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Genoprotective and DNA Repair Activities of Fruit Pulp Ethanol Extract from *Detarium microcarpum* Guill. and Perr. (Caesalpiniaceae)

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Abstract: DNA is continuously degraded by numerous genotoxic agents including intracellular reactive oxygen species produced by cell metabolism and exogenous environmental pollutants. These genotoxic agents destroy the DNA integrity leading to carcinogenesis or cell death. An increased consumption of vegetables, fruits and other foods rich in antioxidant compounds can protect DNA from oxidative damage and prevent cell carcinogenesis. This study was designed to investigate in vitro the genoprotective and DNA repair activities of the fruit pulp ethanol extract from *Detarium microcarpum* against two known mutagenic agents such as hydrogen peroxide and methyl methane sulfonate. To assess the genoprotective and DNA repair activities of extract, human lymphocytes in culture were treated with the extract before or after the genotoxic agent exposing. The amount of DNA damages was assessed by using the standard comet assay. The fruit pulp extract in concentration up to 500 µg/mL, compared to vehicle didn't affect the integrity of DNA. Interestingly, the genotoxic effects of hydrogen peroxide and methyl methane sulfonate on human lymphocytes were significantly reduced by the extract pre-treatment. In addition, the DNA damages induced by hydrogen peroxide and methyl methane sulfonate were repaired further to the extract addition. The fruit pulp ethanol extract from *Detarium microcarpum* contains bioactive compounds that can preserve the integrity of DNA from the deleterious effects of genotoxic agents. The daily intake of this fruit pulp as food supplement could prevent DNA damages and carcinogenesis.

Keywords: *Detarium microcarpum*, Genoprotection, DNA Repair, Hydrogen Peroxide, Methyl Methane Sulfonate

1. Introduction

The human body and cells are daily exposed to the harmful effects of many environmental toxic agents such as ionizing radiations, viruses, dyes, pesticides, smokes and heavy metal [1]. These agents, if they are not scavenged by the cellular antioxidant system, could cause severe damages to cellular biomolecules such as proteins, membranes and nucleic acid. DNA damages include single or double strand breaks, cross links, bases modification and apurinic/apyrimidinic lesions formation [1]. Many cellular dysfunctions result from these biochemical damages including excess in cell proliferation, cell death by apoptosis, lipid deposition and mutagenesis. By creating such disorders, environmental toxic agents are partly responsible for aging and age-related diseases as cancer, cardiovascular disorders and neurodegenerative diseases. In certain situations, endogenous antioxidant systems fails to nullify the environmental toxic agents. In these conditions, exogenous chemopreventive compounds extracted from plant derived foods are required to prevent biomolecules oxidative damages and to maintain cell hemostasis

Two genotoxic agents such as hydrogen peroxide and methyl methane sulfonate involving different oxidative damage mechanisms were used in this study. Hydrogen peroxide produced in cells as a by-product of oxidative metabolism, is normally reduced to water by catalase,

glutathione peroxidases and peroxy-redoxins. When reduction mechanisms are not sufficient, hydrogen peroxide can react with transition metals (iron, copper) and via the Fenton reaction, they together produce highly reactive hydroxyl radical which attacks DNA at the sugar residue of the DNA backbone leading to DNA single-strand breaks [2]. It also transform purines and pyrimidines to their corresponding hydroxyl derivatives, such as 8-hydroxyguanine [3]. According to MMS, it is known as a monofunctional alkylating agent of direct action. This compound interacts directly with DNA and transfer charged methyl group or ethyl radicals leading to base substitution and mutation [4].

