

In vivo antioxidant activity of *Mbuja* oil compare to Palm Olein and Corn Oils in Wister Rat

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ABSTRACT: *Mbuja* (*Bikalga*; *dawadawa botso*; *datou*; *Furundu*) is a food condiment obtained by a traditional uncontrolled fermentation of *Hibiscus sabdariffa* seeds in African countries, including Burkina Faso, Mali Niger, Nigeria, Cameroon and Sudan among others. This condiment is known for its nutritive values and for its health properties. In order to contribute to the amelioration of its nutraceutic valorization, a study on physicochemical characterization and on antioxidant value of its oil was carried out. *Mbuja* was purchased in Mokolo market (Far-North, Cameroon); oil extracted from it was assessed on some of its antioxidant components by using classical methods. Nutraceutic aspect was conducted after feeding male rats for 50 days. The results had shown that *Mbuja* oil contained vitamin E ($13.73 \pm 0.37 \text{ mg}/100\text{g}$), vitamin A ($30.07 \pm 0.11 \mu\text{g}/100\text{g}$); vitamin C ($20.59 \pm 0.85 \mu\text{g}/100\text{g}$), liposoluble phenolic compounds ($2.24 \pm 0.09 \text{ g}/100\text{g}$). Feeding adult male rats with this oil had shown good Antioxidant activities in their blood. Consumption of *Mbuja* oil need to be encouraged and can be advised as nutraceutic and therapeutic diet to persons suffering from hypertension.

Key words: *Mbuja*, Oil, Antioxidant, Nutraceutic activities.

1. INTRODUCTION:

Mbuja (*Bikalga*; *dawadawa botso*; *datou*; *Furundu*) is a food condiment obtained by a traditional uncontrolled fermentation of *Hibiscus sabdariffa* seeds in African countries, including Burkina Faso, Mali Niger, Nigeria, Cameroon and Sudan among others. This condiment is known for his nutritive values and for his health properties [1][2]. In spite of its nutritional and healthy properties the consumption of *Mbuja* is less appreciated in urban areas. This is due to its strong smell, to its bad condition of manufacturing practices which leads to the rapid alteration of nutritive values. The main problem now is how to lead people to consume *Mbuja* which nevertheless contains bioactive molecules, which can help in the treatment or in the prevention of some cardiovascular diseases [3]. Among those bioactive molecules, the lipidic profile is one of the main element which has an impact on hypertension. No scientific study on the impact of the used of *Mbuja* oil in the treatment of some chronic diseases was found in literature review. For this reason the goal of this study target its nutraceutic valorization. The present study was undertaken essentially to investigate the *in vivo* potential effects on antioxidant defenses of *Mbuja* oil compared to corn and palm oils in the aim of the treatment of inflammatory diseases. To overcome this, the composition of different oils on dietary antioxidant compounds, theirs influence on oxidative stresses parameters on male rats were assessed.

2 MATERIAL AND METHODS:

2.1 Oil Sampling and Proximate composition:

The *Mbuja* was purchased from various sellers from the Mokolo market in Far-North (Cameroon). The lipid composition was determined by exhaustively extracting a known weight of sample with hexane using a Soxhlet apparatus [4]. Some Chemical compounds of *mbuja* oil used (Vitamin A [5]; Vitamin E [6]; Total Phenol Compound (TPC) [7]) were analyzed using classic methods.

2.2 Animals and Diets:

7 month old weaned male albinos Sprague Dawley rats (Harlan, France) weighing $260 \pm 20 \text{ g}$ were housed in polycarbonate cages in a controlled environment with a temperature of $25 \pm 2 \text{ }^\circ\text{C}$, relative humidity (40–60%), with a 12-h light–dark cycle (12h/12h : 7 – 19 h light and 19 – 7h dark) [8]. During an acclimatization period of 1 week, the rats received tap water and a commercial rat diet *ad libitu* [9]. At the end of this period, the rats were weighed and randomly assigned to one of the three groups (n = 6 / group) according to diet composition. For 50 days, each group was fed a diet containing one of the following: *Mbuja* oil (MO group), corn oil (Lesieur France) (CO group) and palm oil (Palm'Or, Maya, Douala-Cameroun) (PO). Oil represented 5 % of the composition of the diet as prescribed by American Institute of Nutrition [10]. The diet was reconstituted by using an alipidic diet (moisture content 8.53 %, proteins 21.48 %, dextrose 32.00 %, starch 26.42 %, cellulose 6.35 %, mineral mix 4.58 %, vitamins mix 0.64 %). Animals had free access to water and food. Food was given each week and water twice per week.

