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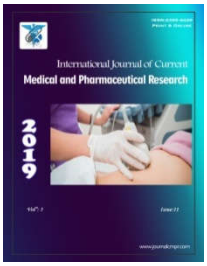

Research Article

BIOLOGICAL ACTIVITIES OF THE PULP OF *DEUTARIUM MICROCARPUM* FRUIT

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ABSTRACT

Deutarium microcarpum is an edible wild fruit that has a high content of nutrients and bioactive compounds compared to that ofcertainsdomestic fruits. Inspiteits virtues, this fruit is still underexploited and undervalued by the nutraceutical industry due to little knowledge about it. The aim of this work is to study the biological properties of the pulp of *Detarium microcarpum* fruit. The bioactive coompounds and the *in vitro* antioxidant activity of the pulp of the fruit were determined. Different doses (250 mgg/kg, 500 mgg/kg and 750 mgg/kg) of pulp powder fruit was given to male rats during 28 days to evaluate the biological activities. At the end, hematological parameters, lipid properties and antioxidant activities were evaluated. Results show that the fruit is rich inphenolic compounds, such as flavonoids and tannins, in vitamin C and fibers. The levels of total cholesterol, LDL-cholesterol and triglyceride observed in animal groups taken fruit were lower than the control group. the most significant reduction was obtained with 750mg/kg dose $p < 0.05$.Compared to control group, concentration of HDL-cholesterol in all animal groups taken fruit was significant higher $p < 0.05$.The fruit also has a good antioxidant activity with an IC50 of 0.33 mg / mL and decreasing of malondialdehyde in all group rats taken fruit. These results show that the consumption of the pulp of *Deutarium microcarpum* fruit would be a good alternative for the treatment of diseases such as anemia, obesity, and oxidative stress.

INTRODUCTION

Non-communicable diseases (NCDs) such as: cardiovascular diseases, cancers, obesity, type 2 diabetes today constitute a huge public health problem. Several risk factors are associated with the occurrence of these diseases, particularly dyslipidemias, hyperglycemia hypertension and obesitty[1]. These risk factors favours the production of free radicals in the body which are the origin of oxidative stress which leads to the alteration of some biological macromolecules and provokes these diseases [2]. Many strategies have been proposed to fight these diseases with a particular emphasis on dietary interventions[3]. For example, health program recommends that, fruits and vegetables be consumed daily because when consumed in sufficient quantities, they will help prevent major diseases, such as cardiovascular diseases and certain metabolic disorders in the body [4]. In addition, the United Nations estimates that globally, up to 2.7 million lives could be saved each year by increasing the consumption of fruit and vegetables [5]. As regards health benefit, fruits and vegetables provides certain nutrients and bioactives compounds such as fiber, protective micronutrients (vitamins C, E and selenium, iron, zinc, manganese ...), phenolic compounds. Which contribute to lowering the cholesterol levels, anti-anemic and antioxidant effects [6,7]. However, there exist a variety of fruits with varingcompositions which content subtances which are beneficial to the body [8]. In recent years, the majority of scientific work in search of bioactive compounds has focused more on domesticated fruits. Nevertheless, some wild edible fruits also have a considerable content of bioactive compounds[9] although lessstudy has been done on them and thus littleis known about them. This lack of information on wild fruits account for them not been included in the nutritional balance classification[10]. This is in the case of Deutarium microcarpum fruit. Detarium microcarpum is a plant which geographically, is naturally distributed all over arid sub-Saharan of Africa. locally the pulp of this fruit is eaten raw or cooked and is used in baby's porridge [11], and the consumption of the pulp is especially recommended in times of meningitis epidemic. This fruit contains a high level of phenolic compounds [12]. Dietary fiber [13,14],vitamin C[15,8],and minerals like iron[8]were also found present. In

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addition, the antioxidant activities of the fruit pulp have already been proven *in vitro* by Abasse *et al* [16].However, very little study has been done in evaluating the*in vivo* effect of pulp of *Detarium microcarpum* fruit consumption on itsbiological properties. Therefore, the aim of this work is to study the biological properties of the pulp of *Detarium microcarpum* fruit.

