dose.

The genotoxic effect of sodium azide on the cell of *Allium cepa* was evident as different forms of chromosomal aberrations such as stickiness, chromosome bridge, colchicine mitosis, vagrant chromosome, nuclear abnormality, disturbed spindle and chromosome break were induced at higher frequencies by the direct mutagen (sodium azide) than those induced by the combination of sodium azide and *Aloe vera* gel extract. The percentage chromosomal aberration recorded with sodium azide was the highest at 0.55%, others were 0.29%, 0.31%, 0.31% and 0.41% recorded at 6.25%, 12.5%, 25.0% and 50.0% doses. However, no chromosomal aberrations were recorded at 100.0% of A. *vera* gel extract in combination with sodium azide because no cell was observed to be dividing. Genotoxicity of sodium azide was suppressed by 47.27% at the least dose of 6.25% of the gel extract of *Aloe vera*. At each of 12.5 and 25.0% doses, 43.64% suppression was recorded, while 50.0% dose suppressed sodium azide genotoxicity by 25.46%. However, there was a complete inhibition of cells division at 100.0% gel extract dose in combination with sodium azide making the calculation of extent of sodium azide genotoxicity reduction impossible at this dose (Table 2).

C-bridge: chromosome bridge, C- mitosis: chromosome mitosis, vagrant C: vagrant chromosome, N.A.: nuclear abnormalities, D.S.: disturbed spindle, C.B.: chromosome break.

The root growth toxicity of sodium azide to A. *cepa* was suppressed by *Aloe vera* gel extract in an inversely proportional to the selected doses except 100.0%. The highest suppression of NaN_3 - toxicity was recorded at 6.25% dose resulting to 64.24%. At 12.5%, 25.0% and 50.0% doses, the suppression was 35.76%, 10.07% and 0.69%, respectively. There was a synergistic reaction between sodium azide and gel extract at 100.0% dose resulting to an increased toxicity to the root growth by -0.69% rather than to suppress it. The root growth obtained with the distilled water was significantly different ($p \leq 0.05$) from that of the sodium azide and those of all the tested doses of *Aloe vera* gel extract (Table 3).

DISCUSSION

Toxicity of the mixture of the gel extract of *Aloe vera* and sodium azide to cell division in the root tips of *Allium cepa* in a dose dependent manner suggests that there was a synergistic inhibition of cell division in *A. cepa*. However, mixture of the absolute gel extract of *A. vera* and sodium azide used in this study was able to arrest mitosis completely, suggesting that each of the two major reactants (gel extract and sodium azide) had cytotoxic effects on mitosis in the test organism. Similar mitotic inhibition caused by *Aloe vera* leaf gel extract alone and mixture of *Aloe vera* leaf extract and sodium chloride in *A. cepa* was reported (İlbaş *et* al., 2012; Çavuşoğlu et al., 2016). Phytochemicals in the gel extract of *A. vera* and sodium azide itself could have prevented synthesis of DNA and nucleoprotein by blocking events at G1, S phases and G2 of the cell cycle from taking place thereby preventing cells from entering mitotic phase (Priyanka et al., 2019). Anthraquinones, aloin, barbalion, anthranol, cinnamic acid, acemannan, aloe emodin, aloetic acid, chysalodin, chrysophanic acid, resistanol, cyclooxygenase, bradykininase (enzymes), vitamins (tocopherol, vitamin C), saccharides, amino acids, carotenoids, falvonoids, tannins, superoxide dismutase and glutathione enzymes are the most common phytochemicals in the leaf and gel extracts of *A. vera* (Kayraldiz et al., 2010; Sapkota et al., 2019; Liu et al., 2019; Kim et al., 2020). Aloe emodin is well known for its cytotoxic

Table 2: Chromosomal aberrations induced by mixture of *Aloe vera* extract and sodium azide in *Allium cepa* cells

Dose (%)	Stickiness	C- bridge	C-mitosis	Vagrant C	N. A.	D.S.	C.B.	Total aberration	% aberrant cells	% reduction of NaN_3 genotoxicity
Distilled water	0	0	0	0	0	0	0	0	0	0
NaN_3(sodium azide)	1	5	5	0	3	4	5	23	0.55	0
6.25	2	2	1	2	1	1	3	12	0.29	47.27
12.5	3	4	1	1	0	2	2	13	0.31	43.64
25.0	2	3	0	1	0	3	4	13	0.31	43.64
50.0	3	4	2	2	0	2	4	17	0.41	25.46
100.0	-	-	-	-	-	-	-	-	-	-

C-bridge: chromosome bridge, C- mitosis: chromosome mitosis, vagrant C: vagrant chromosome, N.A.: nuclear abnormalities, D.S.: disturbed spindle, C.B.: chromosome break.