2.3 Experimental procedure:

At the end of the feeding period (50 days), the rats fasted overnight (12 hours), then were weighed, anaesthetized under chloroform vapor and sacrificed. Blood samples were immediately collected from the heart by cardiac puncture in tubes (heparin tubes). Plasma

was separated by centrifugation at 1500 rpm for 10 min (4°C) and it was used for malondialdehyde determinations. Oxidative stress was performed by measuring glutathione peroxydase (GPx) and superoxide dismutase (SOD) activities in red blood cells.

2.3.1 Malondialdehyde level (MDA):

Plasmatic malondialdehyde, samples were prepared by the method described by Tug *et al.*[11], and analyzed according to the thiobarbituric acid reagent method described by Sheu *et al.*[12]

2.3.2 Parameters of oxidative stress:

Oxidative stress was evaluated through glutathione peroxydase and superoxyde dismutase. The SOD and GPX activities were determined using kits Randox (France) respectively RANSOD (Cat. No. SD 125) for SOD and Cat. No. SC 692 for GPX. The SOD activity was measured in hemolysate using an appropriate whole blood SOD control. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red dye.

The enzymatic activity was then measured by the degree of inhibition of this reaction. The GPX activity in whole blood was measured using a whole blood control (Cat. No. SC 692) as described by Lawrence & Burk [13].

2.4 Statistical Analysis:

Results were expressed as means \pm standard deviation. For each group, the result obtained was the mean for 6 rats. All results were analyzed using a one-way analysis of variance. Duncan's Multiple Range test was performed to evaluate differences between groups. Differences between means were considered to be significant at $p < 0.05$.

3. RESULTS:

3.1 Chemical assessment of liposoluble antioxidant contents:

The chemical analysis of liposoluble antioxidant contents (TPC, Vit E, Vit A and Vit C) of different oils used was assessed (Table 1).

Table 1: Liposoluble antioxidant contents of different oils used.

| Compounds | MO | CO | PO |
|---|--------------------------------|-------------------------------|-------------------------------|
| Total phenolic compounds (TPC) (g/100g) | 2.42 \pm 0.09 ^b | 1.75 \pm 0.44 ^{ab} | 1.18 \pm 0.77 ^a |
| Carotenoids (μ g/g) | 0.62 \pm 0.08 ^a | 0.92 \pm 0.20 ^a | 0.52 \pm 0.12 ^a |
| Vitamin A (caroténoïdes /6) (μ g/100g) | 30.10 \pm 0.11 ^b | 31.12 \pm 0.65 ^b | 28.12 \pm 0.27 ^a |
| Vitamin C (mg/kg) | 20.59 \pm 0.85 ^c | 0.03 \pm 0.01 ^a | 8.47 \pm 0.04 ^b |
| Vitamin E (mg/g) | 15.37 \pm 0.37 ^{ab} | 13.3 \pm 0.60 ^a | 14.51 \pm 0.41 ^b |

All results are means of three replicate experiments; values in the same line followed by the same letter are not significantly different at $p \leq 0.05$. **MO:** Mbuja oil; **CO:** Corn oil; **PO:** Palm oil

The liposoluble antioxidant contents vary less and are ranged between 1.18 to 2.42 g/100g (TPC); 28.12 to 31.10 μ g/g (Vitamin A); 0.03 to 20.59 mg/kg (Vitamin C) and 13.30 to 15.37 mg/g (Vitamin E). In general observation, statistical analyses show that there is no significant difference ($p < 0.05$) between MO and CO values as far as TPC; Vit A and E are concerned. For these elements MO has shown good values compare to the other oils. Presence of TPC, vitamin A, C and E could have an influence on biochemistry parameters [14]. Meanwhile, consumption of these natural antioxidants of *Mbuja* oil can affect the oxidative stress and nutritive properties. It is well known that these two parameters are also affected by the quality of food consumed which impact the good health and body weight.

