Full Length Research Paper

Anti-hemolytic and reduction of lipid peroxidation capacities of *Detarium microcarpum* Guill. and Perr. fruits

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This study was designed to investigate the ability of ethanol fruit extract from *Detarium microcarpum* to protect erythrocytes against hemolysis and lipid peroxidation. To achieve this objective, hemolytic, anti-hemolytic and lipid peroxidation from cell membrane assays were used. Hemolytic and anti-hemolytic activities (regarding H_2O_2 induced hemolysis) were assessed by determination of free haemoglobin at 540 nm. Inhibition of lipid peroxidation was measured at 532 nm, using thiobarbituric acid reaction on sodium nitroprusside and ferric sulfate induced liposome peroxidation models. *D. microcarpum* fruit ethanol extract (100 µg/ml) did not exhibit any hemolytic effect but reduces significantly hemolysis from human and rat erythrocyte with inhibitory percentages more than 50 and 75%, respectively. Furthermore, the extract caused a significant decrease in both ferric sulfate and sodium nitroprusside inducing lipid peroxidation in each rat tissue liposomes investigated. *D. microcarpum* fruit ethanol extract **protects** erythrocytes against hemolysis and lipid peroxidation, probably due to its antioxidant potential. Therefore, animal tissues disorders caused by cell membrane lipid damage could be potentially managed/prevented by dietary intake of *D. microcarpum* fruit pulp.

Key words: *Detarium microcarpum*, anti-hemolytic activity, inhibition of lipid peroxidation

INTRODUCTION

A positive link has been established between fruit consumption and the reduction of chronic diseases (Sumathy et al., 2013). Antioxidant compounds from fruits have been thoroughly studied in this focus, owing to their beneficial effects on human health (Seifried et al., 2007; Rauchovà et al., 2012). Antioxidant molecules protect tissues against oxidative stress and related diseases (Badmus et al., 2013) through several mechanisms, including the elimination of free radicals, the protection and regeneration of other dietary antioxidants (e.g. vitamin E) and the chelation of pro-oxidant metals (Lima et al., 2014).

Detarium microcarpum Guill. and Perr. (Caesalpiniaceae) is a well-known wild edible fruit specie

growing in Saharan and sub-Saharan countries. Its fruits are traditionally consumed as food source and for medicinal purpose (Wahedi et al., 2013). Hence, *D. microcarpum* is used in the treatment of diverse diseases, notably syphilis, dysentery, diarrhea, bronchitis, pneumonia, sore throat, malaria, leprosy and meningitis (Akah et al., 2012) while the fruits are used to treat skin infections, meningitis and malaria (Bamisaye et al., 2014). Fruit pulp extract of *D. microcarpum* showed an inhibition of the growth of the plant pathogenic fungus, *Cladosporium cucumerinum* and of the enzyme acetylcholinesterase, implicated in Alzheimer's disease (Cavin et al., 2006).

Studies on the nutritional values and the antioxidant properties of *D. microcarpum* fruit pulp are well documented. A remarkable nutritional value with important content of protein, vitamins, carbohydrates and mineral nutrients have been reported (Abreu et al., 2002; Bamisaye et al., 2014; Mariod et al., 2009; Oibiokpa et al., 2014). High content of flavonoid and polyphenols have been also reported along with a strong antioxidant capacity (Lamien-Meda et al., 2008). Additionally, *D. microcarpum* fruit pulp is benefitial to food digestibility and body weight while hematological parameters (red and white cells) are ameliorated (Obun et al., 2011; Wahedi and David, 2013).

The present investigation focused on the capacity of the fruit pulp from *D. microcarpum* to protect erythrocytes against hemolysis and lipid peroxidation damage mediated by pro-oxidant.

MATERIALS AND METHODS

Plant material collection and extraction

Fresh fruits from *D. microcarpum* Guill. and Perr. (Caesalpiniaceae) were harvested in January 2013 at Gampela (25 km, east of Ouagadougou, Burkina Faso). Botanical identity was assessed by Professor Jeanne MILLOGO-RASOLODIMBY from Laboratoire de Biologie et écologie végétale (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. Fresh fruits were washed with distilled water and pulp manually scraped prior soaking in ethanol (24 h, 25°C room temperature, continuous stirring). Extract was filtrated, concentrated to dryness in a vacuum evaporator and stored at 4°C until further investigations.

