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A sport drink extracted from *Mondia whitei* roots: Impact on endurance performance

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ABSTRACT

The objective was to make a drink from the roots of *Mondia whitei* similar to the standard required for sport drink. Physico chemical. The root barks were extracted with deionised water during 24 hours. Dried powder was obtained after one week of incubation at 45 °C in an oven. Physicochemical studies were done using standard methods. Twenty height g of the paste obtained was dissolved in 1l of deionised water and adjusted with carbohydrate and salt in strict respect of the international norms for obtaining the test drink. Acute, sub-acute toxicity and the ergogenic property of the test drink were performed to the rats using standard methods. The results showed that the drink contained: 97.53 % of water; 4.04 ±0.6 g/dL of carbohydrate; 80.5 ± 0.21 mg/dl of sodium and has a caloric value of 29.30 ± 0, 21 Kcal/dL. Sub acute toxicity showed that, the Lethal Dose (LD) 50 of the dry mass was above 5000 mg/Kg. The endurance performance of the rats tested was higher than that of the control groups after 08 weeks of consumption of the test drink. These results suggest that this drink is ergogenic and not toxic.

KEYWORDS

Mondia whitei;
Sport drink;
Toxicity;
Ergogenic properties;
Endurance performance.

INTRODUCTION

Fatigue is associated with a feeling of extreme physical or mental tiredness, resulting from severe stress, hard physical or mental work[1]. It is defined as the difficulty of initiating or sustaining voluntary activities[2]. This common condition impairs the daily functions of sportsmen during strenuous exercises. Fatigue has a peripheral and a central origin[3]. During a sustained effort central and peripheral fatigue

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develops gradually but the task is usually terminated when the muscles still possess the capacity of producing force[4]. Enoka and Stuart; Loscher *et al*.; Mc Kenzie *et al*. which indicated that the loss of central nervous system impetus is the point of fatigue[5, 6, 7]. During exercises, there is an increase in the utilization of carbohydrates and lipids in the skeletal muscles. Initially, the supply of muscles with energy comes from immediate sources of energy such as ATP, phosphocreatine, from the anaerobic, glycolysis and other metabolic paths. A rapid depletion of energy reserves in muscles, liver tissues and oxidative stress are caused by an excessive generation of reactive oxygen species (ROS) during exercise induces fatigue[8, 9]. Because of constant activity, fatigue, and/or laziness, a good number of people tend to take 'ergogenic substances' as an alternative[10]. Galenical preparations or herbal mixtures intended for non-specific use were formerly referred to as 'ergogenic substances,' especially when they are in a liquid (beverage or drink). The consumption of supplements containing plants and/or plant extracts is in the form of "energy drinks" and their consumption is a not new phenomenon[11]. Without going into an ethical debate, the purpose targeted by the consumers of these "energy drinks" is to improve their performance, to recover and/or to increase muscle volume. The market of "energy drink" in France and in the world is increasing and there is confusion between energy drinks and beverages known as "energy drinks" as well[10]. The ingredients mostly used in these drinks are caffeine, taurine, glucuronolactone, B, C and E vitamins, herbal extracts, etc[11]. It is still fashionable to wonder about the ergogenic property claimed by the various companies that market this supplement without any scientific justification. However, the expression of 'ergogenic substances' was first considered as a substance that enhances performances[12]. For Goussard, the effects of ergogenic aids can better be observed if an athlete is put under a balanced diet and is trained properly[12]. Otherwise, the properties of the product studied would be offset by the different imperfections of its food and its preparation. Several hypotheses were formulated to justify the allegations of people on some ergogenic plants precisely about their adaptive property[13], their anti oxidant effect, their capacity to store the glycogen[14], to mobilize the free fatty acids[15], to activate the hypothalamic-pituitary-adrenal axis[12], to increase the speed of resynthesis post exercise glycogen[16], to reduce the production of lactic acid for the exercise[17], etc. Pharmacological studies have revealed that the extracts or bioactive compounds of plants such as *Allium sativum* L. (Amaryllidaceae)[18], *Bacopa monniera* L. (Scrophulariaceae)[19], *Panax ginseng* C.A. Meyer (Araliaceae)[20], *Rubus coreanus* (Rosaceae)[21], *Pseudosasa japonica,* (Poaceae)[22], *Ocimum sanctum* L. (Lamiaceae)[23] and *Cordyceps sinensis* Berk (Ophiocordycipitaceae)[24], have increased endurance capacity as well as ameliorated exercise induced in oxidative stress in animal models after a forced swimming test. However, the available supplements for increasing endurance capacity from natural sources are very limited and therefore there has been a significant effort in the search of new anti-fatigue agents as an alternative to their synthetic counterparts. Thus, exogenous dietary substances involved in energy production are also candidate to anti-fatigue substances for fight against physical fatigue. Accordingly, we believe that it is important to develop a safe and effective anti-fatigue food that can relieve daily stress and promote public fitness. *Mondia whitei* is used as a medicine throughout the tropical African regions[25]. It is an aromatic plant of the Periplocaceae family[26] and commonly known as "Nkan si 'among the Beti ethnic group of Cameroun. The roots of *Mondia whitei* have a vanilla-like flavour and tastes like a mixture of liquorice and ginger[27-28]. From the Phytochemical view, *Mondia whitei* contains steroids, teriterpenes (a mixture of α-amyrine and β-acetate, lupeol, β-sitosterol, and β-sitosterol glucosilaldehyde) and aromatic compounds (2-hydroxyl-4-methoxybenzaldehyde, 3-hydroxy-4-methoxy benzaldehyde, and 4-hydroxy- 3-methoxybenzaldehyde), glucose, and polyholosides[29]. Other constituents include Zinc, Iron, Calcium, Magnesium and Vitamins (A, D and K)[30].

Of interest, is the fact that several scientific studies have documented the use of *Mondia whitei* in the treatment of some illness and asthenia[27, 28, 32-34]. It has also been reported that *Mondia whitei* is traditionally used as a spice, to stimulate appetite, to

make an energizing juice (traditional drink) for traditional wedding ceremonies or for sport. Good sport drinks must be hydrating, energetic and electrolytic in nature[35]; so that it can compensate water, carbohydrates and sodium lost during sports activities[36]. Sport drinks are usually very expensive; therefore sportsmen take traditional drinks. Unfortunately, traditional drinks made by many Cameroonians do not follow the physicochemical and nutritional standard composition. These drinks are not properly prepared and their microbiological quality is not known. A quality sport drink to be sold must respect the commercial standard of drinks. However, there is paucity of information about the consumption of *Mondia whitei* like a beverage on several other biological and/or physiological parameters during physical activities. The aim of our study is to prepare a drink from the roots of *M. whitei* with a composition that respect the standard of commercial drinks, to evaluate its toxicity and its ergogenic properties in rats.

METHODS

Preparation of crude extract

Root barks of *M. whitei* were collected in Bafoussam in the western region of Cameroon and identified at the National Herbarium of Cameroon (HNC), Yaounde in comparison with the Herbarium Voucher specimen N°42920/HNC collected by Westphal. Using the specimen after two weeks, air-dried at room temperature, this material was grinded into powder. For the extraction, 166.70 g was macerated in 1.5 l of deionised water for 24 h according to the physico-chemistry and nutritional pretest experimentation. The extract was then filtered through whatman paper N° 4 and evaporated to dryness in an oven during three days at 45° C. The crude extract gave 28 g that was kept at 4°C for the experiment.

