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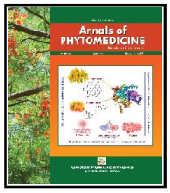

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Evaluation of the antigenotoxic potential of resveratrol and ascorbic acid in MDBK cells through fast-halo assay

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Abstract

The current study was carried out to assess the antigenotoxic potential of resveratrol (RV) and L-ascorbic acid (AA) against hydrogen peroxide (H_2O_2) induced DNA damage using a fast-halo assay. The experiment was carried out using Madin-Darby bovine kidney (MDBK) cells, wherein the cells were pre-treated with three different concentrations (10 µM, 100 µM, and 200 µM) of RV and AA for 30 min and simultaneously exposed with and without hydrogen peroxide (H_2O_2) at 100 µM concentration. Fast-halo assay was performed to access DNA damage after completion of the exposure period. The degree of DNA damage was observed under fluorescence microscopy and scored using a semi-automatic "HaloJ" program. Nuclear diffusion factor (NDF), and percentage halo nucleus DNA (% hn-DNA) were used to express the DNA damage. Hydrogen peroxide-treated cells had significantly (p<0.05) higher NDF values and lower percentage hn-DNA when compared to the control groups, indicating DNA damage. Whereas, the treatment with RV and AA prevented hydrogen peroxide-induced DNA damage indicated by significant (p<0.05) increase in the percentage of hn-DNA and decrease in NDF values when compared to the hydrogen peroxide treatment group. The findings of the experiment indicate the antigenotoxic potential of RV and, AA against H_2O_2, and the assay as a reliable method for rapid detection of DNA strand breakages.

1. Introduction

The functionality of cells includes various processes such as replication, appropriate gene expression *etc*., and the maintenance of these cellular functions depends on the integrity of its deoxyri bonucleic acid (DNA) content. DNA is considered as molecular blueprint essential for the normal function of the cells and any alteration of DNA that changes its properties or impedes with its normal function of transcription or replication is described as DNA damage (Obulesu and Rao, 2010). There are several ways that DNA abnormalities can manifest, including adducts, strand breakages, including both double-strand breaks (DSBs) and single-strand breaks (SSBs), DNA-protein cross-linkages, and base mismatching includes insertions or deletions (Friedberg, 2003). DNA repair is a natural process and occurs regularly within a cell. Failure of DNA repair may lead to a variety of consequences such as cell apoptosis and, necrosis (Roos and Kaina, 2006), carcinogenesis (Barnes *et al*., 2018; Basu, 2018), neurodegenerative diseases (Obulesu and Rao, 2010), and many more. DNA damage occurs by various endogenous and exogenous agents (Shimada *et al*., 2014) and these agents may be responsible for the oxidative cell injury by generation of reactive oxygen species (ROS), DNA alkylation reactions that lead to mismatch of DNA base pairs contribute to the endogenous cause of DNA damage, while the radiation (X-rays, UV rays), toxic chemical agents or biological agents are the exogenous sources of DNA damage (Hakem, 2008). The development of genomic instability and carcinogenesis may occur from oxidative damage caused by ROS (Friedberg, 2003; Martin, 2008; Gonzalez-Hunt *et al*., 2018). Additionally, deamination the complete removal of individual bases add is an other mechanism that damages DNA in a hydrolytic pathway, that is associated with elevated level ROS and the metabolites (Atamna *et al*., 2000).

Nonetheless, the harm caused to the genetic material encompasses not just DNA but also the cellular elements associated with the function and behaviour of chromosomes in the cell (López-Romero *et al*., 2018). Therefore, evaluation for genotoxicity is an imperative part of hazard identification and characterization of a nature of compound which can be done both *in vitro* and *in vivo* methods. The commonly employed techniques include single-cell gel electrophoresis (comet assay), bacterial reverse mutation test (Ames test), mammalian chromosomal aberrationassay, γ-H2AX foci assay, terminal deoxynucleotidyl transferase dUTPnick end labeling assay (TUNEL assay), fast-halo assay (FHA), DNA breakage detection fluorescent *in situ* hybridization (DBD-FISH), real-time polymerase chain reaction (qPCR), radio immune assay, *etc*. Each of these genotoxicity assays has particular benefits as well as inherent drawbacks (OECD, 2017). Most popular technique used for evaluating different types of DNA damage is the comet assay. It is an effective and flexible method for calculating the total amount of DNA damage in individual cells and is

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probably the most commonly used method for genotoxicity testing (Collins, 2004). This assay has limitations such as low throughput of and time-consuming procedures. 8-hydroxydeoxyguanosine (8-OHdG) is also a familiar marker of oxidative DNA damage that can be measured in multiple species as indicators of chemical carcinogenesis (Fenga *et al*., 2017; Rao, 1993). Alternatively, the current study explores the use of a technique called the fast-halo assay (FHA) which is very simple, faster, and cost-effective than the other techniques used for measurement of DNA damage. Though, this assay is not much more popular or extensively used but it is good as high-throughput technique for screening genotoxicants (Sestili *et al*., 2006).

