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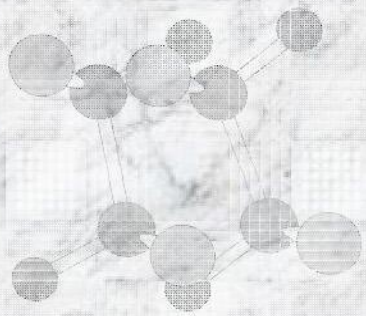

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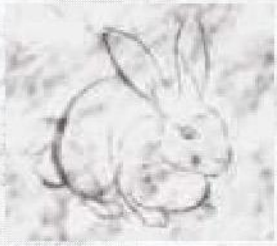

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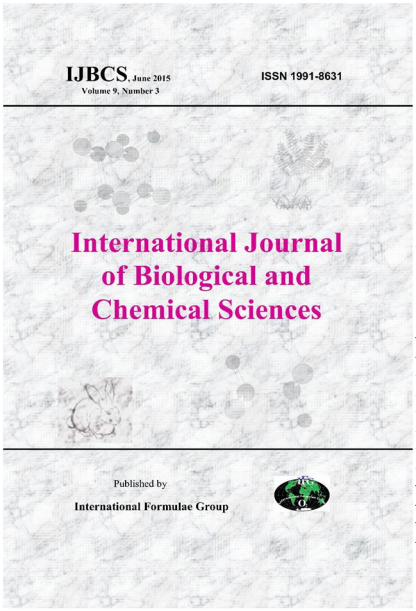

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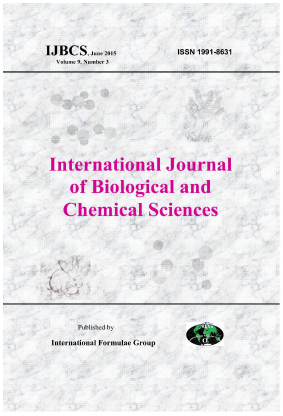

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Antioxidant properties of *Senna siamea* and effects on sports performance in Wistar rats

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ABSTRACT

The objective of this study was to evaluate the antioxidant properties of *Senna siamea* and their effects on sports performance in rats. A total of 15 male Wistar rats were used and divided into 3 groups. The GrpEDSE and GrpED+Entr groups were each subjected to 7 mL/kg of distilled water by body weight, and then the GrpSS+Entr group was treated with 300 mg/kg body weight of aqueous extract of *Senna siamea* leaves. Rats belonging to GrpED+Entr, GrpSS+Entr groups were subjected to swimming for fifteen days. Twenty-four hours after the last training session, the evaluation was made. The results revealed that the studied plant has an antioxidant property with a value of 353.61±11.10 mmol EAA/mg. Then, the training induced a significant increase ($p<0.01$) in swimming performance in treated rats belonging to GrpSS+Entr groups with a running time of 293.452±18.319 min. These extracts of *Senna siamea* leaves represent natural antioxidants and would thus participate in the improvement of performances in sportsmen.

Keywords: Plants, aqueous extract, swimming, wistar rats.

INTRODUCTION

The search for or improvement of physical and athletic performance requires intense and regular training. This improvement in performance requires an increased need for oxygen consumption, which is undoubtedly at the origin of the increase in the production of free radicals (Coisne, 2007). The rate of production of these free radicals during physical effort could exceed the antioxidant capacity of the body (Knez et al., 2007). This leads to oxidative stress resulting from the imbalance between the biochemical processes of free radical production and antioxidant defenses (Powers et al., 2010). Thus, cells become vulnerable to free radical attack, resulting in oxidative damage to cellular components that are responsible for cardiovascular diseases such as: cancer, cataract, amyotrophic lateral sclerosis, acute

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respiratory distress syndrome and especially muscle fatigue (MacLaren, 2007).

