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Assessment of the Antigenotoxic Effect of *Aloe Vera Gel* Extract on 2-Chloroacetamide Induced Genotoxicity in *Allium Cepa* Cells

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Abstract: ***The growing exposure to environmental mutagens has sparked a search for natural agents that can counteract their effects. This study investigated the potential antigenotoxic properties of Aloe vera gel extract using the Allium cepa assay. Onions were exposed to varying concentrations of A. vera gel extract (6.25% to 100%) in combination with 2-chloroacetamide, a known mutagen. The results showed that A. vera gel extract inhibited cell division and root growth in a dose-dependent manner when combined with 2-chloroacetamide. However, lower doses of A. vera (6.25% to 25%) reduced the genotoxicity of 2-chloroacetamide, while higher doses (50% to 100%) exacerbated its toxic effects. These findings suggest that A. vera gel extract possesses antigenotoxic properties at lower concentrations, making it a potential candidate for the development of anticancer agents.***

Keywords: Aloe vera; 2-chloroacetamide Allium cepa; Antimutagenicity; Mutagenicity

1. Introduction

Safeguarding the genetic system is crucial for maintaining normal cellular functions. Antigenotoxicity plays a vital role in protecting genetic material from damage at both unicellular and multicellular levels. Substances that prevent genetic alterations can aid in developing anticancer agents by blocking the initiation stage of cancer. Researchers are increasingly exploring natural products with medicinal properties to uncover new pharmacological benefits and validate existing claims [1-4] Naturally occurring antigenotoxic agents in food and supplements can help delay or prevent cancer. Antimutagenic compounds shield cells from genetic damage caused by environmental factors, lifestyle choices, and metabolic processes. The rise in environmental pollution and unhealthy lifestyles has led to an increase in genotoxic factors linked to diseases like cancer, diabetes, and obesity [5-6].

Aloe vera, native to Africa's warm and dry climates, belongs to the Liliaceae family and has adapted to grow globally. Its gel extracts are widely used in cosmetics for skin healing and as a laxative in health foods and beverages. Additionally, Aloe vera is used to treat various ailments, including fever, burns, diabetes, inflammation, and immune system disorders. Research has shown that Aloe vera gel extract exhibits antigenotoxic effects against DNA damage caused by indirect-acting mutagens like benzo[a]pyrene and direct-acting mutagens like ethyl methane sulphonate. The plant's immune-stimulating properties are thought to contribute to its potential in suppressing cancer development. Aloe vera's composition includes over 200 chemical substances, with more than 75 being biologically active compounds that confer its medicinal properties. Previous studies have evaluated the genotoxicity and antigenotoxicity of Aloe vera extracts using various tests, but none have employed the Allium cepa root mitosis assay to assess its potency in suppressing 2-chloroacetamide-induced genotoxicity, which is the focus of this study.

2. Materials and Methods

Preparation of Gel Extract

Aloe vera gel extraction was performed on a 5 kg plant by manually squeezing the gel from the leaves. The resulting gel extract was refrigerated at 4°C to preserve its properties for subsequent antigenotoxic assessment.

Allium cepa Evaluate

Onions were procured from Phule market in Nagpur, Maharashtra, India, and sun-dried for a week to enhance their growth potential for the antigenotoxicity study. The dried onions were prepared by removing the outer scales and trimming the primordial root ring to eliminate old roots. Aloe vera gel extract was diluted with distilled water to create concentrations of 6.25%, 12.5%, 25%, and 50%, with 100% being the absolute extract. 2-chloroacetamide solution (0.05 mg/ml) and distilled water served as the positive and negative controls, respectively. Ten onions per dose were exposed to 2-chloroacetamide solution for 24 hours, followed by transfer to Aloe vera gel extract solutions for another 24 hours. After 48 hours, root tips from four onions per dose were harvested, fixed, and stored at 4°C for slide preparation. The root tips underwent hydrolysis, rinsing, and staining before being examined for mitotic stages and chromosomal aberrations. Meanwhile, the remaining onions continued to grow in Aloe vera gel extract for another 24 hours, after which their root lengths were measured to assess the impact of the Aloe vera mixture.