Exposure to the genotoxic agents is responsible for the initiation and promotion of many of the diseases in the body, while bioactive phytocompounds has got significance in counteracting these mutagenic and carcinogenic effects. Plant-based bioactive compounds are now gaining credentials in today's world instead of plants as a whole. Such compounds that reduce the mutagenicity are referred as antimutagens. However, the compounds that mitigate the DNA damage associated with genotoxic agents known as antigenotoxic agents (De Flora and Ferguson, 2005; Bhattachar, 2011). The dietary agents such as fruits, vegetables, herbs, spices, *etc*., form a primary source of these antigenotoxic/antimutagenic compounds. Though, there are different classes such as flavonoid, polyphenols, limonoids, terpenes, *etc*., among these phytoactive compounds, the polyphenolic compounds grab the lime light due to their strong antioxidant properties and their preventive role in diseases associated with oxidative stress (Martin, 2008; Sproul *et al*., 2014; Higo *et al*., 2017). The essential function of antioxidants is to shield cells from DNA damage brought through oxidative stress. They work by neutralising ROS, which can harm DNA and other biological components.

Resveratrol (RV) is a natural polyphenol phytoalexin compound known to possess antioxidant and anti-inflammatory effects (Sgambato *et al*., 2001; Lombardi *et al*., 2015). While ascorbic acid (AA) is one of the important micronutrients and a factor for several metabolic reactions. It is known as endo-exo-antioxidant, synthesized in the body, available in various fruits and plants that form a part of our diet and can be supplemented from outside in deficiency (Türkez and Aydýn, 2012). Its deficiency is attributed as a major cause of cancer (Ames, 2001; Khan *et al*., 2023; Surjyo and Rahman, 2004). Published literature indicates that AA and RV are potent antigenotoxic agents and most widely used agents in preventive therapy against genotoxicants (Ames, 2001; Sgambato *et al*., 2001; Surjyo and Rahman, 2004; Lombardi *et al*., 2015).

To the best of our knowledge, there is no study on the effect of selected genotoxicant and anti-genotoxicants interaction on kidney cells and applicability of FHA. Hence, current investigation was carried out with an aim to determine the applicability of FHA in the quantitative evaluation of genotoxicity in Madin-Darby bovine kidney (MDBK) cells induced by H_2O_2 and further to explore the antigenotoxic potential of AA and RV against H_2O_2-induced genotoxicity in MDBK cell line.

2. Materials and Methods

2.1 Chemicals and reagents

The following reagents were procured from Himedia Laboratories, Thane, India: Dubecco'sphosphate buffered saline, Medium-Dulbecco's modified Eagle medium (DMEM), and antibiotic-antimycotic solutions. Whereas, fetal bovine serum (FBS) of MP Biomedicals, Mumbai, India was used. Ascorbic acid (99.9% purity), resveratrol (99.9% purity), trypsin-EDTA, ethidium bromide and trypan blue purchased from Sigma Chemical Company (St Louis, MO, USA). The culture plates and sterile culture flasks were purchased from Nest Biotech Co. Ltd., China. The reagents and various buffers utilized in the experiment were made in autoclaved Milli-Q water and then filtered using Millipore (0.22 µm) membrane filters (Molsheim, France). All of the other analytical reagents used were purchased from reputed manufacturers.

2.2 Cell culture

MDBK cell line was obtained from College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India. Uniform passage number (P47) were used for the study. The cryopreserved cells were removed and thawed immediately at a pre-warmed (37ºC) water. Following thawing, suspended cells were centrifuged for 10 min at 1000 rpm to pellet them. After removing the cryomedia and resuspending the pellet in culture medium, centrifugation was performed at 800 rpm for 10 min at room temperature, followed by three washings with PBS solution. Ultimately, the cell pellet was resuspended in 4 ml of growth medium in 25 cm^3 culture flask. Care was taken to avoid vigorous resuspension of cells during the above-mentioned stages as it could influence the cell viability. The culture flask was incubated in 5% CO_2 incubator at 37°C temperature with >50% humidity. More than 80% confluent cells were passaged after washing with PBS, and trypsinized using trypsin phosphate versine glucose (TPGV) solution (0.25% trypsin, 0.5% PVP, 0.02% EDTA, and 0.05% glucose in PBS). Following the 0.1% trypan blue dye exclusion technique (Strober, 2015), cell viability was evaluated. The live cells were adjusted to a concentration of 1×10^5 cells/ml of growth media and used for subsequent experiments.