According to the spread of environmental genotoxic agents and their harmful effects on biomolecules, it become necessary to find compounds able to protect DNA from the deleterious effects of these genotoxic agents. New ways of research have to be now explored to create more specific and gene targeted molecules able to protect/repair DNA damages. Many epidemiological studies suggested that increased consumption of vegetables, fruits and other foods that contain antioxidant compounds can protect DNA from oxidative damage and prevent carcinogenesis [5]. This biological property of fruits has been attributed to common dietary compounds contained in fruits such as flavonoids, tannins, vitamins, carotenoids and steroids [6,7]. The fruits of *D. microcarpum* are well-known in West Africa for its nutritional and therapeutic values [8]. Fruit pulp is rich in vitamin C, protein, carbohydrate and in mineral contents such as iron, calcium, magnesium and sodium [9]. Antifungal and antioxidant phytomolecules such as clerodane diterpenes have been isolated from the fruit pulp [10]. Water extract of the fruit pulp showed antibacterial activities [11]. Our previous studies demonstrated the free radical scavenging property and the human lymphocytes protective potent of the fruit pulp of D. microcarpum [12] suggesting that fruit pulp of *D. microcarpum* could contain effective DNA protective/repair compounds. The current investigation was designed to evaluate in vitro, the genoprotective and DNA repair activities of the fruit pulp ethanol extract from *D. microcarpum*.

2. Materials and Methods

2.1. Plant Material Collection and Extraction

Fresh fruits of *Detarium microcarpum* Guill. and Perr. (Caesalpiniaceae) were collected in January 2013 in Gampela (25 km, east of Ouagadougou, Burkina Faso). The plant identity was assessed by Professor Jeanne MILLOGO-RASOLODIMBY from Department of Plant Biology and Physiology (Université Ouaga I Pr Joseph KI-ZERBO, Burkina Faso) and a voucher specimen (CI: 15928) deposited in the herbarium of the University Ouaga I Pr Joseph KI-ZERBO. The fruit pulp was powdered and extracted by repeated maceration in ethanol at room temperature under continuous stirring. Extract was concentrated to dryness.in a vacuum evaporator and stored at 4°C until further investigati

2.2. Chemicals and Reagents

Trizma base, triton-X, ethylene-diamine tetra acetic acid (EDTA), low melting point agarose (LMPA), agarose (NMPA), propidium iodide, dimethyl sulfoxide (DMSO), sodium hydroxide, sodium chloride, RPMI 1640, methyl methane sulfonate, histopaque 1077, trypan blue, phosphate buffer saline, gentamycin and fetal bovine serum were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide, ascorbic acid and ethanol were supplied by Labosi (Paris, France).

2.3. Blood Collection and Lymphocytes Isolation

Peripheral blood samples from healthy volunteer donors (with an average age of 28), that did not smoke, drink, or use chronic medication were collected after 12-h overnight fasting by venipuncture using a top Vacutainer® (BD Diagnostics, Plymouth, UK). The study was approved by the Ethic Committee of the Regional Center of Blood Transfusion of Ouagadougou (Burkina Faso) under the reference agreement N°2015-340/MS/SG/CNTS/CRTS-O. Peripheral blood lymphocytes were isolated under sterile conditions by using a density gradient present in the reagent histopaque 1077 according to Gafrikova et al [1]. Blood (5 mL) was taken in eppendorf tubs and mixed with the same volume of phosphate buffer solution. Histopaque 1077 (100 µL) was underlayed and tubs were spun at 800 ×g for 30 min at 4°C. Lymphocytes (100 µL) were retrieved from just above the boundary between the phosphate buffer and histopaque, pipetted into new eppendorf tubes with 1 mL of PBS and spun again at 800 ×g for 5 min at 4°C. Supernatant was removed and the lymphocytes were counted in a Neubauer chamber using trypan blue exclusion principle (0.4%). cell viability of about 98% was accepted for further experiments. Lymphocytes were routinely cultured in culture media containing 1 mL RPMI 1640 supplemented with 10% fetal bovine serum and 1 mM of gentamycin. Cells were maintained in suspension culture at 37°C in a humidified 5% CO_2 atmosphere.

2.4. Genoprotective and DNA Repair Assays

2.4.1. Lymphocytes Treatment

Human lymphocytes were treated according to the method described previously [3].

To assess the genotoxic effect of extract on human lymphocytes, lymphocytes (2.10^5cellules/mL) were incubated with extract at different concentration or with vehicle (Culture medium, RPMI-1640) for 1 h and amount of DNA damage was recorded.

For the genoprotective assay, lymphocytes were pre-incubated with extract at different concentration for 30 min followed by the addition of genotoxic agents (Hydrogen peroxide, 100 µM for 30 min or methyl methane sulfonate, 200 µM for 2 h).