Nutritional composition of *M. whitei extract* and the preparation of the test drink

An analysis of moisture, ash, protein, fat, carbohydrate and sodium was carried out by the Association of Official Analytical Chemist or AOAC methods[38]. Gross energy was calculated according to FAO/WHO/UNU method[38]. The total phenol contained, was determined according to the method of Lim *et al*.[39]. A pre determined dosage indicated the quantity of sugar (saccharin), salt and deionised water that was added to 28g of the crude extract of *M. whitei* to reach the norms recommended for the sport drink (extract of *M. whitei* + salt + sugar) according to the Scientific Committee on Food or SCF[40]. The preparation obtained was made homogeneous to dissolve all its components. The extract of *M. whitei* + salt + sugar was the test treated drink. Other drinks were prepared at the same concentration and contained one or several ingredients found in the test drink (extract of *M. whitei* + sugar, extract of *M. whitei* + salt, deionised water + salt + sugar). Each drink prepared was previously over dried (at 45°C) excepted the negative control drink. The resulting material of each drink was weighed (paste). The treated drink administered by gavage to the experimental groups was obtained by dissolving 400 mg/kg of the paste each corresponding residue of the drink in 1 mL of deionised water. The working positive control drink was obtained by dissolving 400 mg/kg of paste of the deionised water + salt + sugar in 1 mL of deionised water. The working negative control drink was put together with 1mL of deionised water.

Nutritional composition of the drink tested

The phyto-chemical and nutritional composition of the test treated residue drink such as water, carbohydrates, lipids, proteins, sodium were determined using standard methods[37,39]. Gross energy was calculated according to FAO/WHO/UNU method[38].

Toxicity study of the drink

Acute and sub-acute toxicity of the drink were evaluated in rats according to OECD guidelines 423 and 407 techniques[41]. Female rats were used only for acute toxicity and both male and female rats were used for the sub-acute toxicity. Acute toxicity was made for a period of fourteen day meanwhile that of sub-acute toxicity was twenty eight days. In the sub acute toxicity, histopathological analysis of the liver of the rats was done according to the method described by Tedong *et al*[42].

Animals, treatment and substance administration

Male rats weighing 100 -153 g, aged two months

were acclimated and had free access to water and standard diet for eight weeks. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethical Committee (Reg. No. FWA-IRB00001954). Food, water consumption and animal weight were recorded daily. Ninety rats were randomly divided into six groups of 15 rats each. They were given either a physical training on a treadmill and one type of the drinks prepared (the negative control drinks (NC): deionised water, the positive control drinks (PC): deionised water + salt + sugar', extract of *M. whitei*, extract of *M. whitei* + salt, extract of *M. whitei* + sugar and extract of *M. whitei* + salt + sugar at the dose of 400 mg /Kg) for 8 weeks. The rats of the experimental groups received 1ml of 400 mg/kg. The drink for each experimental group was administered orally every day at 7AM for 8 weeks.

Evaluation of the ergogenic properties of the drinks

Training protocol and endurance performance determination

The protocols of habituation and performance determination according to Boutard and Gaston methods[43] were used with some modifications. Briefly, rats were initially acclimated to run 2 consecutive days just before the day of the competition with an increase in velocity (10°, 22 rounds/min) for 60 min on the treadmill. In the morning of the competition, food was removed from the cage at 7: 00 AM and body weight was determined to the nearest gram on weigh triple-beam balance. The endurance test was measured at week 0, 4 and 8. After 0.5 hour of oral administration of the corresponding drink to each group, five accommodated rats were randomly selected per experimental group and were submitted to a race on a treadmill (10°, 33 rounds/min). The time spent on the treadmill during the race of the rats was noted and represented the performance of the rats during the experimentation.

Water mass content and biochemical parameters measurements

Following clearance from the Institutional Ethical Committee, the rats were sacrificed with ether at the end of the competition, each specimen. The Blood (2.5mL) was collected through the jugular catheter for the measurements of glycemia[44] and triglyceridemia by using the standard methods[45]. Dissection was performed to harvest the fresh wet carcass. Abdominal cavity was quickly opened to remove its content (viscera) and the empty carcass was used to determine the quantity of water. The empty carcass obtained was weighed to get the weight of the fresh Carcass. The fresh carcass was then dried in Oven at 105°C for 24 hours and weighed to obtain the dried mass of the carcass. The quantity of water present in the carcass was calculated by subtracting the fresh carcass mass from the dried carcass mass as recommended by the methods of Angeloco, Deminice, Leme, Lataro and Jordao[46]. The results obtained were expressed in percentage.

Statistical analysis

The results are presented as the mean ± S.E (standard error). The normality of the data was confirmed by the Student test. Comparisons between groups were made through an analysis of variance (ANOVA Two-Way) and the Tukey HSD post hoc test when necessary. Pearson's correlation coefficients were used to examine the relation between performance, glycemia, triglycemia, body weight and water content mass of different experimental groups of rats. Pearson's correlation coefficients were used to examine the relationship between endurance performance of different treatments and variables of body weight, aqueous mass and post effort of glycemia and triglyceridemia a statistical difference was accepted at $P < 0.05$.

RESULTS

Physico-chemistry and nutritional characteristics of the drink

The physico chemical and nutritional composition of the aqueous extract of the roots of *M. whitei* and drink testing made from this extract is reported in TABLE 1.

In TABLE 1, the chemical and nutritional composition of the aqueous extract of *M. whitei*, this crude extract without adding some ingredients such as salt and sugar does not meet the criteria for nutrient composition of a drink intended for sportsmen.

TABLE 1 : Physico-chemical characterization of the drink made from *mondia whitei* roots extract

Extract of *Mondia whitei*		Test drink of *Mondia whitei*	
Physico chemical composition	**Quantity**	**Physico-chemical composition**	**Quantity**
Dry matter (g/100 mL)	2.47± 0.03	Dry matter (g/100 mL)	4.98 ± 0.05
Water contents (g/100 mL)	97. 53 ± 0.03	Water contents (g/100 mL)	95. 02 ± 0.03
Total proteins (g/100 mL)	1.06 ± 0.05	Total proteins (g/100 mL)	1.06 ± 0.05
Total lipids (g/100 mL)	< 0.1	Total lipids (g/100 mL)	< 0.1
Total sugar (g/100 mL)	1.2 ± 0. 01	Total sugar (g/100 mL)	4.04 ± 0. 05
Sodium (mg/100 mL)	17.2± 0.21	Sodium (mg/100 mL)	80.50 ± 0.05
Polyphenol (mg/100 mL)	17.2± 0.21	Polyphenol (mg/100 mL)	17.2± 0.21
pH	3.27 ± 0.19	pH	3.48± 0.19
Energy value (Kcals)	45.14± 0.19		28.4 ± 0.21

Test drink of *M.whitei* was obtained after adjustment of the carbohydrate and NaCl to the dry extract of *M.whitei* in respect to the international norms of the Scientific Committee on Food. Values are expressed as mean ± SD

The addition of salt in the extract increased the sodium content of the test drink. Furthermore, adding saccharose in the extract increases its sugar content and energy value for making the test beverage. Saccharose was chosen as an additional sugar in the extract because it is the easily consumed accessible for athletes. This is an equimolar mixture of fructose and glucose. Generally, the aqueous extract of the *M. whitei* roots brings energy and increase mineral level in the test beverage. Only the drink made from the aqueous extract of the roots of *M. whitei* is tested to see if it meets the criteria of composition of a beverage intended for athletes. It is made up of 4.04 ± 0. 05 g / 100g of carbohydrates in a dry matter, 80.50 ± 0.05 mg / 100g dry matter sodium with 28.4 ± 0.21 Kcals as energy value per 100g of dry matter of the beverage. In addition to the three essential elements that are found in a sports drink (water, sugar, sodium), this beverage contains polyphenols and has an acid pH.