2.3 Stock solutions

For preparation of the stock solution of 10 mM concentration, the antioxidants ascorbic acid (AA) and resveratrol (RV) were dissolved in DMEM and DMSO, respectively. From this stock solution the working concentrations were prepared by serial dilution, the final concentration of DMSO in the working solutions was kept at 1%.

2.4 Experimental design

The cells were trypsinized using sterile trypsin phosphate versine glucose (TPGV) solution after which the medium containing suspended cells was aspirated and centrifuged at 1000 rpm at 37ºC in refrigerated centrifuge, followed by serial washing thricely. Finally, cells were seeded into 12 well cell culture plates at a seeding density of 5×10^4 cells per well in Dulbecco's modified Eagle medium (DMEM) with 10% (v/v) FBS. The cells were then incubated at 37°C and 5% CO_2 in a humidified incubator. With the completion of 12 h seeding time, cells were divided into different treatment groups (Table 1). The cells were treated with three different concentrations (10 µM, 100 µM, and 200 µM) of RV and AA, 30 min prior to exposure with H_2O_2. These cells were then co-incubated with H_2O_2 (100 µM) for

60 min concomitantly with RV and AA to study the protective effect against peroxide induced DNA damage due to oxidative stress. The concentrations of the agents in the study were determined based on pilot experiment, wherein the best effective concentrations were determined. The cells treated with the maximum concentration of RV and AA alone were used to observe negative effects, if any, of the phytoactive compounds. Whereas, the cells exposed only to H_2O_2 were considered as a positive control for DNA damage. Cells treated with 1% DMSO were used as vehicle control, and untreated cells served as media control (Table 1).

Table 1: Treatment groups of MDBK cells with genotoxicant and antioxidants

Group	Treatment	Concentration
1	Media control	DMEM + 10% FBS
2	Vehicle control	1% DMSO
3	H_2O_2	100 µM
4	RV_3	200 µM
5	AA_3	200 µM
6	$H_2O_2 + RV_1$	100 µM H_2O_2 + 10 µM RV
7	$H_2O_2 + RV_2$	100 µM H_2O_2 + 100 µM RV
8	$H_2O_2 + RV_3$	100 µM H_2O_2 + 200 µM RV
9	$H_2O_2 + AA_1$	100 µM H_2O_2 + 10 µM AA
10	$H_2O_2 + AA_2$	100 µM H_2O_2 + 100 µM AA
11	$H_2O_2 + AA_3$	100 µM H_2O_2 + 200 µM AA

RV-Resveratrol; AA-L-Ascorbic acid; µM-micro molar, DMEM-Dulbecco's modified Eagle medium, FBS-Fetal bovine serum, DMSO-Dimethyl sulfoxide.

2.5 Preparation of pre-coated agar slides

Precoated frosted end slides were prepared in advance for the experiments and each slide was used for two samples in the current experiment. For preparing the slides, clean grease free microscopic glass slides with a frosted end were taken and the side to be coated was marked. Then, the slides were carefully dipped into 1% (w/v) normal melting agarose (NMA), prepared by dissolving 1g of normal melting agarose in 100 ml distilled water. The glass slides were placed in a horizontal tray on a level surface, their undersides cleaned using lint-free wipes, and they were allowed to dry overnight. The slides were kept in a storage box in dust-free condition, once they had dried.

2.6 Isolation of cells

The cell culture media was aspirated after end of exposure period (60 min) and the cells were then subjected to trypsinization using TPVG solution to dissociate the cells. The activity of trypsin was blocked using DMEM-10% FBS. The cells were subjected to centrifugation, followed by aspiration of the medium and replacing with one ml of PBS solution in which the cells were resuspended. The cell concentration was maintained at approximately 1×10^6 cells per ml in PBS. Trypan blue dye exclusion technique was again used for estimation of viable cell density after which the cells were subjected to FHA as per the protocol of Sestili *et al*. (2006).