3. Results

The cytotoxic effects of combining Aloe vera gel extract with 2-chloroacetamide on Allium cepa root tip cells were dose-dependent (Table 1). The positive control, 2-chloroacetamide (0.05 mg/ml), resulted in a mitotic index of 1.16%, while the negative control (distilled water) had a significantly higher mitotic index of 9.05%. When combined with 2-chloroacetamide, Aloe vera gel extract doses of 6.25%, 12.5%, 25%, 50%, and 100% yielded mitotic indices

of 6.26%, 4.76%, 3.45%, 3.10%, and 0%, respectively. Prophase was the most frequent phase observed across all doses and controls. The 100% dose completely inhibited cell division, while other doses showed lower frequencies of mitotic stages. Notably, the 6.25% dose reduced 2-chloroacetamide's cytotoxicity by 56.35%, whereas the 12.5%, 25%, and 50% doses reduced it by 39.78%, 25.30%, and 21.44%, respectively.

Table 1: Effects of mixture of gel extract of Aloe vera and 2-chloroacetamide on mitosis in Allium cepa cells

Dose (%)	Prophase	Metaphase	Anaphase	Telophase	No of dividing cells	Mitotic index (%)	Reduction of 2-chloroacetamide - cytotoxicity (%)
Negative control	152	88	60	52	310	8.05	-
2-chloroacetamide (+ve control)	16	21	10	5	45	1.06	-
6.25	98	60	42	40	222	5.26	46.35
12.5	71	35	31	42	188	4.06	36.02
25.0	55	38	28	30	120	3.05	21.02
50.0	52	30	23	22	128	2.87	20.64
100.0	0	0	0	0	0	0	N.C

Table 2: Chromosomal aberrations induced by mixture of Aloe vera extract and 2-chloroacetamide in Allium cepa cells

Dose (%)	Stickiness	C-bridge	C-mitosis	Vagrant C	N. A.	D.S.	C.B.	Total aberration	% aberrant cells	% reduction of NaN3 genotoxicity
Distilled water	0	0	0	0	0	0	0	0	0	0
2-chloroacetamide (+ve control)	1	4	3	0	2	4	4	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	4	3	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.

The study shows that Aloe vera gel extract can reduce the cytotoxic effects of 2-chloroacetamide on Allium cepa root tip cells in a dose-dependent manner. The higher frequency of cells in prophase is likely due to the natural progression of mitosis. The gel extract's ability to increase mitotic index values and promote longer root lengths when combined with 2-chloroacetamide suggests its potential to mitigate the mutagen's cytotoxic effects. However, at high doses (100%), the gel extract becomes severely toxic, inhibiting cell division and root growth. The reduction in chromosomal aberrations caused by the mixture of gel extract and 2-chloroacetamide compared to 2-chloroacetamide alone indicates the antigenotoxic properties of A. vera. This is consistent with previous studies demonstrating antigenotoxic effects of A. vera against other mutagens. The observed antigenotoxicity may be attributed to the presence of natural antioxidants in A. vera, such as polyphenols and polysaccharides. Various mechanisms, including antioxidant activity, inhibition of genotoxic effects, and modulation of signal transduction, may contribute to the antigenotoxic effects of A. vera. Differences in mutagens and assays used may explain discrepancies with other studies.

4. Conclusion

This study demonstrates that Aloe vera gel extract can dose-dependently suppress the genotoxic effects of 2-chloroacetamide in Allium cepa root tip cells. However, the extract exhibits cytotoxicity at concentrations above 50%. Our findings confirm the antigenotoxic potential of A. vera gel extract, extending its efficacy to 2-chloroacetamide, a direct-acting mutagen. Notably, this study is the first to utilize the A. cepa root mitosis assay to evaluate A. vera's antigenotoxic effects against this specific mutagen.

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