SCIENTIFIC JOURNAL OF SPORT AND PERFORMANCE

ORIGINAL ARTICLE

Physical performance, haematological and behavioural parameters of five mineral water in rats

Lamou Bonoy . *Department of Physical Education, Health, and Leisure. University of Ngaoundéré. Cameroon.*
Jean-Pierre Mbame. *Department of Biology of Physical Activity and Sports Education. National Institute of Youth and Sports. Cameroon.*
Hamadou André. *Department of Biology of Physical Activity and Sports Education. National Institute of Youth and Sports. Cameroon.*
Pierre Ngarsou. *Department of Biology of Physical Activity and Sports Education. National Institute of Youth and Sports. Cameroon.*
Joséphine Doukoya. *Department of Biology of Physical Activity and Sports Education. National Institute of Youth and Sports. Cameroon.*

ABSTRACT

The objective of this study was to compare the effect of five mineral water mostly consumed in Cameroun on performance, haematological and behavioural parameters in the rats. Twenty-four rats (140 g) were used for this study. Among the groups, there are a control group which received deionized water and five experimental groups which received different mineral water (*Tangui, Supermont, Opur, Aquabelle and Semme*) during 21 days. Rats were allowed for swimming exercise every alternate day between 11h and 17h. On the day 21, rats from each group were subjected to the forced swimming test with tail load. One hour after the forced swimming test, the open field test was carried out to evaluate their behaviour. Water and food consumption, body weight, relative organ weight and haematological parameters were evaluated. Results showed that all the mineral water used during this experimentation did not have any effect on water intake, food consumption, body weight and relative organ weight ($p > .05$). However, significant increase ($p < .01 – p < .001$) in swimming time of rats consuming *Tangui* (445.00 ± 43.60 seconds) and *Opur* (450.80 ± 51.03 seconds) were observed as compared to the control group (325.50 ± 24.37 seconds). In the same way, results of behavioural and haematological parameters showed significant modifications in the same groups in comparison with control group ($p < .01 – p < .001$). Differences of swimming time, hematologic and behavioural parameters observed in rats having consumed *Tangui* and *Opur* could be due to the quantity of some minerals (calcium and magnesium) present in those drinks.
Keywords: Sport medicine, Physical activity, Anxiety, Sports health, Sports nutrition.

Cite this article as:
Bonoy, L., Mbame, J-P., André, H., Ngarsou, P., & Doukoya, J. (2022). Physical performance, haematological and behavioural parameters of five mineral water in rats. *Scientific Journal of Sport and Performance*, *2*(1), 1-8. https://doi.org/10.55860/RMEK4399

Corresponding author. *Department of Physical Education, Health, and Leisure. University of Ngaoundéré. Cameroon*.
E-mail: lamloukessi007@yahoo.fr
Submitted for publication August 15, 2022.
Accepted for publication October 09, 2022.
Published October 25, 2022.
Scientific Journal of Sport and Performance. ISSN 2794-0586.

doi: https://doi.org/10.55860/RMEK4399

INTRODUCTION

Heat resulting during practice of physical exercise is mainly dissipated by evaporation and perspiration. The dehydration which follows block the dissipation of heat, leading to the increase of body temperature, the losses of electrolytic and the reduction of physical performance (Walsh et al., 1994). To prevent these effects, athletes must hydrate themselves as soon as possible. It is thus recommended to the athletes to absorb at least 500 ml of liquid (mineral water, fruit juice, sport drinks) two hours before the beginning of physical activity (Shi, 2004). However, recent studies proved that pure water is not the best solution to be consumed after exercise to replace the sweat water lost (Bigard, 2007).

Recent studies showed that practicing physical activity for more than one hour could cause minerals losses and have a negative effect on athlete's health (IFN, 2010; Gibson et al., 2010; Lazarte et al., 2015). In addition, consumption of 4 litters water and more, practically without sodium to compensate sweat losses, could lead to symptomatic hyponatremia, cerebral oedema, neurological signs, coma and death (Hsieh et al., 2002). In order to replace water and electrolytic losses caused by the practice of physical activity, athletes can consume mineral water. The use of those mineral water was justified by their particular property. Indeed, according to their composition, these drinks are recognized as having a benefit for athlete's health (IFN, 2010).

In Cameroun, several mineral water marks used in training sessions and during competitions exists on the market. However, the choice of these mineral water by athletes does not depend on scientific criteria and there is limited data confirming their efficacy for improving performance. The objective of this study was to evaluate the effect of five mineral water mostly consumed in Cameroun on performance, haematological and behavioural parameters in rats subjected to a training program.