To evaluated the DNA repair activity of extract, DNA

damages were induced by exposing lymphocytes to H_2O_2 (100 µM) for 30 min or to methyl methane sulfonate (200 µM) for 2 h. Extract at different concentrations was added to the medium for 30 mins and DNA damages were analyzed.

2.4.2. Comet Essay

At the end of the appropriate incubation time, lymphocyte samples were collected by centrifugation (800 g, 5 min, 4°C) and incorporated in low melting point agarose (1%) for comet assay as described previously [13]. Eighty microliter of the mixture was embedded on the top layer of a frosted slide with the normal agarose (1%) and covered with a cover glass in duplicate. After solidifying in a refrigerator for 5 min, only the cover glass was removed. Slides were dipped in a fresh lysing solution (1% Triton X-100, 10% DMSO, 2.5 M NaCl, 0.1 M EDTA) and stored at 4°C for 1 h in the dark. Slides were removed from the lysing solution and placed in an electrophoresis buffer during 20 min in the dark to unwind the DNA. Electrophoresis was started at a constant current of 300 mA and 25 V for 30 min. After neutralization in an appropriated buffer (0.4 M trizma base, pH 7.5), the slides were dried and DNA was stained with propidium iodide. Fluorescence-stained slides were examined with a fluorescence inverted microscope (Carl Zeiss, Germany) equipped with a camera connected to a computer. Images of 150 randomly selected nuclei (50 nuclei from three replicated slides) for each treatment were analyzed using CometScore software (version 1.5 of TriTek Corporation) according to Kumaravel and Jha [14]. The amount of DNA damages in nucleus was expressed in percentage DNA in tail (%) and olive tail moment (arbitrary unit).

2.4.3. Statistical Analysis

Experiments were performed in triplicate and data presented as mean value ± standard deviation. GraphPad software (GrapPad Software Inc. San Diego, CA, USA) was used for statistical analyses. The one-way ANOVA for repeated measures followed by Newman-Keuls post-test was used to verify the impact of treatments on DNA damages. P value <0.05 was considered as being significant.

3. Results

To investigate the effect of *D. microcarpum* fruit ethanol extract on DNA integrity (genotoxicity), cells were treated with vehicle or with fruit pulp extract (100 to 500 µg/mL) and comet parameters were reported (Figures 1). Considering the percentage of DNA in tail and the olive tail moment reported in vehicle treatment, a weak DNA damage was observed. Furthermore, the fruit extract of *D. microcarpum* (100 and 200 µg/mL) did not affect significantly ($p>0.05$) the integrity of DNA when the amount of DNA damage was compared to the vehicle treatment. Interestingly, fruit pulp extract (400 and 500 µg/mL) compared to the vehicle, ameliorated significantly ($p < 0.05$) the integrity of DNA. These finding suggested that the ethanol extract of *D. microcarpum* fruit pulp (100 to 500 µg/mL) did not exhibit genotoxic effects in human lymphocytes but reduced the DNA baseline damages caused by the cellular normal metabolism.