Table 3: Effectiveness of *Aloe vera* gel extract in reducing sodium azide - induced root growth toxicity in *Allium cepa*

Dose (%)	Mean Root length (cm)	% root growth	% root growth inhibition	Reduction of NaN_3– root growth toxicity
Distilled water	2.88 ± 0.85^{a}	100.00	0.00	-
NaN_3(sodium azide)	0.21 ± 0.05^{d}	7.29	92.71	-
6.25	2.06 ± 0.55^{b}	71.53	28.47	64.24
12.5	1.24 ± 0.56^{c}	43.06	56.94	35.76
25.0	0.50 ± 0.16^{d}	17.36	82.64	10.07
50.0	0.23 ± 0.14^{d}	7.99	92.01	0.69
100.0	0.19 ± 0.07^{d}	6.60	93.40	–0.69

Values with different superscript alphabets are significantly different at $p < 0.05$.

effect, and ability to induce apoptosis in various cells (Cosmetic Ingredient Review Expert Panel, 2007). The higher frequency of dividing cells at prophase induced at the selected doses of gel extract of *A. vera* and controls could be due to the fact that at the beginning of mitosis large number of cells enter prophase after leaving interphase. Induction of higher mitotic index (MI) values by the mixture of gel extract and sodium azide than the sodium azide alone indicates reduction of cytotoxic effect of sodium azide on mitosis in the root tips of *A. cepa*. This effect of the gel extract of *A. vera* to suppress cytotoxic effect of sodium azide was corroborated by the results of longer root lengths produced by the mixture of gel extract of *A. vera* and sodium azide at the tested doses than the root length caused by sodium azide alone. These results are in accordance with the previously obtained results (Çavuşoğlu et al., 2016). It was revealed that the gel extract at its absolute dose in combination with sodium azide was severely toxic to both cell division in the root tips and root growth in *A. cepa* as all cells were at interphase and the root length obtained from the mixture of 100 % gel extract and sodium azide was lower than that of sodium azide alone. This is an indication that gel extract of *A. vera* can be cytotoxic at a high dose.

Mutagenicity of sodium azide is well established in *Escherichia coli*, fungi, higher plants (such as rice, barley and corn), mammalian cells (National Toxicology Program, 1999). Sodium azide is a direct acting mutagen that reacts directly with a DNA molecule to cause damage, unlike indirect acting mutagens whose metabolites cause DNA damages. The lower frequency of chromosomal aberrations caused by the mixture of gel extract of *A. vera* and sodium azide compared with the aberrations induced by sodium azide alone implies antigenotoxicity of gel extract of *A. vera*. Previously, antigenotoxicity of gel extract of *A. vera* against genotoxicity of a direct mutagen (ethyl-methanesulphonate) induced in *Drosophila melanogaster* following the Drosophila sex linked recessive lethal test was reported (Snežana, 2007). Ogunjobi et al. (2007) also reported antimutagenic and anticarcinogenic activity of aqueous garlic extract and *A. vera* gel extract inhibiting acridine dye – inducing mutation in the tester strain *Escherichia coli* WP2 uvrA. In the present study, our results have also confirmed antigenotoxic potency of *A. vera* gel extract against sodium azide. The observed inhibition of sodium azide-induced genotoxicity in *A. cepa* cells by the gel extract of *A. vera* could be caused by natural antioxidants such as polyphenols, indoles, polysaccharides, and alkaloids in the plant. Antigenotoxic effect of acemannan against benzo [a] pyrene-induced DNA adducts formation in rats by increasing activity of glutathione S-transferase (Liu et al., 2019). Antioxidant activities of acemannan in terms of free radical scavenging, chelating, reduction of iron capabilities have been reported. Relationship between antioxidant activity of shikonin, acetylshikonin and *Arnebia euchroma* callus extracts and suppression of genotoxic effect of 4-nitroquinoline oxide and 2-aminoanthracene was reported (Skrzypczak et al., 2015). Polyphenolic compound like curcumin in its modified and unmodified forms were found to attenuate the cisplatin-induced DNA damage (Mendonça et al., 2015). Other mechanisms of inhibition of genotoxic effect of mutagens are inhibition of genotoxic effect, inhibition of cell proliferation, and modulation of signal transduction (Berti et al., 2016). Contrarily, Anil et al. (2015) reported that *A. vera* leaf extract did not decrease genotoxicity of urethane in the bone marrow cells of rats and mytomycin – C induced genotoxicity in human lymphocytes. This could be due to differences in the mutagens used and the kind of assays adopted for the evaluation.

CONCLUSION

This study has revealed that gel extract of *A. vera* is capable of suppressing genotoxicity of sodium azide in the root tip cells of *A. cepa* in a dose dependent manner. However, the gel extract can be cytotoxic at a dose above 50%. Our results have further confirmed the antigenotoxicity potential of *A. vera* gel extract against sodium azide, a different direct acting mutagen which was used in this study for the first time following the *A. cepa* root mitosis assay.

CONFLICT OF INTEREST

There is no conflict of interest declared.

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