Toxicity study of the drink

Toxicological studies are made on the daily consumption of the drink made from extracts of *M. whitei* at 400 mg/kg body weight. It did not seem to have risks on human health.

Acute toxicity

In acute toxicity evaluation, no animal died during treatment with the test drink containing the extract of *M. whitei*. There were no differences in appearance, for coloration, diarrhea, blood, constipation, anorexia, hydration, and environmentally related changes in the rats.

Sub-acute toxicity

The results obtained on the effect of graded doses of *M. whitei* on the weight, hematological and biochemical parameters are presented respectively in the TABLES 2 and 3.

According to the TABLE 2 and 3, the administration of the drink of *M. whitei* extracts had no effect on the weight of the internal organs (liver, heart, lungs, kidneys, testes and ovaries), hematological and biochemical parameters of rats except to platelets ($P>0.05$; $n=6$). The results obtained on the effect of graded doses of *M. whitei* test drink on the histology of the liver of female rats are shown in Figure 1.

Histological study of the female rats liver in Figure 1, showed no abnormities detected in the treatment of the rats (no cellular infiltration, no edema, no fibrillosys and no hemorrhagy). A similar observation was also made for male rats in the same experiment.

Ergogenic property of the beverage

Endurance performance

Endurance performance of the different groups of the rats is shown in the Figure 2.

According to Figure 2, the mode of treatment influenced the performance (Current effect: $F(5, 24) = 5979.7$, $p = 0.0000$). The Tukey post hoc analysis showed that the average performance was higher, respectively, in the rats treated with the extract of *M. whitei* + salt + sugar (2476.2 Sec), to extract *M.*

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TABLE 2 : Hematological and biochemical parameters of rats in sub-acute toxicity of the drink

Parameters	Rats	Treatments			
	Sex	0 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg
Red cells x 10^{12} (g/l)	M	6.58 ± 0.75	6.70 ± 0.45	6.78 ± 0.9	6.67 ± 0.7
	F	6.1 ± 0.67	6.10 ± 0.90	7.06 ± 0.7	6.5 ± 0.5
Hematocrit (%)	M	40.1 ± 1.6	44.12 ± 5.8	44.20 ± 3.2	44.3 ± 3.4
	F	39.78 ± 1.3	43.3 ± 1.5	43.18 ± 3.6	43 ± 1.34
Hemoglobin (g/dl)	M	13.82 ± 0.52	15.75 ± 1.15	14.5 ± 0.9	13.9 ± 0.72
	F	12.84 ± 0.47	14.48 ± 0.52	14.1± 0.47	12.9 ± 0.73
Blood platelets x 10^{9} (g/l)	M	273.6 ± 42.8	298.6±24.9*	304 ± 42.7*	337 ± 19.23*
	F	249.8 ± 44.7	271.2±56.3*	289 ± 31.8*	321 ± 40.6*
Glycemia (mg/dl)	M	64.6 ± 3.3	65.4 ± 3.6	66 ± 4.8	63.5 ± 3.7
	F	62.6 ± 3.7	63 ± 3.6	64.6 ± 4.7	64.2 ± 4.2
ASAT (U/L)	M	145.4 ± 7.6	144 ± 8.7	146 ± 9.7	146.8 ± 8.6
	F	146.6 ± 7.3	147.2 ±7.3	148 ± 7.9	148.4 ± 7.4
ALAT (U/L)	M	44.6 ± 7.06	45 ± 7.5	46 ± 5.9	47.2 ± 5.8
	F	44.4 ± 8.6	45.2 ± 6.3	45.6 ± 6.8	46,2 ± 7,05
Total Protein (g/l)	M	130.8 ± 10.6	128.2 ± 6.02	126 ± 10.2	122 ± 3.43
	F	134.4 ± 7.5	128.8 ± 4.7	125 ± 8.7	122.4 ± 3.1
Creatinaemia (mg/l)	M	0.67 ± 0.1	0.68 ± 0.06	0.68 ± 0.1	0.69 ± 0.1
	F	0.64 ± 0.08	0.65 ± 0.07	0.68 ± 0.1	0.68 ± 0.1

M: Male, F: Female, ASAT: Aspartate aminotransferase, ALAT: Alanine aminotransferase. Values are expressed as mean ± S.E.M; (n = 6). Treatments consisted in the administration of the test drink (extract of *Mondia whitei* + NaCl + sugar) at 0 mg /kg of the body weight of the rats (untreated group), 400, 600, 800 mg /kg of the body weight of the rats (treated groups) in sub acute toxicity study. *P<0.05 compared with the untreated group.

TABLE 3 : Effect on the weight of internal organs of rats during the sub-acute toxicity

Organs	Treatments				
	Sex	0 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg
body weight	M	195 ± 2.5	205 ± 3.35	215 ± 5.45*	225 ± 4.3*
	F	150 ± 3.7	160 ± 2.7	165 ± 4.25*	175 ± 2.5*
Kidneys	M	1.17 ± 0.12	1.49 ± 0.31	1.37 ± 0.07	1.27 ± 0.47
	F	1.17 ± 0.18	1.16 ± 0.23	1.29 ± 0.11	1.20 ± 0.12
Heart	M	0.71 ± 0.11	0.76 ± 0.14	0.79 ± 0.09	0.78 ± 0.14
	F	0.55 ± 0.06	0.58 ± 0.1	0.61 ± 0.04	0.62 ± 0.07
Liver	M	7.03 ± 0.66	7.26 ± 0.67	7.73 ± 0,99	7.87 ± 1.53
	F	5.07 ± 0.44	5.14 ± 0.85	5.19 ± 0.26	5.3 ± 0.78
Lungs	M	1.69 ± 0.49	1.74 ± 0.19	1.95 ± 0.52	1.99 ± 0.87
	F	1.18 ± 0.15	1.25 ± 0.13	1.38 ± 0.14	1.63 ± 0,59
Testes	M	2.58 ± 0.29	2.64 ± 0.29	2.65 ± 0.19	2.67 ± 0.45
Ovaries	F	0.10± 0.016	0.11 ± 0.03	0.13 ± 0,02	0.14 ± 0.01

M: Male, F: Female, values are expressed as mean ± S.E.M; (n =6). *P<0.05 compared with untreated normal rats (0 mg/kg). Treatments consisted in the administration of the test drink (extract of *Mondia whitei* + sel + sugar) at 0 mg /kg of the body weight of the rats (untreated group), 400, 600, 800 mg /kg of the body weight of the rats (treated groups) in sub acute toxicity study. *P<0.05 compared with the untreated group.

whitei + salt (2344. 5 Sec), to extract *M. whitei* + sugar (1628.1 Sec) and extracted *M. whitei* (1830.5 Sec) that the negative control rats (1161.1 Sec) (P<0.001). In addition, the average performance of the positive control rats (1667.5 Sec) was higher than that of the negative control group (1648.5 Sec).