MATERIALS AND METHODS

Plant material and sample preparation

Detarium microcarpum fruits were harversted in January 2018 from Guindi, Mayo-DallahDivision, Mayo-Kebbi West Region of Chad and transported to the Food Biophysics, Biochemistry and Nutrition Laboratory, of the National Advanced School of Agro-Industrial Sciences (ENSAI) of the University of Ngaoundere. The dried fruits were then lightly pounded (to avoid seed breakage) with a mortar and pistle to separate the seeds from the pulp; the pulp was ground into fine powder using a hammer mill (culattiPolymix France) and sieved through a 500 µm sieve. The powder obtained was stored in sealed polyethylene sachets and stored at 4°C for further analyses.

Proximate composition

The moisture content, crude protein, ash, crude fiber, fat and total cabohydrate was evaluated according to the official analysis method described by the Association of Official and Analytical Chemists [17].

Bioactive compounds

Vitamin C content (Ascorbic acid)

The vitamin C content was determined by titrimetry method according to that described by Tomhiro [18]with some modifications. About 5 g of powder was weighed in a beaker (25mL). 5 mL of acetic acid was added and the whole mixture was triturated to extract vitamin C. Then 20 mL of distilled water was added to the mixture. The mixture was well stirred using a magnetic stirrer and filter. The filtrate was then collected and used to evaluate the vitamin C content. The results were expressed as mg of ascorbic acid / 100 g of dry weight.

Phenolics compounds

Preparation of the extract

The extraction of the phenolic compounds was carried out according to the method of Kim *et al* [19] with some modifications. For this, 0.025 g of the powder was weighed then 25 mL of methanol (70:30, V / V) was added and stirred at room temperature of the laboratory for 24h. The mixture was filtered on Whatmann 1 paper. The extract obtained was used for the determination of total phenolics, flavonoids, and condensed tannins content.

Total phenolics content

Tota phenolicscompoumds (TPC) were deterrmined according the protocol used byWafa *et al*[20]. 40µL of samples was added to test tubes containing 2980 µL of distilled water followed by an addition of 500 µL of folin -Ciocalteu reagent (1N) and 400µL of sodium carbonate (Na_2CO_3 ,20%).Samples and blank were througlhly mixed and vortexed. After 20 min of incubation at room temperature, the absorbance was measured at 760 nm. For the calibration curves, gallic acid solution (0,2g/L) were used and the total content of phenolics compounds was expressed in gallic acid equivalent per 100 g of dry matter (GAE / 100 g of DW). Absorbance measurements were made with a UV-visible spectrophotometer.

Total flavonoid content

Total flavonoid compounds (TFC) were assayed by a colorimetric assay described by Dewanto *et al* [21]. 100 µL of sample was added to a volumetric flask containing 2.4 mL of distilled water and 150 µL of sodium nitrite (Na_2NO_2, 5%). After 5 minutes, 300 µL of aluminum chloride ($AlCl_3$, $6H_2O$, 10%) was added. After 5 minutes, 500 µL of sodium hydroxide (NAOH, 1 N) was added to the mixture. Then, the vortexed samples were stored at room temperature. Absorbance was measured directly at 510 nm. A calibration curve from a rutin solution (0.1 g / L) was used and the total flavonoids were expressed in mg of rutin equivalent per 100 g of dry weight (mg RE / 100gDW).

Condensed tannins content

Condensed tannins compounds were determined according the protocol used by Sun *et al* [22]. 50 µL of samples was mixed with 3 mL of vanilline 4%. 1.5 mL of concentrate H_2SO_4 was added. And the mixture was allowed to stand for 30 min. Absorbance was measured at 500 nm. For the calibration curves, dilluted solutions of catechin (0-0.600 mg/mL) were used and Condensed tannins content was expressed in equivalent amounts of catechin per 100 g of dry weight(mg CE / 100gDW).

In vitro Antioxidant Activities

DPPH tests

The free radical scavenging activity of the samples was measured in accordance with the method of Zhang and Hamauzu [23] with slights modifications. 0.5 mL of each extract solution was added to 2.0 mL of 0.2 g/L DPPH· methanolic. The reaction mixture was incubated in the dark for 60 min at room temperature, and the optical density was recorded at 517nm against a standard of ascorbic acid. For the control, 2 mL of DPPH solution in methanol was mixed with 0.5 mL of methanol, and optical density of solution was recorded after 60 min. The IC_{50} value was used to calculate DPPH value and was defined as the concentration of the sample neccesary to have 50% inhibtion as determined by interpolated linear regression. DPPH value are reported as percentage of reduction of the initial DPPH. The absorbance was measured at 517 nm using a visible spectrophotometer.