3.2. Body Weight:

Figure 1 shows the effect of oils consumed on body weight gain of rats.

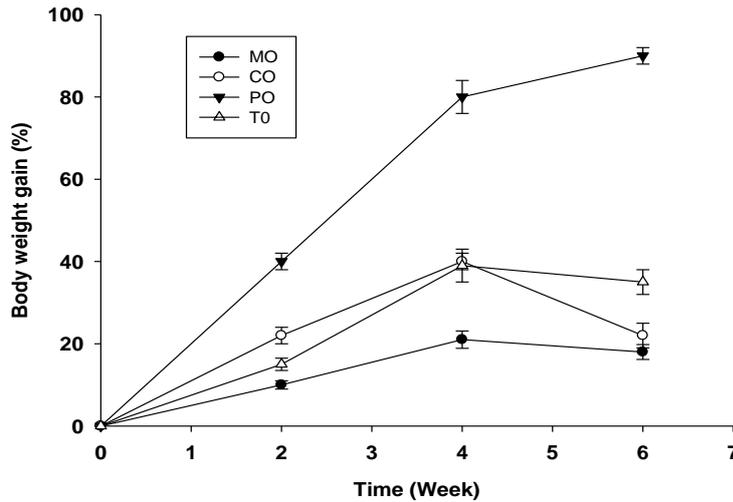


Figure 1: Effect of oils consumed on body weight gain of rats.

T0 : Control ; PO :Palm oil ; MO : Mbuja oil ; CO : Corn oil. N= 6 rats per group

The body weight increased in each group after 6 weeks. Compared to control group (T0), in which the gain is 35±3%, MO (18±2%) and CO (22±3%) have shown less body weight gain, while PO (90±2%) the increase is very important. The body weight gain observed can be an indicator of physiological attitude of the body reaction in response of regime submitted.

3.3. Evaluation of oxidative stresses parameters:

The biochemical assessment is conducted to show the benefic effects of *Mbuja* oil on management of hypertension, inflammatory and cardiovascular diseases which can be linked to the oxidative stresses and blood lipidic parameters [15].

Oxidative stress can be correlated to the lipid molecular peroxydation and can be assessed by determination of inherent products yielded. This can be done by evaluation of MDA which is one of the best indexes of cell destruction due to the fact that this product is rapidly formed in serum than in normal cell. MDA concentration can be then reflected the oxidative stress degree of rats [16]. Antioxidant activities of different regimens were evaluated *in vivo* by determination of SOD and GPX in plasma after 50 days of feeding.

3.3.1 Malondialdehyde (MDA):

MDA constitutes a biological marker of lipid peroxidation. Figure 2 shows the effect of oils consumed on Malondialdehyde (MDA).

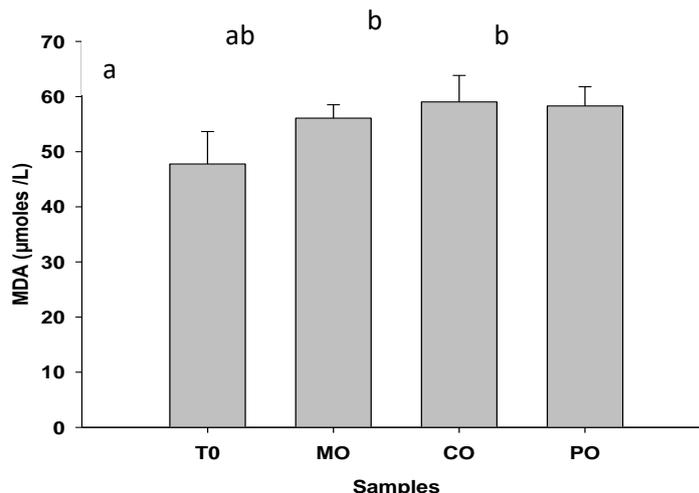


Figure 2: Effect of oils consumed on Malondialdehyde (MDA).

The Plasma MDA of the MO group (55.09±7.42 µmole/l) was lower than that of the CO (57.92±1.35 µmole/l) and PO (58.22±1.48 µmole/l) groups. These values are higher than T0 group (45.76±5.89 µmole/l). The Duncan test shows that there is no significant difference (p < 0.05) between the value of plasma MDA for MO, CO and PO.