Experimental animals

Three-month-old male Wistar rats, weighing between 190 and 250 g were provided by the animal housing facility of Research Institute of Health Science (Burkina Faso). They were maintained at approximately 25°C, on a 12 h light/12 h dark cycle, fed with standard laboratory food and water *ad libitum*. Animals handling procedures strictly conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, publication number 85-25, revised 1996) and approved by our institution, regarding the internationally accepted standard ethical guideline for laboratory animal use and care as described in the European Committee Guidelines (EEC, 1986).

Chemicals and reagents

Gallic acid, sodium nitroprusside, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium phosphate dibasic (Na_2HPO_4), diethyl ether and sodium phosphate monobasic (NaH_2PO_4) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide, ascorbic acid, ferric sulfate and sodium chloride were supplied by Labosi (Paris, France). Ketamine and ethanol were purchased from Prolabo (Paris, France).

Anti-hemolytic activity

Blood collection

Human blood sample was provided by the National Center for Blood Transfusion, Ouagadougou, Burkina Faso. Peripheral blood sample obtained from a healthy volunteer (28 years of age that did not smoke, drink, or use chronic medication) was collected by venipuncture using a top Vacutainer® (BD Diagnostics, Plymouth, UK). Blood collection was in accordance with the declaration of Helsinki (as revised in 64th WMA General Assembly, Fortaleza, Brazil, October 2013). Blood collected in heparinized tubes was store at 4°C for erythrocytes suspension preparation. Rat blood was collected in heparinized tube by heart puncture. Animals were previously anesthetized with ketamine by intraperitoneal injection (60 mg/kg of b.w) before blood collection.

In vitro hemolytic and anti-hemolytic assays

The anti-hemolytic activity of fruit pulp extract was examined *in vitro* as previously described (Sasikumar et al., 2012). Erythrocytes were separated by centrifugation (1500 G, 10 min, 4°C) and washed with isotonic sodium phosphate buffer (pH 7.4) until the supernatant was colorless. Erythrocytes suspensions (2 ml, 2% in isotonic sodium phosphate buffer pH 7.4) were mixed to 0.5 ml of test samples (1 mg/ml in sodium phosphate buffer), 2 ml of sodium phosphate buffer and incubated (37°C, 5 min). Mixtures were subsequently incubated (37°C, 2 h) without or with 0.5 ml of H_2O_2 (100 mM in buffer) for hemolytic and anti-hemolytic investigations, respectively. After centrifugation (1500 G, 5 min and 4°C) supernatants were collected and the extent of hemolysis determined by recording the absorbance at 540 nm, corresponding to hemoglobin liberation. Results were expressed as inhibitory percentage (%) of erythrocytes hemolysis. Ascorbic acid and gallic acid were used as positive controls.

In vitro reduction of lipid peroxidation from cell membrane

Liposomes preparation

Rats were decapitated under mild diethyl ether anesthesia and pancreas, heart, brain, kidney and liver were rapidly dissected, placed on ice and weighed. Each tissue was subsequently homogenized in cold phosphate buffer saline (1/10, w/v). Homogenates were centrifuged (3000 G, 10 min and 4°C) and pellet discarded. Low-speed supernatants containing mainly water, proteins and lipids (cholesterol, galactolipids individual phospholipids and ganglioside) were kept as liposome preparations for thiobarbituric acid assays.
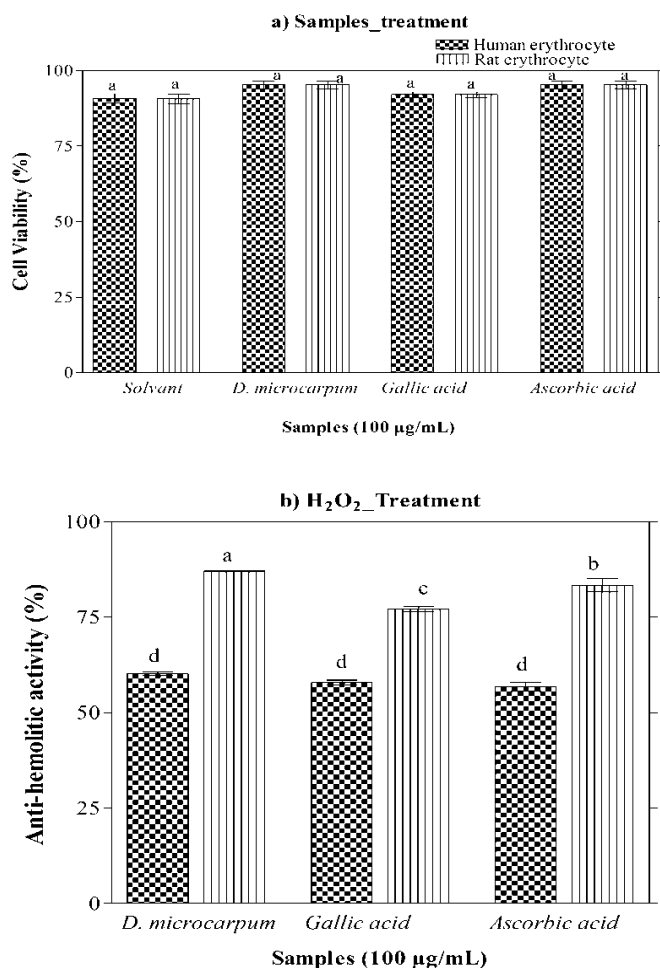
Figure 1. Hemollitic (a) and antihemolitic activity of fruit ethanol extract from *D. microcarpum* on hydrogen peroxide induced human and rat erythrocytes hemolysis (b). Data are expressed as the mean ± SD of triplicate independent experiments (n=3). Data with different superscrit letters (one-way ANOVA followed by Newman-Keuls post-test) are significantly different (P<0.05).