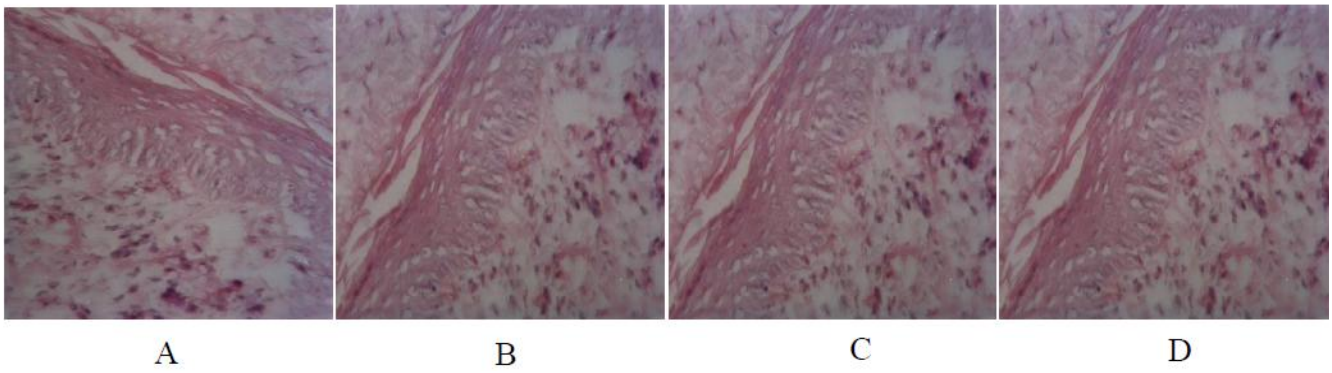
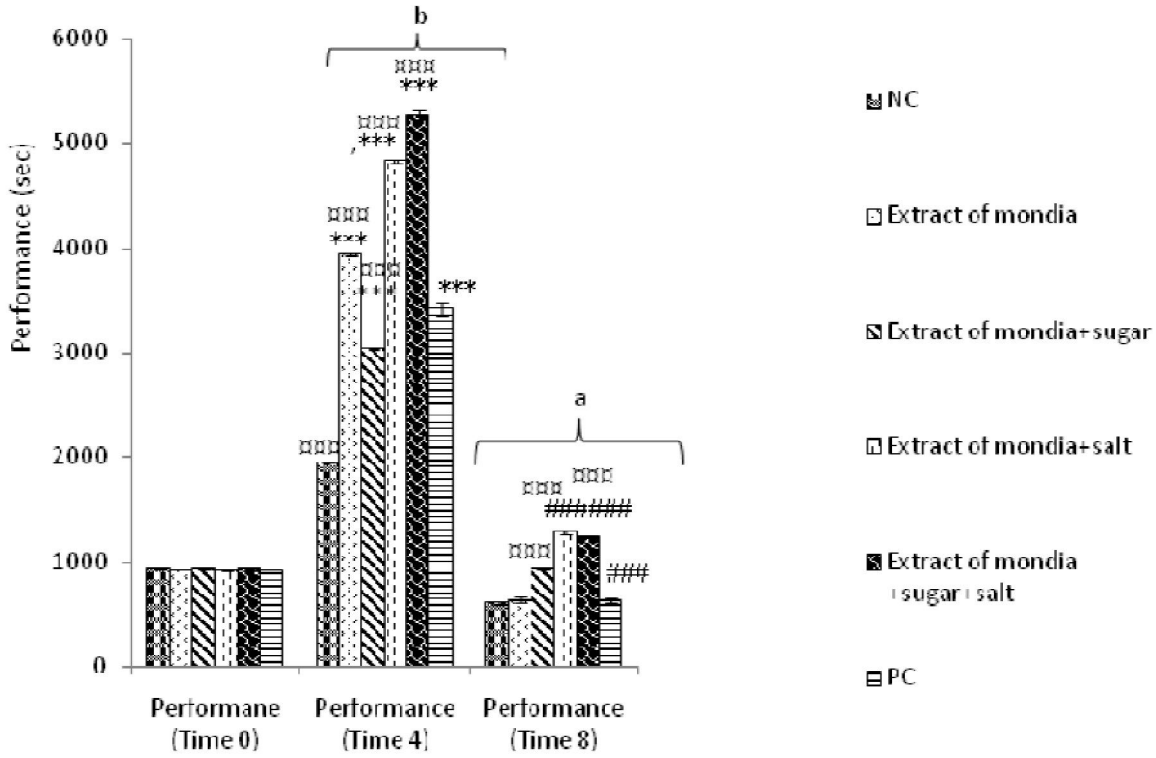


Figure 1 : Photomicrograph of the liver histology of treated and untreated albino rats with the test drink of *Mondia whitei*; A: control; B: 400 mg/kg; C: 600 mg/kg; D: 800 mg/kg of body weight. Liver sections stained with haematoxylin and eosin (100 X)



[a]P < 0,01 compared to the time 0, [b]P<0,001 compared to the time 0;*P < 0.001 compared to control negative group at T4,[###]P < 0.001 compared to the control negative groups at T8, □□□P< 0,0001compared to the control positive group at T4 and T8**

Figure 2 : Variation of the performances of the rats during the experimentation

A time effect on the performance Current effect: F (2, 48) = 1339E2, p = 0.0000 was observed. The post hoc Tukey test showed that the average performance time T0 (917.4 Sec) was smaller than the performance to T4 (3742.8, P <0.001) and was greater than the performance at the time T8 (887, 08 Sec) (P <0.01). The repeated measures ANOVA showed an effect of the interaction between the time of the experiment treatment (Current effect: F (10, 48) = 2329.3, p = 0.0000). Tukey post hoc analysis showed that at time T0, there were no significant differences between the control groups and the other experimental groups of rats. At time T4, the performance of negative control rats (1956.6 Sec) was smaller than that of rats treated with the extract (Sec 3943; P <0.001), with extract + sugar (3037.2 Sec; P <0.001), with extract + salt (4833.8 Sec; P <0.001), with extract + salt + sugar (5267.6 Sec; P <0.001) than the rats in the positive control group (3458.6 sec; P <0.001). At time T8, the performance of negative control rats (608.20 Sec) was smaller than that of rats treated with the extract of *M. whitei* + sugar