Reducing power

Reducing power was investigated using the method developed by Oyaizu [24]. A 2.5mL of each sample extract was mixed with 2.5mL of phosphate buffer (200mM, pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixture was placed in a water bath for 30 min at 50°C. The resulting solution was cooled rapidly, mixed with 2.5mL of 10% trichloroacetic acid and centrifuged at 3,000 rpm for 10min. The supernatant (2.5 mL) was separated in the test tube and added with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (1.0%), and allowed to react for 10 min at room temperature and the absorbance was measured at 700 nm. Ascorbic acid solution was used as standard. The reducing power were expressed in term of mg ascorbic acid equivalent per g of dry weight.

In vivo biological activity

Animals

Spraque-dawleymale rats (200-300g) were bought from the pet shop of the National Advanced School of Agro-Industrial Sciences (ENSAI). They were kept at room temperature (25 ± 2 ° C). With a relative humidity of 44-56%,light and dark cycles of 12 and 12 h. The animals had free access to a standard diet and distilled water *ad libitum*. After a week of acclimatation, the rats were randomly divided into five groups (6 rats per group). The first group served as normal control and only received standard diet. The second group served as a positive control and received in addition to the standard diet of vitamin C (20mg / kg bw in 10mL of distilled water) the third, the fourth and the fifth group received in addition to the standard diet respectively 10mL / kg bw doses 250, 500 and 750 mg / kg bw of *Deutarium microcarpum* powder per day for 28 days. At the end of the experiment, the blood of each animal was collected after sacrifice and centrifuged at 3500 rpm for 10 minutes. Supernatant was collected and used for biochemical analyses.

In vivo antioxidant activity

In vivo antioxidant activity was assessed by measuring the level of MDA in the blood plasma. This was done according to the method described by Yagi [25]. The MDA concentration was expressed in µM / mg protein.

Hematological analysis

Hematological parameters such as white blood cells, lymphocytes, granulocytes, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular concentration in hemoglobin, mean hemoglobin content andblood platelets were automatically determined by the Coulter-Counter automated hematology analyzer of the laboratory of the regional hospital of Ngaoundere.

Evaluation of Lipid Parameters

The lipidemia properties such as: total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were evaluated using the Monlab Test kits. Total cholesterol was evaluated by the enzymatic method described by Naito et David [26];HDL-cholesterol is determined according to the enzymatic method described by Rifai *et al*. [27].The triglycerides were determined according to the enzymatic method described by Naito et David [26]. The LDL-cholesterol is calculated from the formula of Friedewald *et al*. [28].

Evaluation of toxic properties

Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were determined using the Randox kit according to the method of Reitman et Frankel [29]. Creatinine was determined accordint to the method of Henry [30].

Statistical analysis

The results obtained were expressed as mean ± standard deviation calculated using the Excel 2013 software. The analysis of variance (ANOVA) and the DUNCAN multiple comparison test were performed using STATGRAPHICS centurion.16.1 software (Manugistics, Rockville, Maryland, USA, 1997). Values were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Proximate composition of pulp of *Deutarium microcarpum* fruit

Table1 presents the proximate composition of pulp of *Deutarium microcarpum* fruit.

From the values gotten for proteins and lipids, we can observe that the values obtained falls within the same range as those of Florence *et al*. [14] in Nigeria (protein: 4.68 g/100g DW; Lipid: and 2.67± 0.24 g/100g DW) and Thiombiano *et al*. [31] in burkina-Faso (protein: 4.65± 0.10 g/100g DW).The total sugars of *D. microcarpum* pulp (79.47 ± 3.83 g/100g DW) is similarlly equal to that reported by Kini *et al*. [15]. (81.2 g/100g DW) and Thiombiano *et al*. [31]. (74,65 g/100g DW). This level exceeds the values found by Kouyaté *et al*. [11], Bamisaye *et al*. [13], Florence *et al*. [14] and Makalao *et al*. [8] which are respectively 64.5, 54.9, 65.38 and 51.31 g/100g DW. These different values could be explained by the different nature of the soils or the varietal difference. The crude fiber content of the pulp of Detarium microcarpum fruit is quite close to those obtained by Bamisaye *et al*. [13] (10.2 g / 100 g DW) and Florence *et al*. [14] (12.19 g / 100 g DW). Thus, this fruit contains a high crude fiber content. The ash content of the fruit pulp of Detarium microcarpum is 3.46 ± 0.16 g / 100 g DW.