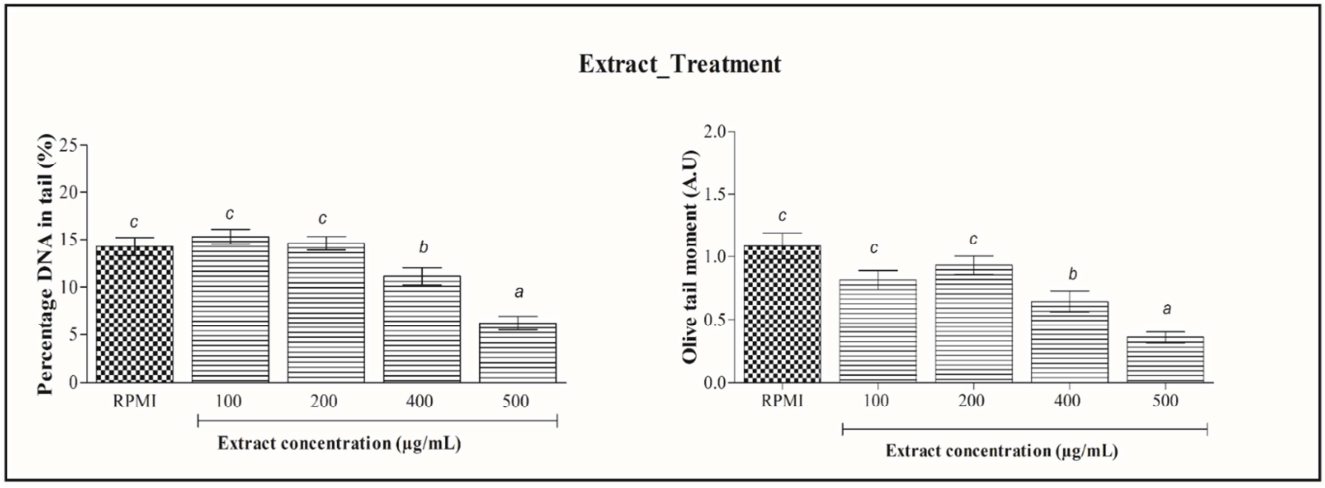

Figure 1. *Genotoxic activity of Detarium microcarpum fruit pulp extract.*

Values are expressed as mean values ± SEM (n=3 independent experiments). abcvalues within each histogram with different superscripted letters differ significantly (P>0.05) as determined by using analysis of variance. A.U = Arbitrary Unit.

To assess the genoprotective effect of the ethanol extract of *D. microcarpum* fruit pulp, DNA damages were induced by hydrogen peroxide or by methyl methane sulfonate followed by vehicle or by fruit extract treatment (Figure 2a and 2b). As expected, significant DNA damages in human lymphocytes ($p < 0.05$) were observed in cells treated with hydrogen peroxide alone (figure 2a) or with methyl methane sulfonate alone (figure 2b). However, when lymphocytes were pre-incubated with extract (100 to 500 µg/mL) followed by hydrogen peroxide or by methyl methane sulfonate addition, the amount of DNA damages decreased gradually with increasing extract concentrations. This finding suggested that extract could prevent the genomic damages induced by hydrogen peroxide and methyl methane sulfonate in human

lymphocytes. Similar results were observed with the standard ascorbic acid. Remarkably, extract exhibited higher genoprotective activity against methyl methane sulfonate than ascorbic acid ($p < 0,05$) while extract and ascorbic acid showed the same genoprotective effect in hydrogen peroxide induced genomic damages ($p > 0,05$). Moreover, extract at 500 µg/mL concentration compared to the control, showed a total protective activity against the genotoxicity of methyl methane sulfonate (figure 2b). All these findings suggest that fruit pulp ethanol extract from *D. microcarpum* protected in dose-dependent manner, the genotoxicity of hydrogen peroxide and methyl methane sulfonate in human lymphocytes in vitro.