Thiobarbituric acid reactions

Liposomes were used to evaluate *in vitro,* the reduction effect of lipid peroxidation on Fe^{2+} induced-lipid peroxidation (Su et al., 2009) and sodium nitroprusside induced-lipid peroxidation (Akomolafe et al., 2012). Reaction mixture, containing test sample (100 μl, 1 mg/mL), hydrogen peroxide (100 μl and 10 mM), liposome preparation (700 μl) and ferric sulfate (100 μl, 10 mM) or sodium nitroprusside (100 μl, 50 mM), was incubated (37°C and 1 h). After incubation, trichloroacetic acid (1 ml, 15 %) and thiobarbituric acid (1 ml, 1%) were added to the mixture and boiled (100°C, 15 min). Mixture was centrifuged (3000 G and 10 min) and absorbance of supernatant measured at 532 nm. Results were expressed as inhibitory percentage (%) of lipid peroxidation. Ascorbic acid and gallic acid were used as positive controls.

Statistical analysis

Experiments were performed in triplicate and data presented as mean value ± standard deviation. GraphPad software (Graph Pad

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 erythrocytes hemolysis and lipid peroxidation. P <0.05 was considered as being significant.

RESULTS AND DISCUSSION

The ability of ethanol fruit extract from *D. microcarpum* to protect the integrity of cell membrane against oxidative damage was assessed using inhibitions of hemolytic and lipid peroxidation assays.

To investigate the effect of *D. microcarpum*, fruit pulp on cell membrane integrity (cytotoxicity), erythrocytes were treated with vehicle (control) or fruit extract (100 μg/mL) and erythrocytes hemolysis was measured (Figure 1a). It was found that *D. microcarpum* fruit pulp ethanol extract did not affect significantly (p>0.05) the integrity of cell membrane when hemoglobin liberation was compared with control. These results suggest that *D. microcarpum* fruit pulp ethanol extract (100 μg/mL) did not exhibit a cytotoxic effect on human and rat erythrocytes *in vitro*.

To assess the anti-hemolytic activity of *D. microcarpum* fruit pulp, human and rat erythrocyte were incubated with hydrogen peroxide after fruit pulp extract application (Figure 1b). It was found that fruit pulp extract exercise some protective activities on rat and human erythrocytes against the hemolytic effects of hydrogen peroxide. Moreover, rat erythrocytes were more protected by extract than human erythrocytes. Interestingly, *D. microcarpum* fruit extract exhibited statically similar anti hemolytic activity than the standard compounds in the human erythrocytes model (p>0.05) while it was more potent in protecting rat erythrocytes against hemolysis (p<0.05).