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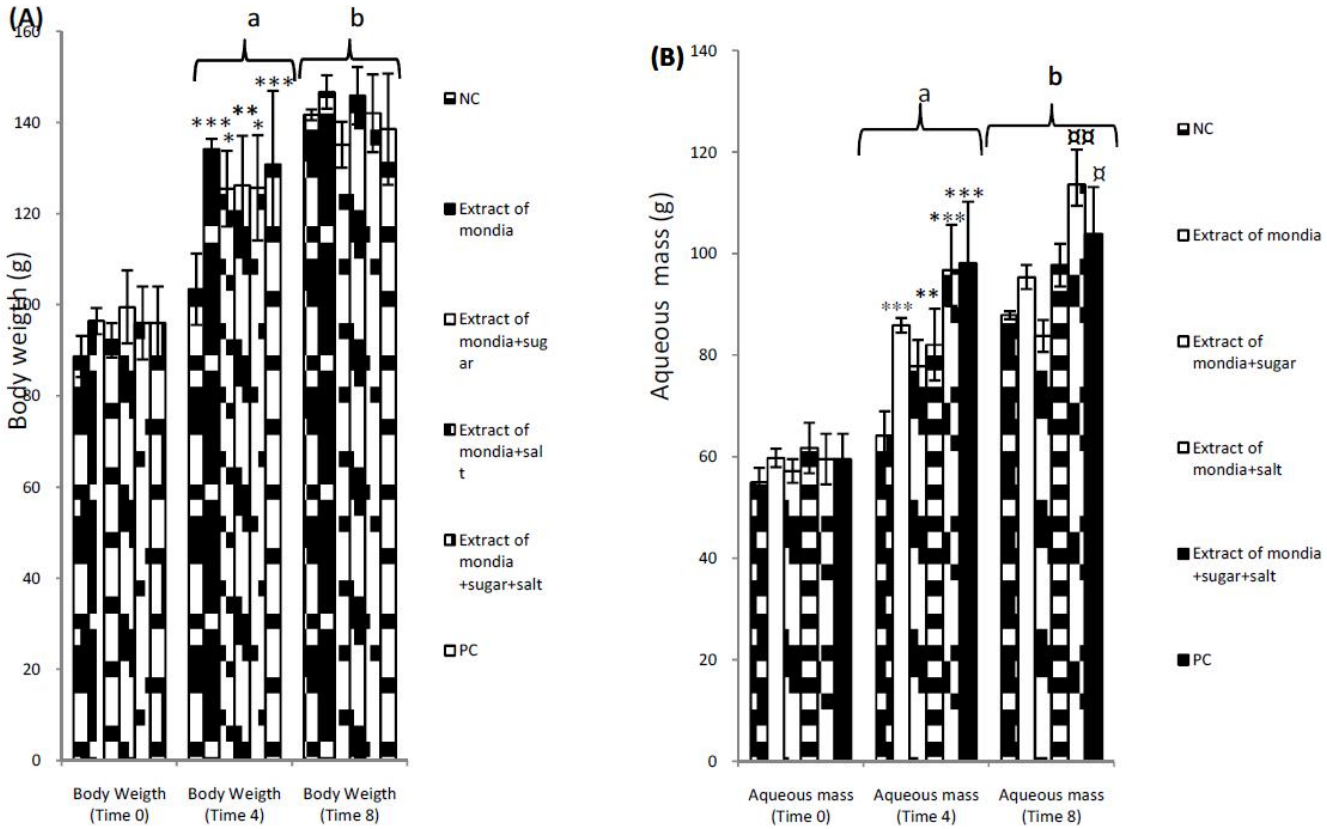


***P<0.05 compared with the time 0 of the experimentation, **P<0.001 compared with the time 0 of the experimentation. Different letters mean P<0.05 compared with different experimental groups at the same period**

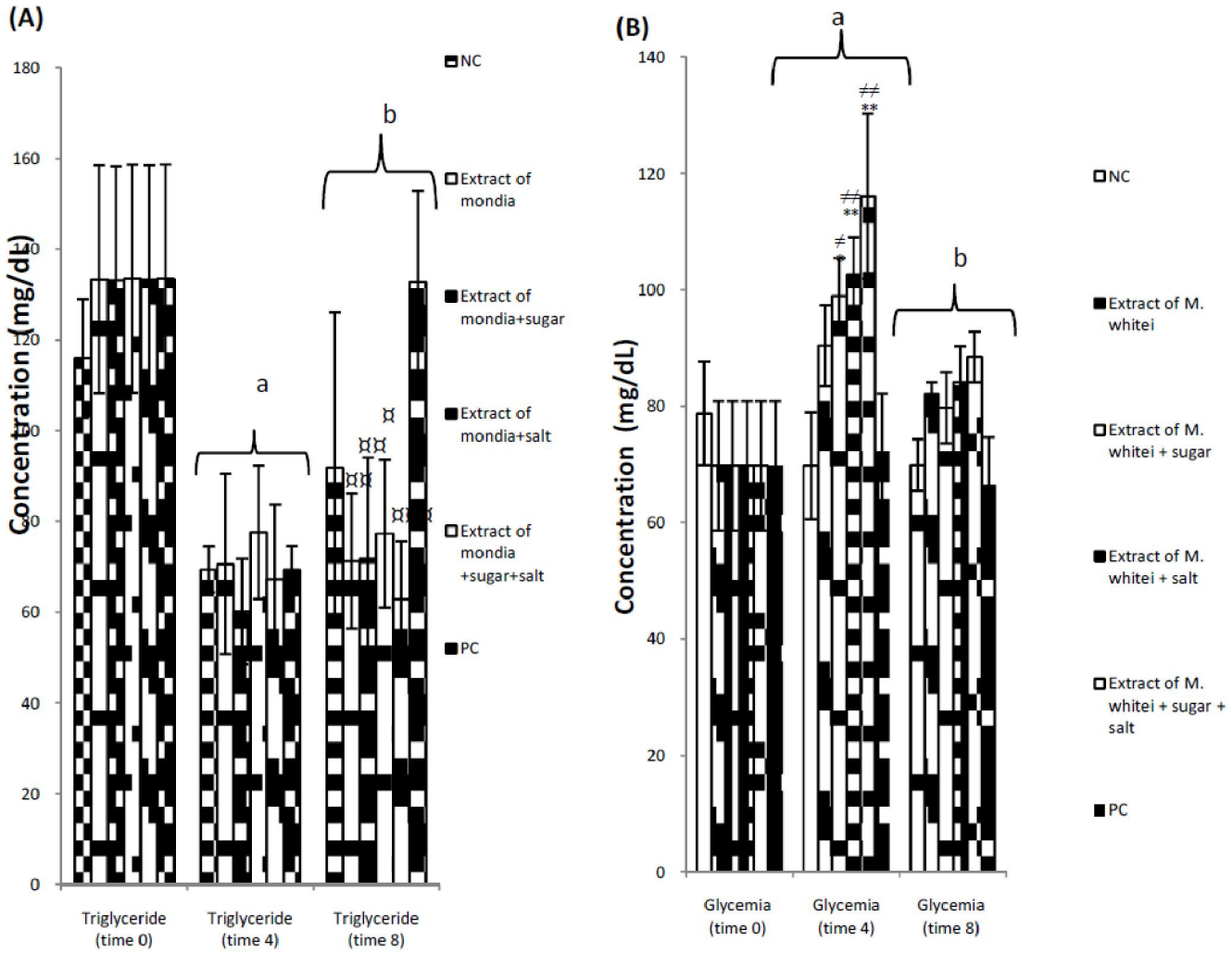
Figure 3 : Variation of the glycemia and the triglycedemia of the rats during the experimentation

(Sec 926; P <0.001), and extract + salt (1284.8 Sec; P <0.001) than those treated with the extract of *M. whitei* + salt + sugar (1243.1Sec; P<0.001).

Body composition of the experimental groups

The body composition of the experimental groups of the rats is illustrated in the Figure 3A and 3B. According to Figure 3A, the mode of treatment showed an effect on body weight of rats in each experimental group (Current effect: F (5.24) = 2.6370, p = 04893). Thus, in general, it has been observed that the rats treated with *M. whitei* aqueous extract had a significantly higher body weight (125.76 g; P≤0.05) to that of the negative control group (111.24 g). The time factor has influenced the weight of rats in different experimental groups (Current effect: F (2. 48) = 955.83, p = 0.0000). The average body weight of rats at time T8 (141.66 g, P<0. 0005) was higher than the average body weight of rats at time T4 (124.30 g) and the time T0 (94.79 g). The average weight of the rats at time T4 (124.30 g; P<0.001) was higher than that at time T0 (94.79g). The treatment time fashion has influenced the average weight of rats Current effect: F (10.48) = 7.9098, p = 00000). At time T0, the average body weight of different experimental groups of rats were not different (P>0.05). At time T4, the average weight of the rats in the negative control group (103.40 g) was smaller than those of the experimental groups treated with extract + Sugar (125.47 g; ≤0.05) in the extract + sugar + salt (125.69 g; P<0.05), to extract + salt (126.24 g; P<0.01) and as well as those treated with the extract (134.16 g; P<0.0005). The group of positive control rats showed an average weight (130.82 g; P<0.005) greater than those of the rats of negative control group (103.40 g). At time T8, there was no significant difference between the different experimental groups.