2.7 Agarose embedding and casting of cells

For agarose embedding and casting of cells, 0.75% (w/v) low-melting agarose (LMA) in PBS solution was prepared. The solution was heated until boiling to completely dissolve LMA. An aliquot of the solutionwas maintained at 40°C. 40-50 µl of the cell suspension (approx. 3 x 10^4 cells) was taken and thoroughly mixed with equal amount of 0.75% LMA solution maintained at 40°C. 40 µl of this mixture was immediately pipetted onto the pre-coated agarose slides and a cover slip was placed. The steps were repeated to prepare slides from different treatment groups. The slides were then placed in slide box and kept at refrigeration temperature until the agarose layer solidifies (approx. 15-20 min).

2.8 Lysis, denaturation and staining of samples

Cell lysis and denaturation of DNA was carried out to measure DNA strand breaks, for this purpose, 300 mM solution of NaOH (pH>13.0) was used. The slides which were kept at refrigeration temperature were taken out and the coverslips were removed while ensuring thatagarose layer remains solidified. They were then placed in horizontal staining trays containing 300 mM NaOH solution for a period of 15 min. After denaturation the samples were stained by placing the slides in the staining jar containing ethidium bromide (25 µg/ml) for 5 min, following proper precaution. Then, the slides were transferred into another coupling jar that was filled with distilled water for destaining. After 15 min of destaining, if immediate visualization is not possible, then the slides can be stored at room temperature for up to 2 h (Sestili *et al*., 2017) in fresh distilled water to reduce EB contamination.

2.9 Evaluation of DNA damage

Phase contrast inverted microscope (Nikon Eclipse Ti, Japan) was used for visualisation of the treated cells for morphological changes of cell damage. Whereas, FHA prepared slides were observed under fluorescence microscope and the images were recorded digitally using software (NIS-Elements Nikon, Japan). The images were then evaluated semi-automatically using HaloJ (Image J plugin software) as described by Maurya (2014). The area of the nuclear remnant to the corresponding area was measured for each cell. The DNA damage

score was expressed as nuclear diffusion factor (NDF) which is the ratio between [(total area of nucleus plus halo and area of nucleus)-1]. The NDF values was calculated for randomly selected cells of different groups. Blinding of the groups was done to prevent biasness while scoring. The DNA damage was expressed in terms of NDF values and percentage halo nucleus DNA (% hn-DNA). Higher values of NDF translate to more diffusion of DNA indicating higher DNA fragmentation whereas more % hn-DNA imply intact nuclear DNA.

3. Results

The morphology of the cells at the end of the treatment was studied under phase contrast microscope (Figure1) and visual scoring of the lesions among different groups was also done (Table 2). Results indicated that treatment with H_2O_2 resulted in development of degenerative changes such as rounding of cells, vacuolation, *etc*. While co-treatment with AA and RV protected the cells against these degenerative changes as observed visually.

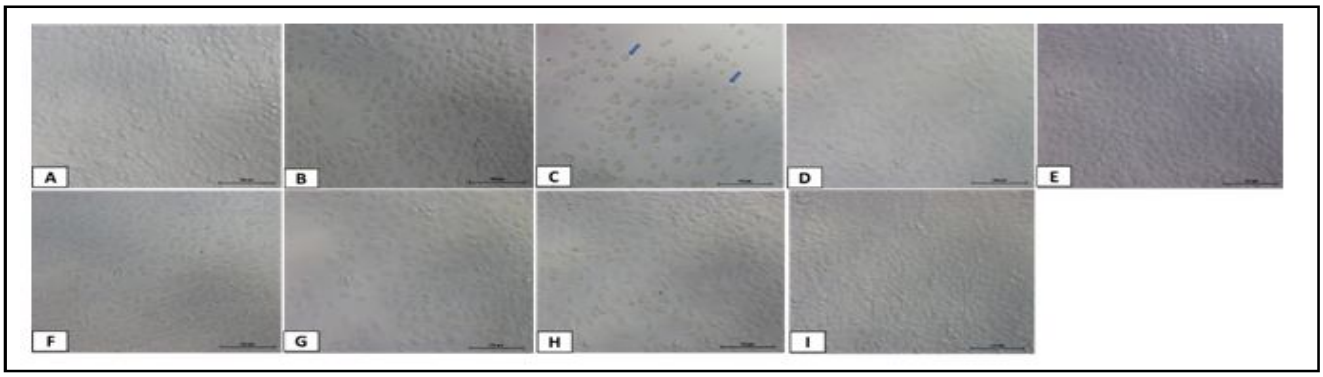

Figure 1: MDBK cells under phase contrast microscope with control (A) and vehicle control; (B) showing normal cells; H_2O_2 treated cells; (C) showing degenerative changes (arrows); RV (10 µM; D);RV (100 µM; E); RV (200 µM; F) and AA (10 µM; G), (100 µM; H), and (200 µM; I) with H_2O_2 showing protective effect (Magnification 200X; scale-100 µm).