Table 1 Proximate composition of the pulp of *Deutarium microcarpum* fruit

Compounds	Values
Moisture content (g/100g DW)	10.24 ± 1.07
Proteins(g/100g DW)	4.26 ± 0.32
Lipids(g/100g DW)	2.67 ± 0.24
Total sugars (g/100g DW)	79.47± 3.83
Crude fibers (g/100g DW)	10.08 ± 0.12
Ash(g/100g DW)	3.46 ± 0.16
Energy values (Kcal/100g DW)	359.00 ± 30.00

DW: dry weight; Values are presented as mean ± SD.

Phytochemical content

The phytochemical content of *Deutarium microcarpum* pulp powder is as shown ontable 3. It can be noted from the table that, the pulp of *D. microcarpum* fruit has a higher total phenolics content (6710.27 ± 182.33 mg GAE / 100g DW), flavonoids content (537 ± 46 mg RE /100 g DW), condensed tannins content (1455 ± 254 mg CE/ 100 g DW) and vitamin C content (51.04 ± 0.42 mg / 100g DW).

Table 2 Phytochemical content of the pulp of *D. microcarpum* fruit

Compounds	Values
Total phenolics compounds (mg GAE/100g DW)	6710.00 ± 182.00
Total flavonoids (mg RE /100 g DW)	537.00 ± 46.00
Condensed tannins (mg CE/100gDW)	1455.00 ± 254.00
Ascorbic acid (mg/100g DW)	51.04 ± 0.42

GAE: gallic acid equivalent, RE: rutin equivalent, CE: catechin equivalent.

Totalphenolics content of pulp of *Deutarium microcarpum* fruit is higher than the values found by Lamien-Meda *et al*. [12]on the methanolic extract (4946.67 ± 79.41 mg GAE / 100g DW) and acetone extract (5978. 33 ± 87.50 mg GAE / 100 g DW) in Burkina Faso. The flavonoids content is higher than that obtained by Lamien-Meda *et al*.[12] on the methanolic extract (116.05 ± 3.04 mg EQ / 100gDW) and acetone (155.90 ± 1.89 mg EQ / 100gDW) of the fruit pulp of *Detarium microcarpum*. This variation could be explained by the standard used, which is quercetin in the study of but also

by the level of maturity of the fruit as well as the extraction solvent used. The condensed tannins contentis much higher than that obtained by (Obum, 2010)(23 mg QE / 100gDW). With regard to vitamin C, it is apparent from this table that the fruit pulp of *D. microcarpum* has a high vitamin C content which is similar to that found by Florence *et al*.[14] (55.10 mg / 100gDW).

In vitro antioxidant activities

DPPH test

From Table 3 we note that the pulp powder of *D. microcarpum* fruit has a strong anti radical activity with an IC50 of 0.33 ± 0.06mg / mL. The strong inhibition of DPPH could be explained by the high content of phenolic compounds and vitamin C in the pulp of *D. microcarpum* fruit. Indeed, its compound are recognized for their ability to stabilize free radicals [32].

Table 3 Values of DPPHradical percent inhibition (%)of different concentrations of extracts and vitamin C.

	Percent inhibition (%)	
Concentration (mg/mL)	**Vitamin C**	***D. microcarpum***
0.025	84.73	11.99
0.05	91.64	19.18
0.1	95.18	27.19
0.5	94.64	64.32
1	93.73	71.10

IC50 Vit C = 0.0280 ± 0.00 mg / mL; IC50 D. microcarpum = 0.33 ± 0.06 mg / mL; IC50 = small concentration that inhibits 50% DPPH

Total reducing power of the pulp of *D. microcarpum* fruit
The total reducing power of the powder is 51.94 mg EQAA / g DW. The reducing activity of the fruit pulp powder is much greater than that obtained by Lamien-Meda *et al*. [12](42.35 mg EQAA / g of DW). The high number hydroxyl groups found in the fruit pulp of *D. microcarpum* would be responsible for this reducing power. Indeed, its compounds are able to give up a proton and therefore stabilize free radicals [33].