MATERIALS AND METHODS

Mineral waters

Five most consumed mineral waters (*Tangui, Supermont, Opur, Aquabelle and Semme*) were bought in the local market (Yaoundé) and used for this study. The choice of these mineral water is justified by fact that they were mostly consumed in Cameroon. The mineralogical composition of those mineral water was obtained from the labels present on each bottle.

Animals

Male albino rats of Wistar strain weighing 140 g were obtained from the Animal house of the National Institute of Youth and Sports in Yaoundé. They were placed in plastic cages in a room under standard Laboratory conditions (temperature 20 to 30°C, relative air humidity 45 to 55%, and 12/12 h light/dark cycle). The rats were fed with a basal diet and water *ad libitum*. The feed was a standard rat chow (National Veterinary Laboratory (LANAVET), Cameroon) composed of carbohydrates (52%), protein (22%), fat (6.5%), water (12%), ash (6%), and fibre (4.5%). The authorization for the use of laboratory animals in this study was obtained from the Cameroun National Ethics committee (Reg. No FWA-IRB00001954). The use, handling and care of animals were done in adherence to the European Convention (Strasbourg, 18.III.1986) for the protection of vertebrate animals used for experimental and other purposes (ETS-123), with particular attention to Part III, articles 7, 8 and 9. The animals were transferred to the laboratory at least 1 hour before the start of the experiment. The experiments were performed during the day (11:00-17:00hr).

Animal grouping

After two weeks of acclimatization, thirty rats (six groups of five rats) were used for this study. Among the groups, there are a control group which received deionized water and five experimental groups which received five different mineral drinks (*Tangui, Supermont, Opur, Aqua-Belle and Semme*) during 21 days. The quantity of food and water consumed by each group of rats, as well as body weights were measured every two days during 21 days, between 11:00 AM and 12:00 AM.

Swimming training program

Swimming training program was performed as describe previously (Lamou et al., 2016) but with some modifications. The rats of the experimental and control groups were allowed for swimming exercise every alternate day for a period of 21 days. Each animal was placed individually in a swimming pool (90cm × 45cm × 45cm), filled with water to a depth of 35 cm (Matsumoto et al., 1996; Kamakura et al., 2001) and maintained at 25 ± 1°C (Qi et al., 2014). The initial swimming time of the rats was 15 minutes, then 5 additional minutes were added every two days. The rats were then removed from the pool, dried with a paper towel, and returned to their original cages. The pool water was replaced after each session. After the last training session, the rats were prepared for the open field test.

Open field test

Following 12 hour of food and water deprivation, animals of all groups were submitted to open field test as described by Belzung (1999). The rats were individually placed at the centre of a clean open field apparatus (40×40×15 cm, divided into 25 squares), and the activity of each was video recorded for 5 minutes and analysed later. The latency period (time in the centre square), defecation, crossing (number of squares crossed indicating spontaneous locomotor activity), grooming and rearing (exploratory activity) for individual rat were scored. The arena was cleaned after each test using a 70% v/v ethanol.

Weight loaded force swimming test

The weight loaded force swimming test was performed as described previously (Lamou et al., 2016) but with some modifications. Briefly, one hour after open field test, rats taken from each group were subjected to the force swimming exercise. Each animal was supplied with a constant load (corresponding to 10% of the body weight) tagged to the tail and placed individually in a swimming pool (90 cm × 45 cm × 45 cm), filled with water to a depth of 35 cm and maintained at 30 ± 1°C (Qi et al., 2014). Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 seconds (Qi et al., 2014) and swimming time was recorded immediately. The rats were then removed from the pool and dried with a paper towel. At the end of the swim, the rats were rested for an hour and then sacrificed (under ether anaesthesia) by cutting through the jugular vein, blood samples were taken into bottles containing EDTA as anticoagulant was used to determine blood cell count.

Statistical analysis

Except mineralogical composition results of each drink, other results are expressed as mean ± standard error of mean (SEM). Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons and *p*-values less than .05 were considered significant.

RESULTS

Mineralogical composition of mineral water

Mineralogical composition of mineral water obtained from the labels present on each bottle was presented in Table 1. Observation of those results indicated that all mineral water used in this study have not the same mineralogical composition. Calcium and magnesium were most found in *Tangui* (32.00 mg/l and 21.00 mg/l for calcium and magnesium respectively) and *Opur* (45.00 mg/l and 16.00 mg/l for calcium and magnesium respectively). Potassium were most found in *Tangui* (10.00 mg/l), while chlorides, sodium and bicarbonate were most found in *Aquabelle* (4.00 mg/l) *Semme* (12.00 mg/l) and *Opur* (268.40 mg/l).