Table 2: Scoring of toxico-morphological changes in MDBK cells post exposure to H_2O_2, RV and AA, alone and in combinations

Morphological features	Groups										
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
Cellular swelling	1	1	5	2	1	4	3	4	4	2	2
Chromatin condensation	1	2	4	1	1	3	2	3	3	1	1
Nuclear fragmentation	1	1	4	1	1	2	2	3	2	2	1
Loss of plasma membrane	1	1	3	2	1	2	3	3	3	1	2
Release of cytoplasmic content	1	1	4	1	1	2	2	3	2	1	1

1: Normal morphology; 2: Low level changes; 3: Moderate level changes; 4: High level changes and 5: Very high-level changes

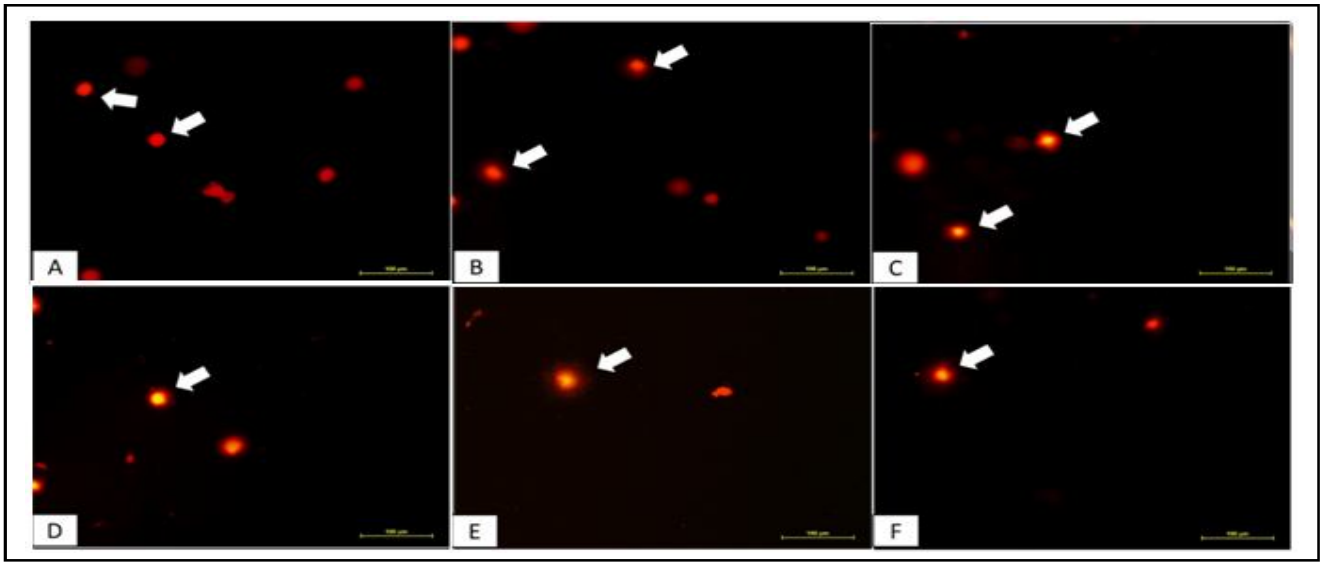

Figure 2: MDBK cells in control (A), mild DNA damage (B), moderate DNA damage (C), High DNA damage (D), and cells of H_2O_2 treated group with probable apoptosis (E,F) indicated with arrows (magnification 200X; scale-100 µm).

After completion of treatment, the cells were harvested, embedded in agarose, casted on to microscopic glass slides, subjected to lysis, denaturation and staining. The cells were observed under fluorescent microscope as represented in Figure 2. The cells of control group (group1) showed no damage (no halo formation), but the cells that are damaged in treatment groups showed different levels of halo formation depending on the extent of damage (mild, moderate and high).