3.3.2 Superoxide dismutase (SOD):

SOD role is to accelerate dismutation of toxic superoxide radical (O_2^-) produced during the oxidation process which yields energy, hydrogen peroxide and oxygen molecular. This test permits to evaluate the effect of *Mbuja* oil consumption on the dismutation of free radicals. Figure 3 shows the effect of oils consumed on Superoxide dismutase (SOD).

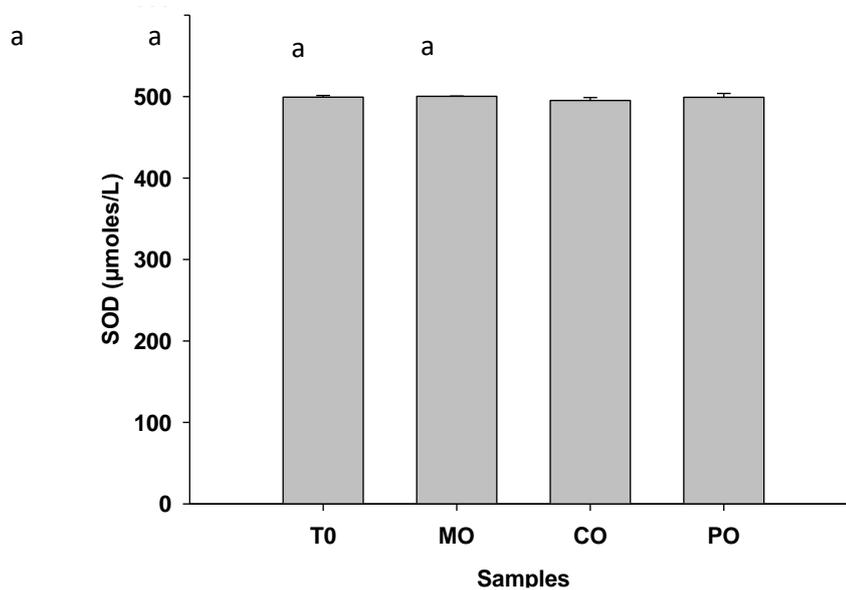


Figure 3: Effect of oils consumed on Superoxide dismutase (SOD).

The Plasma SOD of the MO group ($499.28 \pm 0.38 \mu\text{mole/l}$) was higher than that of the CO ($493.32 \pm 3.63 \mu\text{mole/l}$) and PO ($496.19 \pm 4.75 \mu\text{mole/l}$) groups. SOD of these two groups (CO; PO) are lower than T0 group ($498.53 \pm 2.08 \mu\text{mole/l}$). According to the Duncan test there is no significant difference ($p < 0.05$) between the value of plasma SOD for different groups studied. Dietary lipids have not significantly affected the activity of SOD in red blood cells ($p < 0.05$).

3.3.3 Glutathion peroxidase (GPx):

Glutathion peroxidase is an enzyme which destroys the free radicals, takes free radical from cell and protects the cell membrane against oxidation. GPX allows the conversion of oxygenate water into water. Figure 3 shows the effect of oils consumed on Glutathion peroxidase (GPx)