Biological activities of the pulp of *Detarium microcarpum* fruit Effect of the consumption of pulp powder of *Detarium microcarpum* fruit on lipidemia

Table 4shows the levels of total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) in rats that received *D. microcarpum* pulp powder at different doses. It can be noted from Table 4 that there are no signaficative differences (p> 0.05) in the total cholesterol level between the groups of rats consuming pulp of *D. microcarpum* at the different doses thus used in the normal control groups. In term of LDL cholesterol, we found that the consumption of *Detarium microcarpum* pulp powder resulted in a decrease in LDL-cholesterol levels compared to normal control. However, this decrease was more marked in the groups that received the powder at doses of 750 mg / kg and 500 mg / kg. This decrease could be explained by the presence of significant amount of fibers and phenolic compounds in the pulp powder. Indeed, dietary fiber reduces the adsorption of lipids by binding to bile salts present in the intestine and eliminating them in the stool [34]. They are also fermented by the microbiota of the colon inducing the synthesis of short chain fatty acids, therefore propionic acid, which inhibits the synthesis of the enzyme HGM-CoA reductase responsible for the endogenous synthesis of cholesterol [35] and some mechanism including increased LDL receptors in hepatocytes [36]. In addition, Bok *et al*. [37] and Lee *et al*. [36] showed that consumption of flavanones for 2 to 6 weeks by normolipidemic or hyperlipidemic rats induced a reduction in blood plasma total cholesterol and LDL cholesteerol.

Table 4 Total cholesterol, HDL-cholesterol triglycerides and LDL-cholesterol levels of rats receiving pulp powder of *Detariummicrocarpum* at different doses.

	Doses			
Parameter	**CNo**	**250 mg/kg**	**500 mg/kg**	**750 mg/kg**
CT (mg/dL)	69.89 ± 6.55	58.61 ± 10.38	56.54 ± 9.58	53.13 ± 11.51
C-HDL (mg/dL)	31.92 ±0.18	40.12 ± 4.71*	37.14 ± 3.07*	44.90 ± 3.77*
TG (mg/dL)	32.84±6.75*	39.99 ± 12.64*	39.99 ± 5.40*	22.25 ± 6.11
C-LDL (mg/dL)	19.50±1.35*	15.87 ± 4.71*	11.07 ± 1.08	13.26 ± 3.33

CNo: Control Normal CT: Total Cholesterol, HDL-C: HDL cholesterol, LDL-C: LDL cholesterol, TG: triglycerides, (n = 3). Values on the same line with * are significantly different to others (p <0.05).

HDL cholesterol is known as a protective marker of cardiovascular disease, thus preventing the accumulation of cholesterol in the blood vessels and thus avoiding the risk of atherosclerosis. Thus, it can be seen from Table 4 that consumption of *D. microcarpum* pulp powder resulted in an increase in HDL-cholesterol levels compared to normal control which did not receive *D. microcarpum* powder. The most remarkable results were that gotten from 750 mg/kg dose. This increase blood level of HDL cholesterol could be explained by the action of the phenolic compounds present in this powder in a significant amount. Indeed, several studies have reported that the consumption of tea and fruit rich in polyphenols prevents cardiovascular diseases and especially leads to an increase in HDL cholesterol[38,39].

Table 4 shows that consumption of *D. microcarpum* powder at a 750 mg / kg dose resulted in a significant decrease in blood triglyceride levels compared to other doses and normal control. This decrease could be explained by the action of phenolics compounds. Indeed, several authors have reported that phenolics compounds have the ability to inhibit pancreatic lipase, an enzyme that hydrolyzes triglycerides to monoglycerides, diglycerides, and free fatty acids to be absorbed by the intestine. Inhibition will cause malabsorption of lipids in general and those of triglycerides in particular and therefore their excretion and decreased plasma levels[40,41].