The level of damage was quantitatively measured using HaloJ software, the results of the which are reported in Table 3, Figures 3 and 4. Analysing the calculated results, it can be observed that the group 3 (H_2O_2, 100 µM) showed lowest mean %hn-DNA values which was significantly low ($p<0.05$) compared to other groups, indicating highest DNA damage. This can also be correlated with NDF values, wherein the same group showed highest mean NDF value compared to other treatment groups ($p<0.05$). The control group (group 1), containing growth media with no treatment, showed mean NDF values statistically similar ($p<0.05$) to the groups that received treatment with antioxidant agents alone (groups 4 and 5) and also the vehicle control group (group 2). While the treatment with RV (groups 6, 7 and 8 significantly ($p<0.05$) improved the %hn-DNA as compared to the cells H_2O_2, there was no statistical significance ($p<0.05$) in protective effect of RV with increasing dose. Looking at the cells that received treatment with AA prior to exposure to H_2O_2 (groups 9, 10 and 11), it can be observed that even the lowest dose (10 µM) of AA had significantly ($p<0.05$) improved %hn-DNA, compared to cells that were exposed to H_2O_2 alone (group 3). However, a significant ($p<0.05$) increase in protective effect was observed at higher doses of AA (100 and 200 µM) as compared to lower dose (10 µM). The same is reflected in %hn-DNA as well as the NDF values.

Table 3: Comparison of % halo nucleus DNA (%hn-DNA) and nuclear diffusion factor (NDF) between treatment groups of MDBK cells

Groups	1	2	3	4	5	6
% hn-DNA	73.92 ± 1.76^{def}	74.68 ± 1.54^{ef}	30.46 ± 3.74^{a}	64.78 ± 2.34^{cd}	81.71 ± 2.31^{f}	42.03 ± 4.12^{b}
NDF	0.76 ± 0.06^{a}	0.87 ± 0.07^{a}	10.67 ± 2.43^{d}	1.59 ± 0.16^{ab}	0.63 ± 0.06^{a}	4.21 ± 0.54^{c}
Groups	7	8	9	10	11	
% hn-DNA	50.20 ± 4.56^{b}	45.60 ± 4.30^{b}	41.68 ± 2.41^{b}	63.53 ± 2.86^{c}	67.43 ± 2.56^{cde}	
NDF	3.64 ± 0.81^{c}	2.89 ± 0.36^{bc}	3.54 ± 0.34^{c}	1.52 ± 0.18^{ab}	1.23 ± 0.14^{ab}	

Values (Mean ± SEM, n=3) bearing different superscripts in the same row differ significantly ($p<0.05$) in one-way ANOVA with Duncan's post hoc analysis.

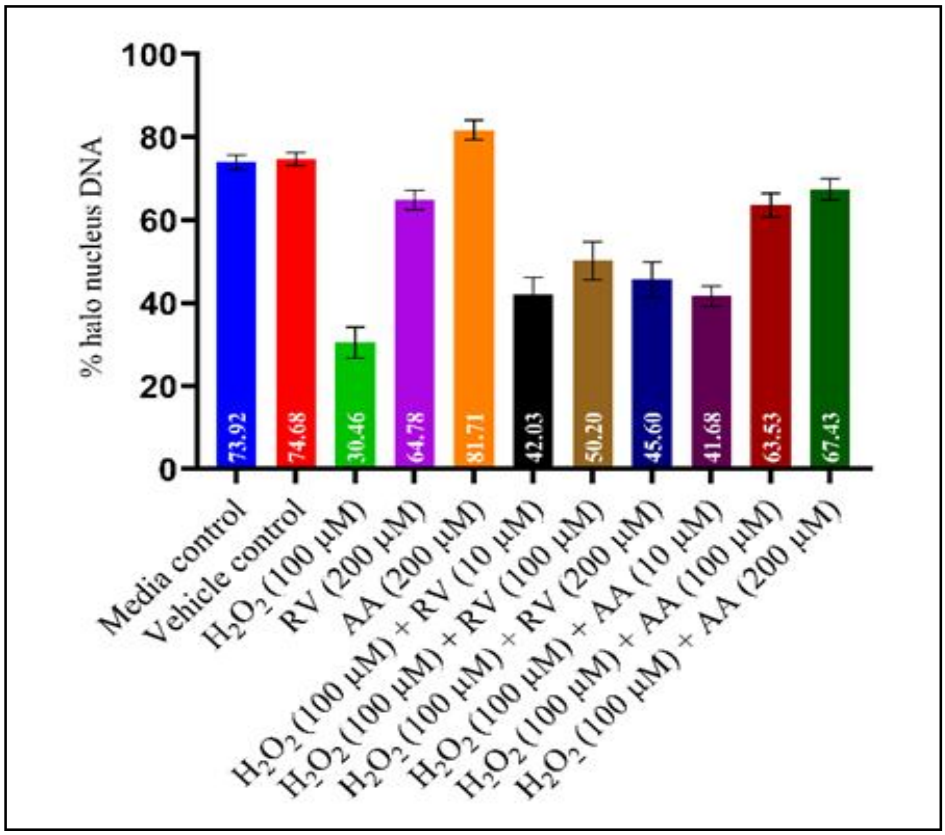

Figure 3: Comparative histogram (Mean ± SEM) %hn-DNA values among different treatment groups.