According to Figure 3B, the method of treatment has had an effect on the water mass (Current effect: F (5.24) = 12.778, p = 00000). The Tukey post hoc analysis showed that water or aqueous mass of negative control rats (68.97 g) was lower than that of rats treated with the extract (80.33 g; P<0.05) to extract + salt (80.5g; P<0.05), to extract + sugar (82.057g; P<0.05) in water (87.17 g; P0<0.0001)



[a]P < 0,05, [b]P<0,001 compared to the time 0; ***P < 0.001, **P < 0.0001 compared to control negative group at T4; ¤P<0,05, ¤¤P<0,001compared to the positive control group at T8

Figure 4 : Variation of the triglycedemia (A) and the glycemia (B) of the rats of the rats at 0, 4, 8 weeks of the experimentation

and that those of rats treated with the extract + sugar + salt (89.97g; P<0.0005 The time factor influences the water percentage in different batches of rats (Current effect:. F (2.48) = 1074.1, p = 0.0000). The post hoc Tukey analysis showed that the body water in rats at time T8 (97.04, P<0, 0001) was higher than the average weight of rats at time T4 (84.12 g) and at time T0 (58.77g). The average weight of the rats at time T4 (84.12 g. P<0.0001) was greater than the time T0 (58.77g).

The treatment time fashion has influenced the average body water Current effect of rats: (Current effect: F (10.48) = 21.140, p = 00000). At time T0, the body water of different experimental groups of rats were not different (P>0.05). A time T4, body water rats from the negative control group (64.11 g) was smaller than the experimental groups treated with extract + Sugar (77.79 g; P<0.05) than those treated with extract + sugar + salt (96.78 g; P≤0.0005) than those treated with the extract + salt (82.05 g; P<0.001) and as well as those treated with the extract (85.86; P<0.0001). The positive control group of rats had an average body water (98.11g; P<0.0001) than negative control rats (64.11). At time T8, the average body water of the negative control group (87.84 g) was smaller than the group taking the drink + salt + sugar extract (113.61 g; P<0.0001) and that of S positive control group (103.91g; Pd"0.01) in different batches (Current effect: F (2. 48) = 1074.1 p = 0.0000). The post hoc Tukey analysis showed that the body water in rats at time T8 (97.04, P<0.0001) was higher than the average

weight of rats at time T4 (84.12 g) and at time T0 (58. 77g). The average weight of the rats at time T4 (84.12 g; P<0.0001) was greater than the time T0 (58.77g). The treatment time fashion has influenced the average body water Current effect of rats: (Current effect: F (10.48) = 21.140, p = 00000). At time T0, the body water of different experimental groups of rats were not different (P>0.05). At time T4, body water rats from the negative control group (64.11 g) was smaller than the experimental groups treated with extract + Sugar (77.79 g; P<0.05) than those treated with extract + sugar + salt (96.78 g; P<0.0005) than those treated with the extract + salt (82.05 g; P<0.005) and as well as those treated with the extract (85.86; P<0.0005). The positive control group of rats had an average body water (98.11g; P<0.0001) than negative control rats (64.11). At time T8, the average body water of the negative control group (87.84 g) was smaller than the group taking the drink + salt + sugar extract (113.61 g; P<0.0001) and that of positive control group (103.91g; P<0.01).

Biochemical parameters post exercise measurements

The biochemical parameters post exercise measurements of the experimental groups of the rats is illustrated in the Figure 4A and 3B. According to Figure 4A, the treatment affects the rate triglyceride (Current effect: F (5.24) = 2.6497, p = 04.812). The Post hoc analysis showed that only the positive control group had higher triglyceride levels (111.85 mg / dL) than rats taking the full test drink (87.82 mg / dL; P≤0.05). Time influence on triglycerides (Current effect: F (2.48) = 82.631, p = 00000). The post hoc analysis showed that the average rate was triglycerides High time T0 (130.45 mg / dL) compared to the time T4 (69.06 mg / dL; p <0.0001) and time T8 (84.64 mg / dL; P<0.0001). The average triglyceride level at time T8 was higher than that at time T4 (p ≤ 0.01). Comparing the interaction time * triglycerides showed effect (Current effect: F (10.48) = 3.3318, p = 0.0235). The post hoc analysis showed that at time T0, there were no significant differences between the experimental groups. At time T8, there was no significant difference between the negative control group rats and the rats of the other experimental groups (P>0.05). However it can be noted that at time T8, triglyceride levels were higher, respectively, in rats the positive control group (132.79 mg / dL) compared to rats treated with the extract (71.23 mg / dL; P<0.005) by extract + sugar (71.78 mg / dL; P<0.005) and to extract + sugar + salt (62.849mg/dL; P<0.005).

According to Figure 4B, Statistical analysis repeated measures ANOVA showed a treatment effect (Current effect: F (5.24) = 20.674, p = 00000). The post hoc Tukey analysis showed that the mean glucose levels were elevated post stress in rats took to extract (80.70 mg / dL; P<0.05), to extract + sugar (82.88 mg / dL P <0.01), to extract + salt (85.53 mg / dL; P<0.001), and the extract + sugar + salt (91.48 mg / dL; P≤0.0005) that the negative control rats (72.84 mg / dL). No significant difference between the rats negative controls with the control rats controls positive (69.50 mg / dL; P >0, 7). The rats given the beverage have a higher average blood glucose (91.48 mg / dL) than those taking only the extract (80.70 mg / dL; p ≤ 0.005) or while those taking the extract + sugar (82.88 mg / dL; p <0.05). The positive control rats have an average blood glucose (69.50 mg / dL) than rats who took the extract (80.70 mg / dL; P<0.005), the extract + salt (85.53 mg / dL; P<0.0001), the extract + Sugar (82.88 mg / dL; P <0.0005) and extracts + sugar + salt (91.48 mg / dL; P<0.0005) the rats negative (72.84mg / dL). The comparison of the average blood glucose over time showed that there was an effect of time on treatment (Current effect: F (2.48) = 30.229, p = 0.0000). The post hoc analysis showed that average blood glucose was higher in T4 time (91.71 mg / dL; P<0.0005) and T8 (78.48 mg / dL; p < 0.05) than T0 (71.30 mg / dL). Glucose T4 was higher

Than that to T8 (p<0.0005). Comparing the interaction time * shows a treatment effect (Current effect: F (10, 48) = 5.0164, p = 00006). The post hoc analysis showed that at T0, there is no significant difference between the experimental groups. At time T4, blood glucose was higher respectively in rats treated with the extract + sugar (99.00 mg / dL; P<0,001), to extract + salt (102.60 mg / dL; P<0.0005), and extract sugar + salt + (116.10 mg / dL; P <0.0005) compared with positive control group rats (69.80 mg / dL). At time T8, there is no significant difference between the negative control rats and other experimental groups of rats (P>0.05).