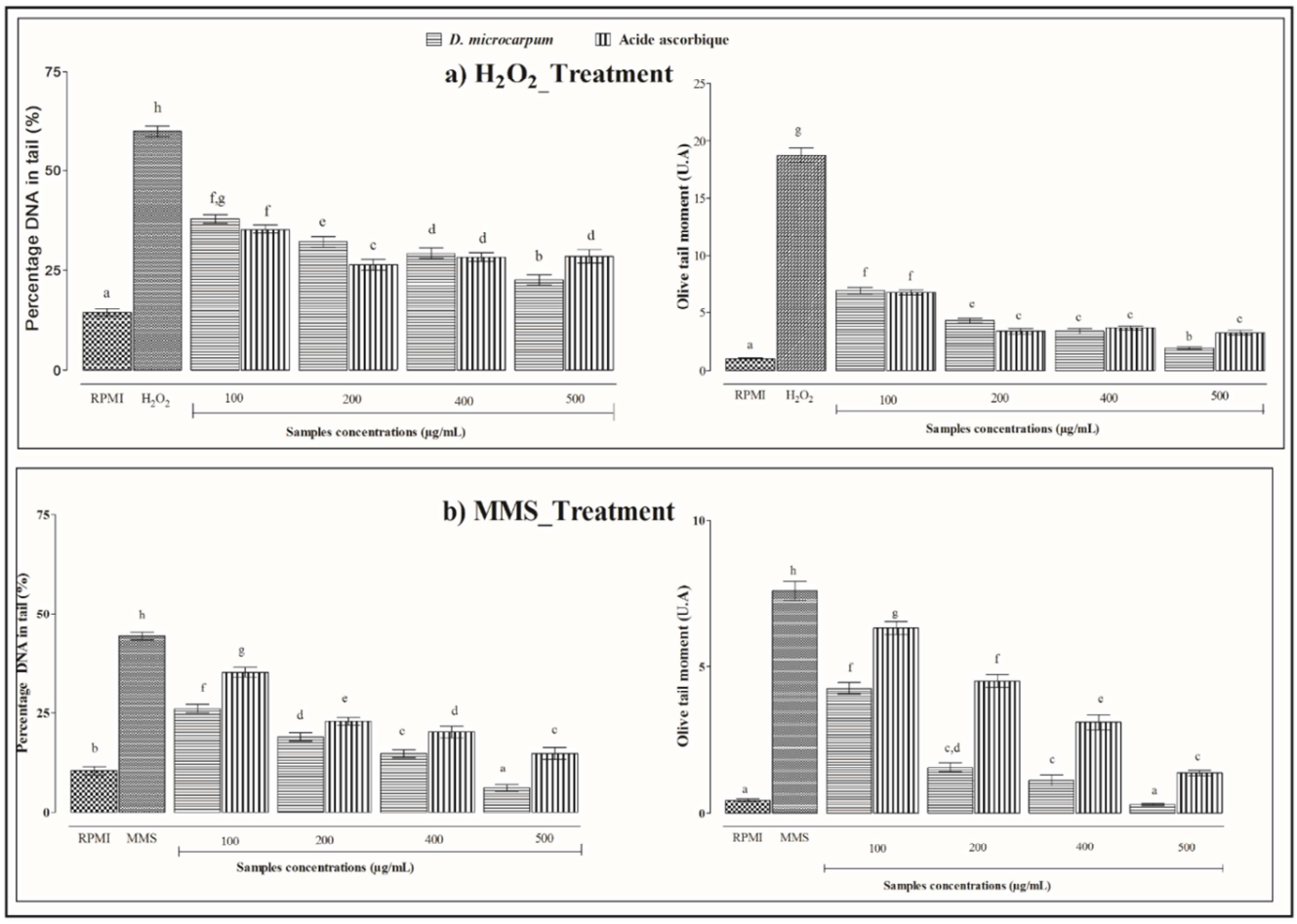

Figure 2. *Genoprotective activity of Detarium microcarpum fruit pulp extract.*

Values are expressed as mean values ± SEM (n=3 independent experiments). ^{abcdefgh}values within each histogram with different superscripted letters for each genotoxic agent applied differ significantly ($P>0.05$) as determined by using analysis of variance. A.U = Arbitrary Unit; H_2O_2 = Hydrogen peroxide; MMS = Methyl Methane Sulfonate.

To investigate the DNA repair activity of extract, genomic damages were firstly induced by hydrogen peroxide or by methyl methane sulfonate followed by extract addition and DNA damages were reported considering percentage DNA in tail (%) and Olive tail moment (A.U) (Figure 3). DNA damages induced by hydrogen peroxide (figure 3a) or by methyl methane sulfonate (figure 3b) were repaired by extract treatment. According to the amount of genomic damages reported in vehicle treatment (Figure 3a and 3b), a total repair activity of extract at 400 and 500 µg/mL was observed. In general, ascorbic acid induced more DNA repair process than extract. Extract at 500 µg/mL ameliorated the integrity of DNA compared to vehicle ($P < 0.05$) suggesting that in absence of exogenous genotoxic agents, extract could repair the genomic damages caused by the normal metabolism of lymphocytes. Furthermore, DNA damages induced by methyl methane sulfonate were more repaired by extract than those induced by hydrogen peroxide.