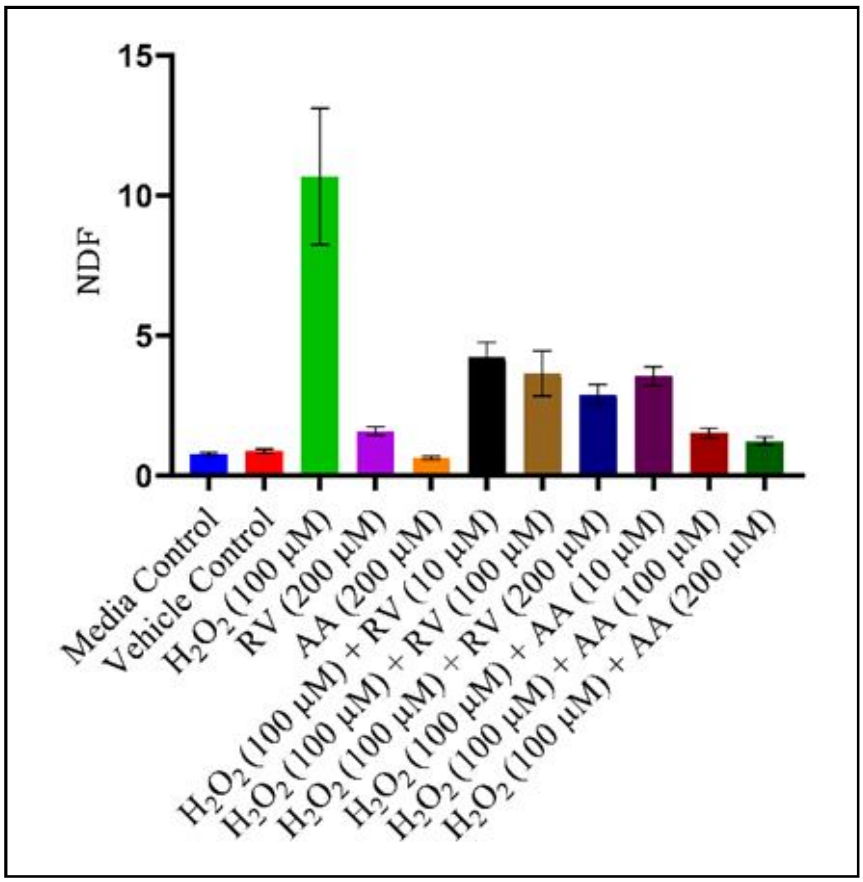

Figure 4: Comparative histogram (Mean ± SEM) NDF values among different treatment groups.

4. Discussion

Antigenotoxic potential of RV against xenobiotics has been reported and the results are consistent with earlier published data, where a study by Konyalioglu *et al*. (2013) demonstrated that the RV treatment can help to prevent the H_2O_2 induced DNA damage at different concentrations (100, 150 and 250 µM). Further, RV at 0.5 µM was effective in protecting human lymphocytes against radiation induced DNA damage (Dobrzyñska and Gajowik, 2021). When HL-60 cells were co-treated with genotoxicants nitroquinoline-1-oxide (NQO) and mitomycin C (MMC) with three concentrations of RV. RV showed maximum genotoxic inhibition with lowest test concentration against NQO and MMC mediated genotoxicity (Abraham *et al*., 2016). RV exposure antagonize the ability of AFB_1 to cause DNA damage such as sister chromatin exchange and chromosomal aberration in cultured human lymphocytes (Türkez and Sisman, 2012). RV administered intraperitoneally in adult male mice two hours prior to subjecting them to a single dose of γ-irradiation (2 Gy) to the whole body, protected them from the harmful effects of radiation caused DNA damages as evident from the alkaline comet assay of their peripheral blood lymphocyte (Koohian *et al*., 2017). Administration of RV also showed significant positive effects inprevention of Zearalenone (ZEA)-induced cell damage. The mechanism behind this involves the activation of $FOXO_3$a-mediated pathways and also upregulation of SIRT1 gene there by augmenting the resistance of cells against the oxidative stress caused by ZEA exposure (Lv *et al*., 2024). These effects $FOXO_3$a of RV could be due to the free radical scavenging activity, thereby further supporting reported literature on its antioxidant, antiproliferative, and anti-inflammatory properties (Vasanthkumar *et al*., 2024).