Inhibition of lipid peroxidation by fruit pulp extract on liposomes from rat organs using ferric sulfate and sodium nitroprusside induced-lipid peroxidation models were demonstrated (Figure 2). It was found that fruit pulp extract at 100 μg/mL reduce more than 60% lipid peroxidation of tissues homogenate in both iron ion and sodium nitroprusside induced lipid peroxidation assays. Regarding Figure 2a, lipid peroxidation of heart, kidney and brain liposomes (separately) induced by iron ion were more inhibited by fruit pulp extract than liver and pancreas liposomes. Similar results were observed with standard compounds. When sodium nitroprusside was used as toxin (Figure 2b), lipid peroxidation of brain, liver and pancreas liposomes were less inhibited by extract than heart and kidney liposomes. Similar results were also observed with gallic acid and ascorbic acid, used as positive control. Furthermore, extract showed some similar lipid peroxidation inhibition (p>0.05) than standards compounds. In general, heart liposome was the most protected against both iron ion and sodium nitroprusside induced lipid peroxidation and liver liposome was the list protected. Moreover, all tissues liposomes were more protected by extract against iron
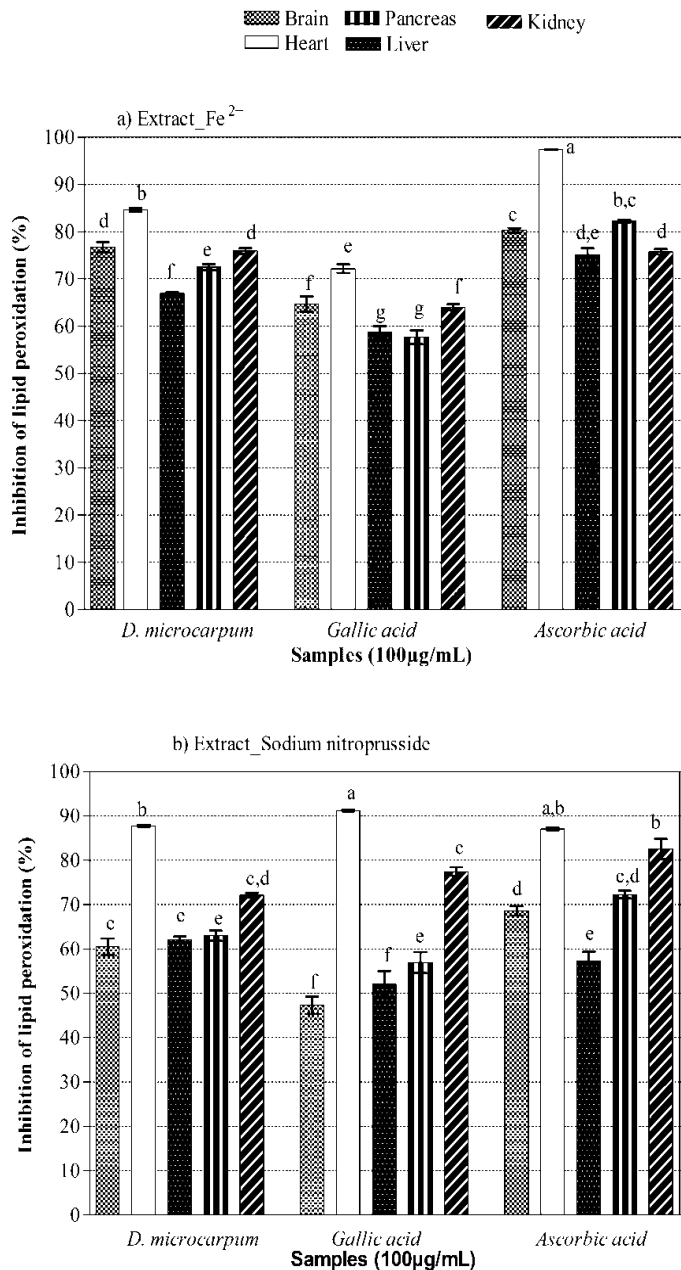
Figure 2. Protective effects of fruit ethanol extract of *D. microcarpum* on ferric sulfate (a) and sodium nitroprusside (b) induced lipid peroxidation in rat tissues homogenate. Data are expressed as the mean ± SD of triplicate independent experiments (n=3). Different letters in each toxin applicated assay represent significant differences using analysis of variance followed by Newman-Keuls post-test (*p* values < 0.05 were considered as significant).

ion induced lipid peroxidation than sodium nitroprusside. Together, these finding suggest that the capacities of fruit pulp ethanol extract from *D. microcarpum* to protect bio membrane from lipid peroxidation depend on the nature of pro-oxidant and the source of liposomes.