In vivo antioxidant activity

Table 5 shows the level of malondialdehyde (μM / mg protein) in animals. We generally observe a decrease in the level of MDA in the kidney, heart and liver in animals consuming the pulp of *Detarium microcarpum* compared to those control normal. At the level of the heart, the MDA level in the groups of rats fed at the dose 250 mg of the pulp of *D. microcarpum* fruit and those of normal control are not statistically significant thus those of groups of 500 mg and 250mg of the liver. The 750mg dose showed the highest reduction (heart: 0.66 ± 0.08 μM / mg protein; liver: 0.25 mg ± 0.04 μM / mg protein; kidney: 0.34 mg ± 0.02 μM / mg protein). In general, we can say that there was a generally decrease with dosages.

Table 5 Rates of malondialdehyde (μM / mg protein) of the rats subjected to the pulp powder of *D. microcarpum* fruit at different doses

	Doses			
Organs	CNo	250 mg/kg	500 mg/kg	750 mg/kg
Heart	2.12 ± 0.06*	2.07 ± 0.03*	1.71 ± 0.12*	0.66 ± 0.08
Liver	3.84 ± 0.10*	1.49 ± 0.05*	1.39 ± 0.02*	1.25 ± 0.04
Kidney	1.06 ± 0.01*	0.62 ± 0.03*	0.48 ± 0.03*	0.34 ± 0.02

CNo: Control Normal, Values on the same line with * are significantly different to others (p <0.05).

This proportional dose reduction, would be due to the action of the phenolic compounds and vitamin C present in the pulp of *Detarium microcarpum* fruit. Indeed, these bioactive substances are recognized for their ability to trap and neutralize free radicals that are responsible for lipid peroxidation [42].They prevent the oxidation of membrane phospholipids and thus prevent the loss of polyunsaturated fatty acids from cells [43].

Effect of consumption of pulp powder of *D. microcarpum* fruit on hematological properties

The results on hematological parameters are given in Table 6.In general, there was no significant difference ($p > 0.05$) in the white blood cell count between the different groups at different doses of *D.microcarpum* pulp powder and normal control. Our results are in agreement with those of Wahedi and David [44] who found no significant change in white blood cell count between the control group and the 500 mg / kg dose group of the *D. microcarpum* fruit pulp powder.

Table 6 Hematological properties of rats subjected to the powder of the pulp of *D. microcarpum*at different doses

	Doses			
Paramètres	**CNo**	**250 mg /kg**	**500 mg/kg**	**750 mg/kg**
WBC (10^3/µL)	6.47 ± 1.01	5.27 ± 0.70	5.63 ± 1.37	5.03± 1.54
GRA (%)	25.23 ± 1.77*	13.3 ± 0.85	22.8 ± 0.69*	23.47 ± 0.23*
LYM (%)	61.10 ± 6.09	78.2 ± 7.26*	63.5 ± 0.60	63.03 ± 1.59
MON (%)	13.37 ± 0.98	11.67 ± 0.23	12.60 ± 0.35	12.63 ± 1.78
RBC(10^6/mm^3)	4.46 ± 0.59	4.50 ± 0.37	5.81 ± 0.19*	8.29 ± 0.40*
Hb (g/dL)	9.17 ± 1.67	9.03 ± 0.65	12.53 ± 0.85*	14.60 ± 0.34*
Hct(%)	21.43 ± 0.84	23.73 ± 0.40	30.30 ± 2.52*	33.80 ±1.39*
MCV(fL)	48.73 ± 0.98	50.30 ± 0.46	52.93 ± 1.27*	54.27 ± 0.12*
MCHT(pg)	18.73 ± 0.46	20.47 ± 0.21*	21.83 ± 0.23*	22.97 ± 0.12*
MCHC (%)	39.07 ± 0.92	40.63 ± 0.60*	39.77 ± 0.85*	38,93 ± 0.84*
PLT (10^9/L)	165.33 ± 18.47*	129 .00 ± 32.90	182.67±10.97*	207.00 ± 1.73*

CNo: Normal control; WBC: White Blood Cell, LYM: lymphocytes, GRA: granulocytes, MON: monocytes, RBC: Red blood Cell, Hb: hémoglobin; Hct: hématocrit; PLT: platelet; MCV: mean corpuscular volume, MCHC: mean corpuscular concentration in hemoglobin, MCHT: mean hemoglobin content. Values on the same line with * are significantly different to others ($p < 0.05$).