Table 1. Mineralogical composition of mineral water.

Minerals (mg/l)	**Mineral water**				
	Tangui	*Supermont*	*Opur*	*Aquabelle*	*Semme*
Calcium	32.00	30.00	45.00	20.00	13.00
Magnesium	21.00	5.90	16.10	9.00	12.00
Potassium	10.00	3.80	0.58	1.50	5.00
Chlorites	1.00	1.30	00	4.00	00
Sodium	1.00	00	2.02	2.00	12.00
Bicarbonate	217.00	134.00	268.40	96.00	161.00

Source. Labels obtained from each bottle.

Body weight, food and water intake

Consumption of different mineral water has no significant effect on body weight (Table 2) and food and water intake (Table 3) as compared to control group (p > .05).

Table 2. Body weight change of the rats during experimentation.

Body weight (g)	**Week 1**	**Week 2**	**Week 3**
Control group	140.00 ± 17.94	160.50 ± 15.69	168.75 ± 15.65
Tangui group	139.50 ± 19.12	159.50 ± 8.70	165.00 ± 5.29
Supermont group	139.75 ± 20.29	156.50 ± 20.17	163.25 ± 17.17
Opur group	141.00 ± 20.48	155.25 ± 13.96	168.75 ± 18.71
Aqua-Belle group	141.50 ± 20.09	157.50 ± 20.73	161.25 ± 13.00
Semme group	141.75 ± 19.70	161.25 ± 18.01	165.75 ± 14.20

Note. Each value represents the mean ± SEM, n = 5.

Table 3. Food (g/group/week) and water (ml/group/week) intake in rats.

Food intake (ml/group/week)	**Week 1**	**Week 2**	**Week 3**
Control group	207.75 ± 24.66	190.25 ± 41.04	197.00 ± 44.65
Tangui group	231.75 ± 18.84	192.50 ± 60.23	197.25 ± 44.75
Supermont group	204.75 ± 19.72	201.25 ± 62.36	186.00 ± 49.81
Opur group	206.00 ± 16.35	178.25 ± 55.31	184.75 ± 21.70
Aqua-Belle group	207.75 ± 32.49	191.00 ± 73.03	188.75 ± 22.19
Semme group	216.00 ± 21.60	196.00 ± 40.11	164.75 ± 13.43
Water intake (ml/group/week)	**Week 1**	**Week 2**	**Week 3**
Control group	145.76 ± 20.24	140.76 ± 18.86	238.51 ± 73.78
Tangui group	105.86 ± 20.37	127.86 ± 10.63	244.61 ± 82.86
Supermont group	144.97 ± 34.65	133.22 ± 6.34	270.22 ± 96.09
Opur group	132.42 ± 25.37	121.92 ± 17.47	267.42 ± 69.84
Aqua-Belle group	122.16 ± 16.75	106.66 ± 10.60	265.16 ± 83.17
Semme group	121.06 ± 25.49	128.31 ± 31.16	257.29 ± 92.45

Note. Each value represents the mean ± SEM.

Behaviours scored

Results of behavioural parameters (latency period, defecation, crossing, grooming and rearing) were shown in Table 4. Significant decrease of defecation and latency period were observed in Tangui, Opur and Semme groups as compared to control group ($p < .05 – p < .001$). In the same way, significant decrease of grooming was observed in *Tangui, Supermont, Opur* and *Aqua-Belle* groups as compared to control group ($p < .05 – p < .001$). However, number of squares crossed and exploratory activity (rearing) increased significantly in *Tangui* and *Opur* groups as compared to control group ($p < .01 – p < .001$).