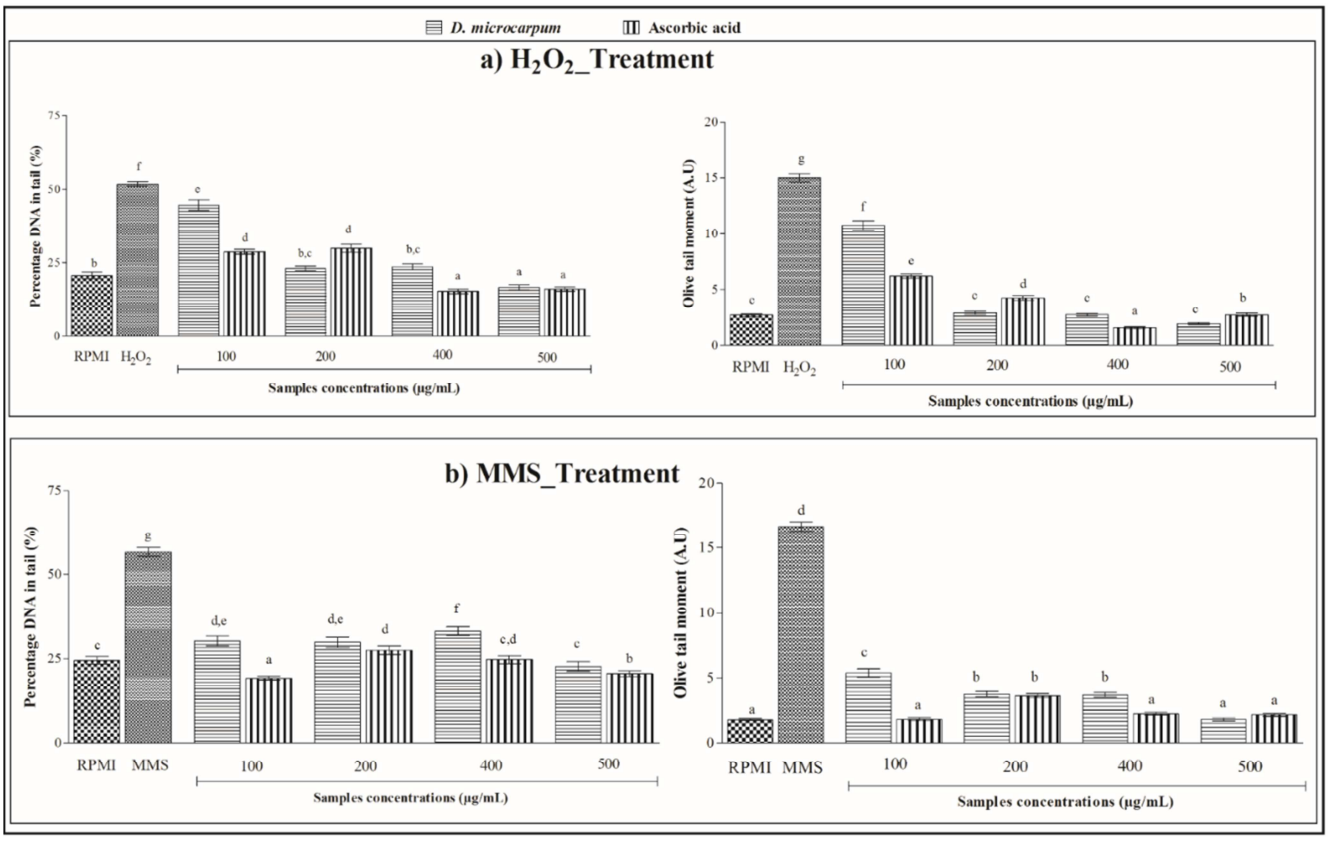

Figure 3. *DNA repair activity of Detarium microcarpum fruit pulp extract.*

Values are expressed as mean values ± SEM (n=3 independent experiments). ^{abcdefg}values within each histogram with different superscripted letters for each genotoxic agent applied differ significantly (P>0.05) as determined by using analysis of variance. A.U = Arbitrary Unit; H_2O_2 = Hydrogen peroxide; MMS = Methyl Methane Sulfonate.