TABLE 4 : Correlation between endurance performance, aqueous mass, body weight, triglyceridemia and glycemia post effort at different times of the experimentation

	Correlation indices		
Study variables	**Performance (T0)**	**Performance (T4)**	**Performance (T8)**
Body weight (T0)	-0.204(0.279)	0.434 (0.0165)*	0.282(0.131)
Body weight (T4)	-0.252(0.000)***	0.460 (0.010)*	0.0635(0.739)
Body weight (T8)	-0.365(0.048)*	0.325 (0.079)	0.0877(0.645)
Aqueous mass (T0)	-0.204(0.279)	0.434 (0.016)*	0.282(0.131)
Aqueous mass (T4)	-0.0961(0.614)	0.974 (0.0063**)	0.124(0.515)
Aqueous mass (T8)	-0.175(0.354)	0.687 (0.0000)***	0.345 (0.0618)*
Glycemia (T0)	-0.50 (0.0117)*	-0.0676 (0.723)	-0.263(0.177)
Glycemia (T4)	-0.176 (0.353)	0.694(0.000)***	0.740 (0.000)***
Glycemia (T8)	-0.197(0.296)	0.699 (0.000)***	0.620 (0.000)***
Triglyceridemia (T0)	-0.114(0.550)	-0.095 (0.617)	-0.0256(0.893)
Triglyceridemia (T4)	-0.219 (0.245)	0.141 (0.456)	-0.623 (0.0000)***
Triglyceridemia (T8)	-0.0085 (0.0 964)	0.103(0.589)	-0.427(0.0188)*
Performance (T0)		-0.177 (0.348)	-0.140 (0.460)
Performance (T4)	-0.118 (0.533)		-0.757 (0.000)***
Performance (T8)	-0.140 (0.4660)	0.124 (0.515)	

Values in brackets represent the P-value while the others represent the correlation coefficient R *; P < 0.05, **P < 0.01, ***P < 0.001. Drink was obtained after adjustment of the carbohydrate and NaCl to the dry extract in respect of the international norms. Values are expressed as mean ± SD

Correlation between endurance performance time, body composition and some biochemical post exercise parameters of the rats at different times of the experimentation

The TABLE 4 shows the correlation between different parameters studied. According to the TABLE 4, at the time T0, the endurance performance was negatively correlated to alls parameters but the correlation were strong and with the glycemia at T0 ($P < 0.05$). At the time T4, the endurance performance is negatively correlated to the triglyceridemia and is positively correlated with the body weight at T0 ($P < 0.05$), at T4 ($P < 0.05$) and T8 ($P > 0.05$). It was also correlated with the aqueous mass at T0 ($P < 0.05$), at T4 ($P < 0.01$) and T8 ($P < 0.001$) and with the glycemia at the times T4 and T8 ($P < 0.001$). At the time T8, the endurance performance was positively correlated with the aqueous mass ($P > 0.05$), with the glycemia ($P < 0.001$) and negatively correlated with the triglyceridemia at times T8 ($P < 0.05$), and T4 ($P < 0.01$).

DISCUSSION

From TABLE 1, the aqueous extract of the roots of *Mondia whitei* does not respond to the standard in terms of quantity to the chemical composition of a beverage intended for athletes. According to the AFSSA[48], the drink designed for athletes must be between 460 mg / l and 1150 mg / l sodium, having an osmolality between 200 and 300 m Osmol / kg water, having between 80 kcals / 1000 mL and 350 kcals / 1000 mL of energy and have at least 75% of metabolic energy from carbohydrates (glucose, maltose, dextrin, sucrose). When *M. whitei* roots are added into aqueous extract salt and carbohydrates such as saccharose, it enables compliance with standard for nutritional composition of sports drinks. Selecting sucrose as a type of carbohydrate added to the aqueous extract of the roots of *M. whitei* is justified by its easy accessibility in homes and it is an equimolar mixture of glucose and fructose which oxidizes easily. The review of related literature reveals that, the oxidation rate of glucose and fructose as a mixture is higher than that of an equivalent amount of glucose alone[47]. In addition to that, the beverages containing the extract of the roots of M. whitei + sugar + salt is energetic and possess an energy value within the standard energy drinks with 28.4 ± 0.21 kcals / 1000 mL. This energetic prop-

erty is mainly related to its high sugar content (endogenous and exogenous) as shown in TABLE 1. Except the essential elements of a drink for athletes should have sugar, water and salt, TABLE 1 shows that *M. whitei* test beverage contains polyphenols. With specific reference to the literature, components such as phytosterols, glucosinolates and phenol contained in the plant extract have antioxidant properties and may contribute to less protection against oxidative damage induced by strenuous exercise[48-50], especially as nowadays the trend is to develop protecting and toning properties that can reduce oxidative stress and promote the practice of physical activity without secondary effects on health. Sports drinks are dietetic in nature that is to say it obeys to the norms[35, 51]. From recommendations made by the Scientific Committee on Food or SCF[40], beverages must not be harmful to health and must be geared towards effective recovery as advocated by Bilzon *et al*.[52], on hydration and the performance of the athlete during a physical effort of long duration[53]. All these properties have been verified in drink from a beverage test of the aqueous extract of the roots of *M. whitei* + sugar + salt.

In acute toxicity following the 2000 mg / kg of test drink consumed by the rats of the test group for 14 days, no death was recorded within the group and therefore we deduce that the lethal dose 50 (LD50) of this beverage is higher than 5000 mg / kg and thus it is non-toxic[54]. However, in TABLE 2 and 3, the administration of the beverage *M. whitei* respectively had no effect on the hematological and biochemical parameters, on the body weight or on the internal organs of the rats ($P > 0.05$) except the high level of platelets. However, according Ansahl[55], Oluyemi, Omotuyi, Jinoh, Adesanya, Sarahu[56], reduced body weight and internal organs are considered as toxicity indicator after exposure to toxic substances, which is not the case with *M. whitei* + sugar + salt drink. The high quantity of platelets may be a drawback of consuming said beverage to high quantity of drink around 800 mg/kg. In sub acute toxicity of the drink, the recent work of Okon, Bankole, Eneasato, Ezezeah and Bankole on the histological changes in the hearts of rats fed diets containing the aqueous extract of the roots of *M. whitei* have shown that a high consumption of *M. whitei* (4.5 and 13.5 g Kg body weight) and an extended period (3 weeks), the extract of *M. whitei* had toxic effects on the heart[57]. For this reason, they suggested to regulate the incorporation of this extract in the consumable food products. Unlike these studies, Figure 1 rats consuming the test beverage at doses (0, 250, 500, 1000 and 2000 mg / kg of the body weight / day for 28 days) attest the safety of the said beverage as it is noted an absence of lesions in the liver of the rats eating the different doses of the test beverage (absence of oedema, congestion, inflammation, or hemorrhage, etc.). An examination of histological sections of the liver was made since the liver has gone a biotransformation of organs of the body. Indeed the lesions sought in the liver take the available literature which states that the volatile oil contained in the roots of *M. whitei* causes inflammation, irritation of mucous membranes at high doses[57]. Liver pain after a poisoning occurs in terms enzymatic by higher elevated plasma alanine aminotransferase (ALT) and aspartate aminotransferase of (AST) spleen and the reference values of AST and ALT are respectively (96 - 200 U / L) and (21-52 U / L) in the rat[59]. According to the results reported in TABLE 2, the values of AST and ALT in rats sub acute toxicity were normal. Therefore, the safety of this drink would be attributed to the low concentration of the aqueous extract of the roots of *M. whitei* it contains and its moderate consumption.