Indeed, fatigue is one of the main factors that threatens human health (Ishi et al., 2014). In this sense, various researches have proved in the last decades, that it is triggered peripherally but centered and always accompanied by emotional and cognitive disorders. It is generally defined as a voluntary limitation by the impairment of cognition due to a dysfunction of the transmission of impulses in the central nervous system (CNS) (Chandhuri and Behan, 2004). At this level, central fatigue focuses on changes in the central nervous system, as well as the resulting emotional/behavioral disturbances, including depression, anxiety, cognitive impairment, and memory loss. There are many factors that can induce central fatigue, among which excessive physical activity or mental stress are quite essential (Baston, 2013). In addition, it is also defined as a feeling of weariness, lack of energy, a sense of exhaustion (Amtmann et al., 2012). Fatigue can be accompanied by a decline in physical performance and somatic, psychosomatic, endocrine, and immunological symptoms (Gremion & Kuntzer, 2014). To address this, several authors agree on the use of antioxidants.

For example, Bryant et al. (2003) showed that administration of a combination of antioxidants (vitamin C and E, vitamin E and ß-carotene) reduces oxidative stress-induced damage. Therefore, prevention, drug treatment and the use of additives such as synthetic antioxidants are widely used.

Given the adverse health effects on consumers, several questions have been raised regarding the efficacy and safety of these chemicals. To answer these questions, other authors propose the use of natural antioxidants obtained from food plants. It is probably for this reason that in the context of improving athletic performance and combating oxidative stress, the populations of the Kara region of Togo use food plants (Kpatcha et al., 2016a). Specifically, this population uses the pulps and leaves of Adansonia Digitata L. which have antioxidant capacity as they also have antifatigue and anti-stress effects. According to the study conducted by Kpatcha et al. (2016b), this plant can therefore be considered as potential sources of natural antioxidants for therapeutic or industrial purposes and as an alternative to synthetic products.

In Benin, many traditional pharmacopoeia works highlight the antioxidant properties of several plant species. Among these, we have Liliospsida, Torilis leptophylla, Cleome ibercia, *Senna siamea* (Farimani et al., 2016). Despite their wide use by the population, to our knowledge, the antioxidant properties of these plants have been the subject of very little scientific work on improving physical and sports performance.

Thus, in an approach of valuing medicinal plants useful for physical activities while securing athletes from doping phenomena, the present study aimed at evaluating the effects of *Senna siamea* on sports performance.

MATERIALS AND METHODS Method

Preparation of crude extracts

The extraction of total chemical principles was performed by the decoction method. Based on the extraction techniques described by Houngbeme et al. (2014), 50 g of powder was dissolved in 500 mL of distilled water. The mixture was brought to a moderate boil for 30 min. After cooling, the resulting mixture was filtered (3 times in a row) through absorbent cotton and the filtrate was transferred to a 1000 mL flask and then subjected to evaporation at 40°C using a rotavapor ((Heidolph Laborota 4000 efficient) coupled to a water cooler (Julabo FL 300). The dry residue thus obtained is weighed and the yield (Rdt) is calculated according to the expression : Rdt= (Weight of dry extract/ Initial weight of powder) x 100.

Evaluation of the antioxidant power of extracts

The antioxidant activity of the extracts was evaluated according to the method used by Gandonou et al. (2018) on the stable 2,2diphenyl-1-picrylhydrazyl (DPPH) radical. The DPPH radical is one of the most widely

used substrates for the rapid and direct assessment of antioxidant activity due to its solubility and simplicity of analysis. The principle of the test is based on the measurement of the capacity of the test substance to reduce the DPPH° free radical. The reduction of the DPPH° (2,2'-diphenyl-1picryl hydrazyl) free radical can be followed by UV-Vis spectrophotometry, by measuring the decrease of the absorbance at 517 nm caused by the antioxidants. In the presence of the free radical scavengers, the purple-colored DPPH (2,2 Diphenyl 1 picryl hydrazyl) is reduced to the yellow-colored 2,2 Diphenyl 1 picryl hydrazine. For this purpose, a stock solution of each extract is prepared at Cm=10-2 mg/mL of analytical grade methanol (Sigma-Aldrich). The methanolic solution of DPPH is prepared at 0.4 mg/mL. Then, 1.5 ml of the extract solution is mixed with 3 ml of the DPPH methanolic solution. The mixture is incubated for 30 minutes at room temperature and the absorbance is read at 517 nm against a blank. Thus, the positive control is represented by a solution of a standard antioxidant; ascorbic acid (purity 99.5%, Sgma-Aldrich) is also prepared under the same conditions as the samples but with a variable concentration range 0.10 mg/mL. The antioxidant activity of the extract was determined using the calibration curve established with ascorbic acid. Each assay is performed in duplicate. The antioxidant activity is expressed as mmol ascorbic acid equivalent per gram of extract (mmol EAA/mg).