Antigenotoxic potential of ascorbic acid also have been reported through different study parameters by earlier co-workers. A study in H2T9 cells showed that preincubation with vitamin C at 10 µM and 25 µM for 1 h decreased genotoxic effect induced by H_2O_2 (Kontek *et al*., 2013), whereas, 100 µM of ascorbic acid alone or in combination with vitamin E was more effective in mitigating H_2O_2 induced oxidative DNA damage (Milev *et al*., 2022). Antigenotoxic effect of ascorbic acid and resveratrol against glyphosate-induced genetic damage has been studied in human lymphocytes and in erythrocytes of *Oreochromis niloticus* and *Ambystoma mexicanum*. Simultaneously, exposure with various concentrations of ascorbic acid and RV in glyphosate toxicity, these antioxidants preserved the integrity of DNA through antioxidant capacity and contributed antigenotoxic property (Alvarez-Moya *et al*., 2022)

In cyclophosphamide (CP)-induced genotoxic damage mice model, vitamin C and N-acetylcysteine (NAC) showed protective effects in exfoliated bladder cells. Both the agents decreased the CP-mediated micronuclei frequencies in bladder cells by 41-71%. This could be due to their ability to act as precursors of glutathione, antioxidant and, nucleophilic properties (Gurbuz *et al*., 2009). In a study, human hepatic carcinoma (HepG2) cells exposed to triphenyltin (TPT) and ascorbic acid (50, 100, and 200 µM) for 24 h. Results of study revealed that ascorbic acid mitigate the cytotoxicity, oxidative stress,

apoptosis, and genotoxicity associated with TPT (Alkahtane, 2020). Ascorbic acid has got free radical scavenging capacity, which helped in protecting the lymphocytes from radiation-induced DNA damage and subsequent genotoxicity in X-ray-irradiated mice (Koohian *et al*., 2021). Coming to the present study, the exposure to H_2O_2, a popular method to induce oxidative DNA damage, was used and the successful induction of DNA damage in cells was evident by the increase in NDF values and decreased %hn-DNA. Moreover, treatment with antioxidants (RV and AA) was found to be mitigate the oxidative stress, thereby decreasing DNA damage and conserve DNA integrity.

The identification of DNA breaks and measurement of the repair in mammalian cell represent primary endpoints for genotoxicity testing. Many sensitive techniques have been introduced for this purpose to increase the knowledge in the area of genetic toxicology (Sestili, 2009). This is due to introduction of large number of new synthetic molecules and adoption of more stringent chemical regulations and their risk reduction policies (Sestili *et al*., 2017). These new techniques are more sensitive, flexible and less complicated. The FHA is a very recent assay method introduced to detect DNA-strand breakage and DNA repair at the single-cell level. FHA is comparatively a reliable, sensitive and flexible assay as that of its well-established counterpart, the comet assay, but it is more rapid, less expensive thereby allowing oneself to perform a large number of genotoxicity tests for a number of compounds at once. Sensitivity and rapidity of FHA has been studied by checking genotoxic effect of inhalation anaesthetic sevoflurane (SVF) in peripheral blood lymphocytes (PBL) using comet assay and FHA. It has a completely different principle than comet assay, even then it was conveniently utilized for the assessment of DNA single strand breakage in individual mammalian cells (Kadioglu *et al*., 2009). FHA was found to be an effective and rapid tool for detecting DNA breaks in a high-throughput manner, even though it is less popular than established method, the comet assay.

5. Conclusion

It is pretty clear from present study FHA is suitable for initial screening of large number of genotoxicants where detailed information about DNA damage is not required. For more in-depth analysis, researchers have to rely on methods that provide higher resolution and specificity, such as the comet assay, γ-H2AX foci assay, and next-generation sequencing techniques. All of the mentioned studies used the well-established alkaline comet assay for evaluation of the protective efficacy of the antioxidants and the use of fast-halo assay seems to be unpopular for evaluation of genotoxicity even though was found to be reliable fast screening and time saving study tool. Our current study proposes that FHA can be considered as a screening tool for genotoxicity study. Preliminary evidence from this study also demonstrates the antigenotoxic effects of RV and AA and proposes their usage as promising candidates for controlling environmental and non-environmental chemical exposure mediated DNA damage. Further studies involving multiple cell types along with varied genotoxicants and understanding the molecular mechanisms behind the protective effect may further cement the role of RV and AA as antigenotoxic agents.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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