4. Discussion

Hydrogen peroxide is a potent genotoxic agent able to induce oxidative DNA damage, including DNA-strand breakage and bases modifications [3].The genotoxic effects of ROS, in particular H_2O_2 that induces lesions similar to those resulting from ionizing radiation in human lymphocytes, has been well documented [2].Hydrogen peroxide is converted to the hydroxyl radical which is expected, due to its high reactivity, to cause oxidative damage close to the site of its formation [15]. Oxidative damages can occur to macromolecules such as proteins, DNA and lipids. DNA base alterations, strand breakage, and mutations are the results of free radical attacks on DNA [16]. In previous studies, it has been demonstrated that these damages can be stopped, reduced, and even reversed with antioxidant supplementation [5]. The protection of biomolecules from oxidative damages induced by free radicals is vital for the cell hemostasis. Crude extracts, fractions or purified antioxidant compounds from plant have exhibited in previous studies, DNA protection activities against genomic oxidative damages [7]. The antioxidant properties of the fruit pulp of D. microcarpum are well documented that could justify its genoprotective effect in this study against genomic oxidative damages induced by hydrogen peroxide [17]. The genoprotective activity of ascorbic acid against hydrogen peroxide observed in this study is an accord with previous studies [18]. Fruit pulp of *D. microcarpum* is rich in ascorbic acid that could be in partly responsible of the genoprotective activity of extract.

The MMS is a well-known DNA alkylating agent that induces mono adducts and crosslinks on DNA causing base substitution and mutations [19]. It is a mono functional alkylating agent of direct action. This chemical interacts directly with DNA and transfers charged methyl group and ethyl radicals [19]. MMS showed high SN2 reactivity causing N-alkylation of purines (N7-methylguanineand N3-methyladenine) [20]. This finding suggested that N-methylation events are responsible of the clastogenic effects of MMS. The protective effect of fruit pulp extract against the genotoxicity induced by MMS may be related to the ability of extract to induce enzymes expression that inactive the mutagen before it comes into contact with DNA. Furthermore, previous studies reported that the alkylating agents provide in mammalian cells, the depletion of the enzyme glutathione S-transferase leading to oxidative stress [21]. The depletion of glutathione S-transferase can cause the accumulation of reactive oxygen species (ROS) as by-products of normal cellular function which can compromise cellular antioxidant defenses [21].The generation of ROS may represent an alternative pathway in the genotoxicity

induced by MMS. In this respect, the antioxidant property of fruit pulp extract may involve a reduction of the alkylation damage induced by MMS. Extract could reduce the alkylating damages of DNA by intercepting the free methyl groups produced by MMS. So, it has been demonstrated that polyphenols contained in fruits may play the role of methyl or ethyl group's interceptors and therefore protect DNA against alkylating agents [16].

Fruit pulp extract exhibited also a DNA repair activity on genomic damages induced by hydrogen peroxide and methyl methane sulfonate suggesting that extract can enhance the DNA repair process in human lymphocytes. Polyphenols in blueberry such as anthocyanins, through the ATM/ATR pathway induced the expression of P_{53} and P_{21} proteins and therefore increase DNA repair activity in vertebrate somatic cells [22]. The ethanol extract from *D. microcarpum* fruit pulp may increase the expression of P_{53} by a post or a pre transcriptional regulation. DNA repair mechanisms differ according to the nature of damages. Previous studies reported that DNA polymerase β is required for efficient DNA strand break repair induced by MMS but not by H_2O_2 [23]. The DNA repair activity of extract in MMS induced genomic damage may be due to the propriety of extract to increase the activity of DNA polymerase β. Further investigations are necessary to understand the molecular mechanism of DNA repair activity of the fruit pulp of *D. microcarpum*.

5. Conclusion

This study highlighted the DNA protective/repair activity of the fruit pulp ethanol extract from *D. microcarpum*. Extract didn't affect the DNA integrity of human lymphocytes in vitro but protected in dose-dependent manner, the genomic damages induced by hydrogen peroxide or by methyl methane sulfonate. Furthermore, fruit pulp extract stimulated DNA repair enzymes expression. The dietary intake of *D. microcarpum* fruit pulp could prevent DNA damages and carcinogenesis induced by genotoxic agents.

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Author Disclosure Statement

The authors declare that no competing financial interests exist.

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