In contrast to white blood cells, red blood cells, hematocrit, hemoglobin, and erythrocyte constants (VGM, TCMH, MCHC) increased significantly ($p < 0.05$) in rats fed with fruit pulp powder of *D. microcarpum*at doses of 500mg / kg and 750mg / Kg compared to the 250mg / kg dose and normal control. However, this increase was more marked in the rats administered with 750 mg / kg dose of the *D. microcarpum* powder. Thus, the intake of *D. microcarpum* at a dose of 750 mg / kg increased by 85.87% the red blood cells and 57.72% the hematocrit level compared with normal control. This increase of red blood cells could be explained by the presence of significant amount of iron in the pulp powder of *Detarium microcarpum*. Makalao *et al*.[8] and Obum [45] have shown that the pulp of *D. microcarpum* fruit is an important source of iron. Iron deficiency in humans leads to the development of iron deficiency anemia. In addition, the high values of red blood cell, hemoglobin and hematocrit observed after consumption of *Detarium microcarpum* pulp powder could also be explained by the presence of vitamin C, the content of which increases with increase in the administrated dose. Indeed, vitamin C, promotes the absorption of iron in intestinal cells [46].These results are in agreement with those obtained by Wahedi and David [44] who showed that the consumption of the pulp of *D. microcarpum* fruit at a dose of 500 mg / kg resulted in an increase in red blood cells and hematocrit. Moreover, Ablasse *et al*. [16] showed that the ethanolic extracts of the pulp *Detarium microcarpum* at a dose of 100 µg / mL protect erythrocytes against hydrogen peroxide-induced hemolysis. For platelets, there was a significant increase ($p < 0.05$) in the *Detarium microcarpum* pulp powder group compared with the 250 and 500 mg / kg pulp groups compared with the control normal group. This increase could be explained by the fact that blood platelets and red blood cells originate from the same stem cell of the myeloid lineage which is the hemocytoblast [47].

Effect of consumption of pulp powder of *D. microcarpum* fruit on some toxicity properties

Alanine amino-transfersase, aspartate aminotransferase, and creatinine are parameters that respectively gives an indication of the state of good functioning of the liver, heart and kidneys. Blood levels of creatinine, ALT, and ASAT from rats receiving different doses of the powder of *D. microcarpum* pulp are reported in Table 7, the creatinine and ALT levels of the groups of animals subjected to the different doses of *D. microcarpum* fruit pulp powder and those of normal control showed no significant difference ($p > 0.05$). This therefore allows the consumption of the powder of the pulp of *D. microcarpum* has no deleterious effect on the renal and hepatic function up to the dose of 750 mg / kg.

Table 7 Creatinine, ALT, ASAT levels of the rats receiving *Detarium microcarpum* pulp powder at different doses.

	Doses			
Parameter	CNo	250 mg/kg	500 mg/kg	750mg/kg
Creat(mg/dL)	0.77 ± 0.06	0.83 ± 0.15	0.80 ± 0.10	0.93 ± 0.06
ALAT (UI/L)	5.33 ± 2.08	5.67 ± 0.58	5.33 ± 2.52	6.67 ± 1.53
ASAT (UI/L)	16.33 ± 5.77	30.00 ± 2.52*	45.67 ± 0.58*	15.26 ± 0.62

AlAT: alanine aminotransferase, ASAT: aspartate aminotransferase, Creat: creatinine, (n = 3). Values on the same line with * are significantly different to others ($p < 0.05$)

Blood levels of ASAT from groups receiving different doses of the pulp of *D. microcarpum* are also not very significant ($p > 0.05$), the 500mg dose significantly increases the ASAT rate ($p < 0.05$).In general, the consumption of pulp powder of *Detarium microcarpum* has no deleterious effect on the functioning of the liver, heart and kidney.

CONCLUSION

It appears that the pulp of *Detarium microcarpum* fruit is an important source of bioactive compounds and nutrients. The consumption of fruit in general improves lipid parameters of rats and increases the rate of red blood cells, hemoglobin and hematocrit. Theses increase and improvement was more marked in the rats which received 750 mg / kg dose. The consumption of the pulp of fruit does not show any deleterious signs on the functioning of the organs. This work opens great prospects for the use of the powder from the pulp of *Deutarium microcarpum* fruit in the formulation of nutraceuticals with high biological activities.

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