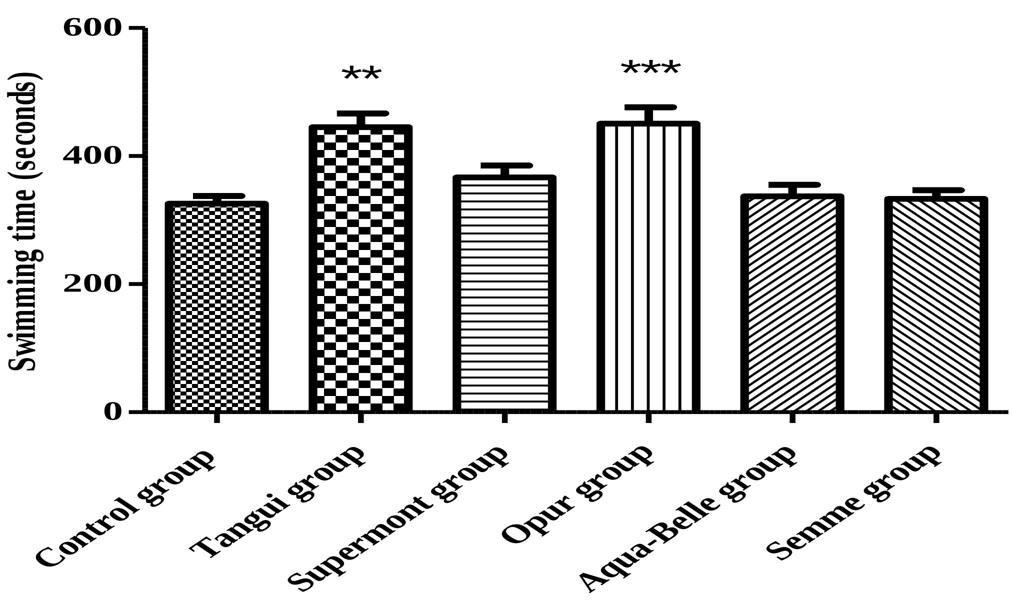
Table 4. Behaviours scored of rats during experimentation.

Treatment	Defecation (g)	Latency period (sec)	Crossing	Grooming	Rearing
Control group	0.32 ± 0.19	11.00 ± 3.56	19.00 ± 3.16	6.75 ± 1.71	3.00 ± 1.41
Tangui group	0.12 ± 0.07*	5.00 ± 1.41**	34.50 ± 10.02**	2.50 ± 1.29***	8.00 ± 2.58***
Supermont group	0.25 ± 0.07	7.00 ± 2.16	24.50 ± 5.32	4.50 ± 1.29*	4.75 ± 1.50
Opur group	0.06 ± 0.04**	6.00 ± 2.94*	37.75 ± 8.73***	2.00 ± 0.82***	7.50 ± 1.29**
Aqua-Belle group	0.21 ± 0.10	9.75 ± 2.22	25.00 ± 5.48	3.75 ± 1.26**	5.50 ± 1.29
Semme group	0.25 ± 0.10	6.00 ± 2.16*	20.25 ± 2.06	5.50 ± 1.29	5.50 ± 2.08

*Note. Each value represents the mean ± SEM, n = 5. *p < .05, **p < .01 and ***p < .001 compared with control group.*

Swimming time to exhaustion

Swimming time to exhaustion of rats were presented in Figure 1. Results showed that swimming time of Tangui and Opur groups were significantly higher than that recorded for the control group ($p < .01 – p < .001$). Maximum swimming time was recorded in Opur group (450.80 ± 51.03 seconds) with a rate of increase of 38.49% as compared to control group (325.50 ± 24.37 seconds).



*Note. Data are presented as mean ± SEM, n = 5. **p < .01 and *** p < .001 compared with control group.*

Figure 1. Swimming time of rats subjected to FSET.

Haematological parameters

As shown in Table 4, no significant difference of number lymphocytes, red blood cells, haematocrit and haemoglobin between the control group and the test groups was observed ($p > .05$). While a significant increase in the white blood cells number of *Tangui group* was observed as compared to control group ($p < .01$ – $p < .001$). The rate of granulocyte (in the *Tangui group*) and the percentages of lymphocyte and monocyte (*in Opur group*) increased significantly ($p < .05$; $p < .001$) in comparison to control group. However, significant decrease ($p < .01$ – $p < .001$) of the rate of monocyte in four test groups (*Tangui, Supermont, Aqua-Beautiful and Semme groups*) and percentage of granulocyte in two test groups (*Tangui and Supermont groups*) were observed as compared to reference group.