Concerning the ergogenic property of the drink, Figure 2 shows that at the time T4, the extract of *M. whitei* only increases the endurance performance of the rats that consume it as compared to those of rats in positive control groups ($p < 0.01$) and those in the negative control group ($p < 0.001$). Adding ingredients such as salt or salt + sugar both potentiates the ergogenic effect of the extract and that is why the experimental groups extract *M. whitei* + salt and extracted *M. whitei* + sugar + salt have time endurance to physical exertion on the treadmill higher ($p < 0.01$). The fact that the performance in endurance rats falls at T8 time in all experimental groups compared to the time T0 and T4 at the times would be attributed to the high intake of body weight of rats that would interfere with their performance on treadmill. However, the groups extracted *M. whitei* + salt

and extracted *M. whitei* + sugar + salt recorded again this time T8, the highest performance. According Xiafeng Shen[60] (2012), the treadmill is the instrument best suited to measure the rats in endurance performance and increase endurance time in rats consuming the drinks; one drink containing at least the aqueous extract of *M. whitei* roots, attests the ergogenic nature of this extract. The ergogenic property of the beverage is due to its physico-chemical and nutritional composition which include the bioactive substances like polyphenols, as many studies have revealed that bioactive substances of plant extracts (polyphenols) as Cordyceps sinensis Berk (ophiocordycipitaceae) according Koh *et al*.[23], *Allium sativum* L. (Amaryllidaceae) according to Morihara *et al*.[64], *Japonica pseudosasa* (Poaceae) according to You *et al*.[2], the Rubus coreanus (Rosaceae) according to Jung *et al*.[21], *Panax ginseng* CA Meyer (Araliaceae) by Tang *et al*.[20], the *Bacopa monniera* L. (Scrophulariaceae) by Anand *et al*.[19] and *Ocimum sanctum* L. (Lamiaceae) according Prasad and Khanum[22] increased the physical endurance capacity of rats. The mechanisms by which the bioactive substances in plants could have an ergogenic action are diverse. Thus, according to the literature, ergogenic plants such as Ginseng, Ginkgo biloba, Gamma oryzanol and Tribulus terrestris, Kava and St. Johns Worth have been described by Williams and Brand[63], by William[10]. To Chee *et al*.[64], it would increase the availability of energy substrates (glucose, fatty acids, etc.). Yeomans *et al*.[65] believe that, it would cause changes favorable metabolic functions, hematological and cardiovascular[10]. Moreover, some ergogenic substances increase the body recovery ability by fighting against oxidative stress caused by intense physical effort as demonstrated by the study of coenzyme Q10 supplementation in rats trained to swim[66] and the polyphenol supplementation in athletes[67]. As regards drinks containing the extract of *M. whitei,* their ergogenic properties would be allocated to the mobilization of substrates energy during and after physical exertion especially with regards to glucose (Figure 4). Therefore glycemia post effort is greater in the different experimental groups of rats regardless of the experimental period in contrast to controls (positive and negative). The positive control group recorded a higher performance than the negative control group. These findings support the work of Maugham[68] (2001) who found that the ingestion of water and carbohydrates are separate and additive effects on performance. However, several factors influence the endurance performance (TABLE 4), that is why at time T4, the performance is positive and low correlation to body weight ($R = 0.460$) and triglyceridemia ($R = 0.103$) post effort ($p > 0.05$) post effort it is strongly and positively correlated with body water ($R = 0.974$) ($p < 0.01$) and post exertion blood glucose ($R = 0.694$) ($p < 0.001$). However, it is extremely difficult to prove scientifically that the gene ergo reality of a substance especially for researchers from poor countries where methods and advanced measurement instruments used are not peak. Given the results presented in Figure 4, ergogene effect of the test beverage containing the aqueous extract of the roots of *M. whitei* + sugar + salt would be attributed to bioactive substances contained in the aqueous extract of the roots of *M. whitei* which would act on glycogenolysis and gluconeogenesis resulting from the high levels of sugar in blood after efforts that would act effectively and positively on recovery according Bilzon *et al*.[52]. As such, the drinking-water extract of the roots of *M. whitei* + sugar + salt would be a subject recovery beverage to doping. Liquid containing glucose consumed during exercise reduces the risk of dehydration, hyperthermia, provides energy to prevent premature fatigue and thus enhances performance[69]. It is sometimes difficult to separate the effects of water replenishing those of the substrate and electrolyte after ingestion of a solution enriched with carbohydrates and electrolytes. However, according to Rochcongar[35], the causes of poor physical performance of athletes are attributed to the depletion of glycogen stores and dehydration. The positive and strong correlation recorded both in time T4 and T8 post effortlessly between blood sugar and body water in TABLE 4 are consistent with the causes of such performance as stipulated by Rochcongar[35]. Hydration is therefore a key determinant of performance; this is the reason why the hydrant property of the drinking-water extract of the roots of *M. whitei* + sugar + salt was studied.

However, according to Figure 3, the rats that

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consumed the drink containing the aqueous extract of the roots of *M. whitei* have a higher body water than that of rats in the negative control group ($P < 0.05$) and the rats that consumed the drink containing the aqueous extract of the roots of *M. whitei* + sugar + salt has a higher water percentage ($P < 0.001$) and similar to that of the positive control rats control group ($P > 0.05$). This increase in body water suggests that the aqueous extract of the roots of *M. whitei* has a moisturizing property which is potentiated by the addition of both sugar and salt, wherein the water percentage higher by the group of rats who consumed the drink containing the aqueous extract of the roots of *M. whitei* + sugar + salt. The results in Figure 3 would be justified by the fact that the sodium (content in these beverages, see TABLE 1) involved in the cellular water retention as supported by Costill[69] in the active transport mechanism glucose by Wright *et al*.[70]. Salt and sucrose in the beverage containing the aqueous extract of the roots of *M. whitei* + sugar + salt promote the absorption of glucose, sodium and water and therefore induce an increase in body water consumer rats. These elements are put together to ensure oral rehydration during childhood diarrhea for example, or other gastroenteritis, where maximum stimulation is desired of sodium + absorption of water by adding glucose[70].

CONCLUSION

This study was carried out to prepare a drink from roots of *M. whitei* with a composition similar to the standard commercial drink. To evaluate its toxicity and its ergogenic properties in rats, a test drink was made with extracts of *M. whitei* + salt + sugar respects the standards composition of the sport drink and gets energetic, hydrating and ergogenic properties. It is not also toxic. The *M. whitei* plant has been proven to contain great and diverse nutritional and medicinal values and then the test drink prepared with its roots could be considered as a nutraceutic beverage. But it is important to mention something about doping, anti oxidant, microbiological and organoleptical aspects of this drink before its promotion.

ETHICAL CONSIDERATIONS

All procedures in this study followed the clearance from the Institutional Ethical Committee and were approved by the Animal Ethical Committee of the Laboratory of Animal Physiology of the Faculty of Sciences (Reg. No. FWA-IRB00001954), University of Yaoundé I Cameroon.

AUTHORS' CONTRIBUTIONS

Mibo'o P, Ngogang Yonkeu J, and Mbofung Fontang CM, and Nso E conceived the study. Fouda Omgbwa Nsi L participated in its design, performed statistical analyses and drafted the manuscript. Pieme A C and Robert Germain Beka performed nutritional and biochemical study, statistical analyses and have been involved in drafting the manuscript. All authors read and approved the final manuscript.

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COMPETING INTEREST

Authors have declared that no competing interests exist.

HIGHLIGHTS

- The composition of the formulated drink made from extract of *Mondia whitei* was similar to a standard commercial sport drink.
- The formulated drink made from roots of *Mondia whitei* is not toxic
- The endurance performance of the rats which consumed the drink made from roots of *Mondia whitei* was the highest.
- The drink of *Mondia whitei* is ergogenic, energetic and hydrating.

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