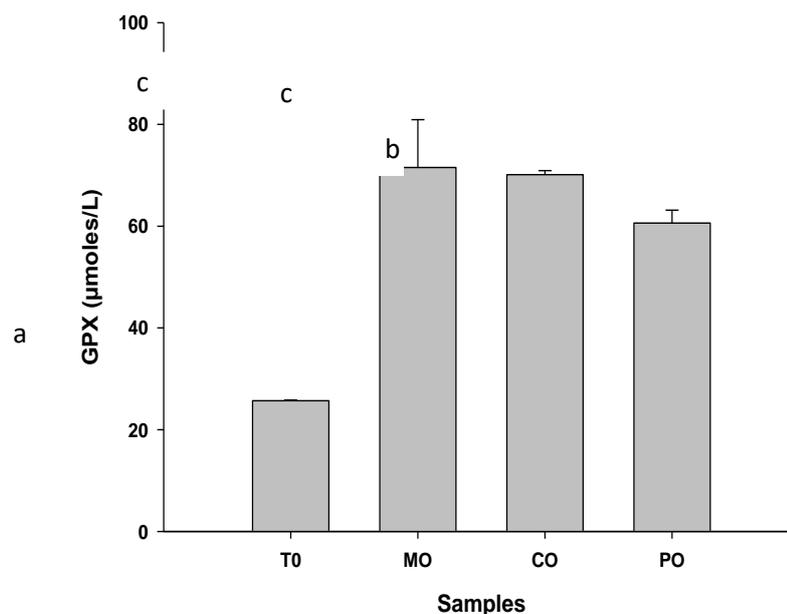


Figure 3: Effect of oils consumed on Glutathion peroxidase (GPx).

GPx activity of MO ($70.54 \pm 9.39 \mu\text{mole/l}$) was also higher than that of CO ($59.16 \pm 0.79 \mu\text{mole/l}$) and PO ($69.21 \pm 2.78 \mu\text{mole/l}$). Compared to T0 ($23.46 \pm 0.15 \mu\text{mole/l}$), are higher. The Duncan test shows that there is no difference ($p < 0.05$) between GPx levels for rats fed with MO and CO diets.

4. DISCUSSION:

Aerobic organisms produce reactive oxygen species (ROS) as a consequence of aerobic respiration and substrate oxidation. Low levels of ROS are helpful in many processes including cell differentiation, cell growth, apoptosis, immunity and defence against micro-organism. In contrast, high levels of ROS result in oxidative stress, which may cause metabolic malfunction and macromolecular, cellular and tissue damages. In order to deal with damaging activities of ROS, aerobic organisms process antioxidant defence systems. The enzymatic antioxidant defences include SOD, GPX and CAT [17]. These activities play an important role in the progress of the disease, therefore in the treatment of chronic diseases, food antioxidant treatment may useful and should be added to combined therapy for these patients. The reports from several studies have produced a clear evidence that there exist a good correlation between type and severity of disease and antioxidant level in the blood. Such relations have been documented in many diseases including cardiovascular diseases, neurological diseases, pulmonary diseases and various types of malignancies [17].

Mbuja is used as food antioxidant in the treatment of some chronic diseases like hypertension. The oil of this condiment contains bioactive substances. Interpretation of the distribution of oil contents and the effects observed in rat blood fed with different oils allows saying that the antioxidant activities of MO, CO and PO regimens are linked to presence of Vit A; Vit E; Vit C and TPC. It is well known the presence of TPC, vitamin A; C and E in food can impact the biochemistry parameters [14]; [18]; [19]; [20]. Meanwhile, consumption of these natural antioxidants of *Mbuja* oil can affect the oxidative stress and nutritive properties. These two parameters are also affected by the quality of fatty acids. Assessment of *Mbuja* oil has shown the good quality of oil (riches in PUFA, ω -6: 57.37%). These constituents could be responsible to eradicate free radicals in cell [14]. However with PO regimen, the antioxidant activities can be due to the presence of MUFA, VitE and important secretion of GPX which can reduce free radicals production through the oxidation process of palm oil and transform them into MDA. Base on this result it is possible to say that MO has antioxidant property. This interpretation of the different interactions confirms that MO can help to treat or to prevent inflammatory. These results are in agreement with the traditional medicine in which *Mbuja* is used to treat and to prevent hypertension, inflammatory and cardiovascular diseases [21]. The fact that body weight increase less means that *Mbuja* can be used to control overweight from obese patient.

5. CONCLUSION:

The results of this study indicate that *Mbuja* oil concentrate may be a dietary supplement with the potential properties of improving antioxidant status. Its consumption by rats reduces oxidative stress.

ABBREVIATIONS:

T0 : Control ;

PO :Palm oil ;

MO : Mbuja oil;

CO : Corn oil;

MUFA: Mono-unsaturated fatty acids;

PUFA; polyunsaturated fatty acids;

SFA: Saturated fatty acids;

Vit A: Vitamin A;

Vit E: Vitamin E;

TFC: Total phenol compounds;

MDA: Malondialdehyde;

SOD: Superoxide dismutase;

GPX: Glucathione peroxidase;

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