This study data demonstrated that fruit ethanol extract from *D. microcarpum* was an interesting radical fighter and seems benefitial in preventing cell membrane against oxidative damage. In the *in vitro* iron ion induced erythrocyte hemolysis model, hydroxyl radical is formed by hydrogen peroxide via Fenton reaction. This radical initiate the lipid peroxidation of bio membrane by electrophile attack, and the propagation of this peroxidation is conducted by alkoxyl and peroxyl radicals engendered, leading to haemoglobin liberation. Inhibition of hydrogen peroxide induced erythrocyte hemolysis by extract could be explained by the properties of extract to scavenge directly hydrogen peroxide by turning it into water molecule (James and Alewo, 2014) or to inhibit hydroxyl radicals formation via Fenton reaction, impeaching lipid peroxidation initiation (Su et al., 2009). Fruit extract could also stop lipid peroxidation propagation by alkoxyl and peroxyl radical scavenging. Verstraeten et al. (2004) reported in liposomes stability study, that flavonoids such as flavanols and related procyanidins can prevent the ferrous iron-mediated increase in membrane permeability. Together, these finding supported the hypothesis that these flavonoids can interact with the polar head group of lipids and consequently limit the incorporation of certain deleterious molecules that could affect membrane integrity and function. These finding suggested that the antihemolitic property of *D. microcarpum* fruit may be due in part to it richest in flavonoids content (Lamien-Meda et al., 2008). Rat erythrocytes were more protected than human erythrocytes against hydrogen peroxide toxicity according with previous studies (Pekiner, 2002). This author reported that membrane of rat erythrocyte was richer in lecithin contents than human erythrocyte, but the last one contained most rate of sphingomyelin. These finding suggested that sphingomyelin is more sensible to lipid peroxidation than lecithin. Regarding rat erythrocyte hemolysis test, extract showed higher erythrocyte protection than ascorbic acid. This finding suggested that ascorbic acid in certain conditions can exercise adverse effects on cell membrane stability. So, ascorbic acid in the presence of transition metals can react in turn to form radical, leading to cell membrane lipid peroxidation contributing to attenuate its membrane protective effect.

In iron ion and sodium nitroprusside induced liposomes lipid peroxidation model, iron ion can initiate liposomes lipid peroxidation by a direct one electron transfer on an unsaturated site in lipid carbon skeleton. Iron ion can also cause indirectly lipid peroxidation by hydroxyl radical formation via Fenton reaction and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Ojo et al., 2014). In the *in vitro* sodium nitroprusside induced lipid peroxidation model, the hydrolysis of sodium nitroprusside in physiological middle releases Fe^{2+} causing lipid peroxidation via Fenton

Reaction and the concomitant formation of oxide nitric radical can react together with other reactive oxygen species amplifying lipid peroxidation by secondary radical species formation (Akomolafe et al., 2012). The protected activity of extract against these two pro-oxidants could be explained by the properties of extract to chelate iron ion or to trap radical species formed by these pro-oxidants. Previously, demonstrated radicals scavenging effect of fruits from *D. microcarpum* could justify its lipid peroxidation inhibition properties (Lamien-Meda et al., 2008). Extract could also stop the propagation of lipid peroxidation by alkoxyl and peroxyl radicals' neutralization. Extract inhibited more lipid peroxidation induced by iron ion than sodium nitroprusside (Figure 2a and b). This finding may be due to the probable synergic effect of Fe^{2+} and nitric oxide released from the hydrolysis of sodium nitroprusside on lipid peroxidation mediation (Khan, 2014). The highest sensibility of liver and brain to these pro-oxidants toxicity may be due to the presence of abundant polyunsaturated fatty acids in these organs. So, brain and liver contains higher amount of polyunsaturated fatty than heart, pancreas and kidney justifying their vulnerability to lipid peroxidation (De et al., 2008). These two organs are equally, potential sources of reactive oxygen species with a considerable reduction of antioxydant level comparatively to the other organs exposing their liposomes to lipid peroxidation (De et al., 2008).

The use of many drugs is limited because of their cytotoxicity effects associated with cell membrane degradation and these actual results could encourage the dietary and medicinal intake of *D. microcarpum* fruit.

Conclusion

The ethanol extract of fruit pulp of *D. microcarpum* reduces significantly *in vitro*, lipid peroxidation of all liposomes mediated by iron ion and Sodium nitroprusside. It also reduces significantly the hydrogen peroxide-induced erythrocyte hemolysis. A part of the mechanisms through which the ethanol extractable phytochemicals in these fruits protect the testes from oxidative stress may be due to the presence of antioxidant compounds. The use of *D. microcarpum* as food additive could protect consumers against tissues chronic diseases associated with cell membranes destruction caused by free radicals mediated-lipid peroxidation.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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