Plant material

It was composed of leaves of *Senna siamea* (F.S.S) harvested in January 2020 in Benin precisely in Parakou in the department of Borgou. They were dried and powdered. The dose of 300 mg/kg was administered according to the results of the work of Agbodjogbé (2013) which indicate that this dose is the most effective.

Animal material

Albino rats, all males of Wistar strain, weighing 170 ± 10 g were obtained at the animal house of the Exercise Physiology Research Unit in Porto-Novo. The animals were housed in wire mesh cages equipped with feeders and drinkers. Access to food and water was ad libitum. They were fed wheat bran, corn meal, and running tap water. They were subjected to a regime of 12 hours of light, 12 hours of darkness at a temperature of 28 ± 1°C and 60 ± 5% humidity.

Parameters that influenced the swimming protocol

The swimming time of each rat was determined using a stopwatch.

Choice of the tank

A circular, inverted cone-shaped basin with a small radius of 20 cm (base), a height of 120 cm, and a large radius of 40 cm (surface) was selected according to the protocol of Kpatcha et al. (2016b). It was filled with water to a height of 100 cm and maintained at a temperature between 35 and 36°C (Figure 1).

Rat swimming protocol

Constitution of the experimental groups

Animals were randomly divided into three groups (n = 5). All groups were treated at a dose of 300 mg/kg body weight per day. Oral administration was performed via a gastric tube for 15 days. Thus, the GrpEDSE group consisted of the rats treated with 7 mL/kg of distilled water per body weight and not subjected to training. The GrpED+Entr group was also composed of rats treated with 7 mL/kg of distilled water per body weight and then subjected to training. Finally, the GrpSS+Entr group was composed of rats treated with the aqueous extract of *Senna siamea* leaves and then subjected to training.

Habituation of rats to swimming

Rats belonging to GrpED+Entr, GrpSS+Entr groups were subjected to swimming for 15 days according to the protocol of Kpatcha et al. (2016b) and modified by us. It was performed in batches of five in the selected pool. In order to address stress-related issues and to get an idea of their natural performance, the rats belonging to these different groups were subjected to the swimming exercise under the same conditions one week before the experiment. They were

subjected to 15 minutes of swimming per day for five days. ***Training of the rats to swim***

The training was performed according to the protocol of Kpatcha et al. (2016b) and modified by us. Thus, the rats were trained to swim without load for 15 days, six days per week, and then were at rest on the seventh day but with gavage. During training, they were subjected to 30 minutes of swimming on the first day of the experiment, and an increase of 10 min/day is made on the following days to reach one (01) hour on the fourth day. From the fourth to the fifteenth day, the rats were subjected to one hour of swimming per day. The administration of the plant was done one hour before the beginning of the exercise. To avoid any confounding effect and to exclude potential exertion, the GrpEDSE (no training) group was kept in a pool containing 3 cm of water at the same temperature. Twenty-four hours after the last training session, all rats belonging to the three groups GrpEDSE, GrpED+Entr, GrpSS+Entr were subjected to swimming. During this session, uncoordinated movements and remaining underwater for 10 seconds without surfacing were considered as the criteria for rat exhaustion. At this point, the swimming was stopped, the swimming time was recorded in minutes for each rat, and then the average per group was calculated. To avoid the influence of circadian variations in physical activity, the swimming exercise was performed from 9:00 am to 5:00 pm.