Table 4. Haematological parameters of rats

Haematological parameters	Control group	Tangui group	Supermont group	Opur group	Aqua Belle group	Semme group
WBC (10^3/µl)	7.11 ± 1.33	11.45 ± 3.30*	9.89 ± 2.43	9.70 ± 2.65	8.50 ± 2.33	8.15 ± 2.22
LYM (10^3/mm^3)	3.17 ± 0.57	2.24 ± 0.20	3.43 ± 0.79	2.88 ± 0.63	3.38 ± 1.02	3.26 ± 0.20
MON (10^3/mm^3)	1.65 ± 0.50	0.47 ± 0.17***	0.70 ± 0.21***	1.30 ± 0.07	1.03 ± 0.12**	0.84 ± 0.07***
GRA (10^3/mm^3)	3.79 ± 0.61	9.45 ± 5.91*	6.20 ± 2.83	7.05 ± 1.64	4.53 ± 0.91	4.77 ± 0.66
LYM (%)	34.35 ± 9.57	37.95 ± 6.85	41.07 ± 10.59	58.10 ± 17.18*	36.65 ± 5.12	37.57 ± 6.02
MON (%)	16.62 ± 2.97	8.827 ± 4.44	16.12 ± 4.39	34.60 ± 9.47***	16.60 ± 2.65	12.30 ± 1.41
GRA (%)	72.95 ± 10.72	43.45 ± 9.11**	38.70 ± 7.14***	62.95 ± 13.82	80.45 ± 3.40	57.70 ± 8.35
RBC (10^6/mm^3)	4.52 ± 0.55	5.12 ± 0.34	4.42 ± 0.51	4.61 ± 0.44	4.35 ± 0.48	4.40 ± 0.30
HTC (%)	45.05 ± 7.63	41.32 ± 3.92	38.02 ± 2.67	48.05 ± 5.44	37.95 ± 7.82	40.65 ± 3.95
HGB (g/dL)	13.54 ± 1.86	14.20 ± 1.29	12.45 ± 0.62	12.57 ± 0.40	12.32 ± 2.03	13.64 ± 0.76
PLA (10^3/mm^3)	395.00 ± 78.74	406.75 ± 54.83	395.00 ± 78.74	417.75 ± 62.82	313.00 ± 46.08	378.00 ±61.74

*Note. Each value represents the mean ± SEM, $n = 5$. *$p < .05$, **$p < .01$ and ***$p < .001$ compared with control group. WBC: White Blood Cells, LYM: Lymphocytes, MON: Monocytes, GRA: Granulocytes, RBC: Red Blood Cells, HTC: Haematocrits, HGB: haemoglobin, PLA: platelets.*

DISCUSSION

The present study was undertaken to compare the effect of five mineral drinks mostly consumed in Cameroun on performance, hematologic and behaviour parameters in the rats subjected to a training program. Results showed that all the mineral drinks used during this experimentation did not have any effect on water and food consumption ($p > .05$). Moreover, body weight and relative organ weight did not present any significant difference between tests groups and control group ($p > .05$). However, significant increase in swimming time of rats consuming Tangui ($p < .01$) and Opur ($p < .001$) were observed as compared to the control group. Values of swimming time are 445.00 ± 43.60, 450.00 ± 51.03 and 325.50 ± 24.36 seconds respectively for Tangui, Opur and control group. In the same way, results of behavioural and haematological parameters showed significant modifications in the same groups in comparison with control group ($p < .05$ – $p < .001$). Those results can be explained by the fact that all mineral drinks used in this study have not the same mineralogical composition. In fact, potassium was most found in Tangui (10.00 mg/l), while sodium was found in sufficient quantity in Opur (2.02 mg/l) (Table 1). Moreover, calcium and magnesium were most found in Tangui (32.00 mg/l and 21.00 mg/l for calcium and magnesium respectively) and Opur (45.00 mg/l and 16.00 mg/l for calcium and magnesium respectively) (Table 1). Similar results have been reported by Misner (2006) and Bigard et al. (2007), who shown that pure water can't be the best solution to replace water lost in sweat. Minerals are involved in hundreds of biological processes relevant to exercise and athletic performance, such as energy storage/utilization, protein metabolism, inflammation, oxygen transport, cardiac rhythms, bone metabolism and immune function (Kerksick et al., 2018; Heffernan et al., 2019). They were essential to prevent water lost, decrease in muscular and nervous functions and to facilitate recovery. Recent studies showed that practicing physical activity for more than one hour could cause sodium, calcium, magnesium, potassium loss and could have a negative effect on athlete's performance and health, leading to fatigue, cerebral oedema, neurological signs, coma and death (IFN, 2010).

CONCLUSION

Our data suggest that rats consuming Tangui and mostly Opur have benefit effects on endurance capacity, behavioural and haematological parameters. Those effects could be due to the presence, in sufficient quantities, of electrolytes in these mineral drinks. However, further investigations are necessary to elucidate the molecular mechanisms.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology and writing original draft were made by Drs Bonoy Lamou, Mbame Jean-Pierre and André Hamadou. Formal analysis was made by Mr Ngarsou Pierre. Editing were made by Dr Bonoy Lamou.Dr Jean-Pierre Mbame, Dr André Hamadou ans Ms Doukoya Joséphine have been involved in data collection and curation. All authors have read and agreed to the published version of the manuscript.

SUPPORTING AGENCIES

No funding agencies were reported by the authors.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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