Statistical analysis

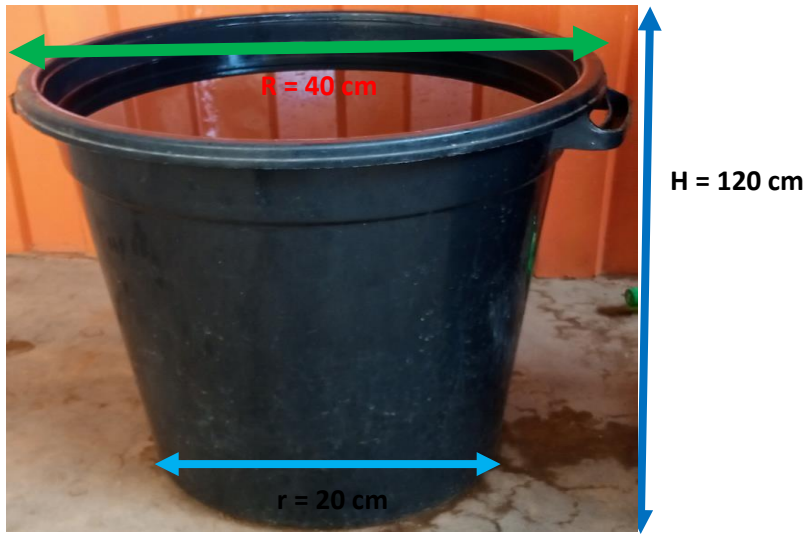


Figure 1 : Circular tank

Results were processed using Stat View software (version 5) from Abacus concepts Inc. (Berkeley, CA, USA). Descriptive statistics were performed and mean values were expressed as mean plus or minus standard deviation. The Kruskall Wallis test was used to compare the mean values of running times performed by the rats. The Mann Whitney U test, a binary test, was used when the Kruskall Wallis test was significant. For all tests, the significance level was set at $p < 0.05$.

RESULTS Yields of the aqueous extract of *Senna siamea* leaf

Figure 2 shows the yield of the extraction of the leaves of *Senna siamea*. From the analysis of the figure, it appears that the best yield is obtained by the aqueous extract of *Senna siamea* leaves with a rate of 17%.

Chemical constituents of the leaves of *Senna siamea*

According to the colored reactions in the tube

Phytochemical analysis of the aqueous extract of Senna siamea leaves

The phytochemical screening reveals the presence of major chemical groups. From the observation of Table 1, it appears that alkaloids, phenolic compounds, coumarins, anthracenics C-heterosides, quinone derivatives, saponosides, terpenes, steroids, mucilages, reducing compounds are present in this plant contrary to cardenolides and cyanogenic derivatives which are absent. The free anthracenics are also present in the leaves of *Senna siamea*. ***Antioxidant activity***

The calibration curve of the antioxidant activity exhibited by vitamin C is presented in Figure 3.

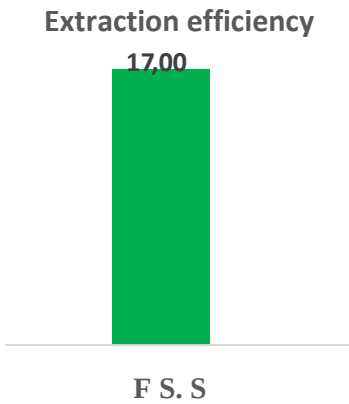
The application of the regression equation to the different OD measurements of the extract-DPPH. binary, allowed to find the values recorded in Table 2. The analysis of this table reveals that the studied plant has a significant antioxidant property with a mean value of 353.61±11.10 mmol EAA/mg.

Effects of plant administration combined with training on the performance of rats

Analysis of Table 3 shows that all rats that were trained significantly improved their swimming performance as $p < 0.01$.

Effectiveness of the plant on sports performance

Analysis of Table 4 shows that rats treated with *Senna siamea* leaves and undergoing training have a significantly greater swimming time ($p < 0.01$) than those treated with distilled water and undergoing the same training load.



FS.S = *Senna siamea* leaf

Figure 2 : Extraction yield of *Senna siamea* leaves.

Table 1: Results of the screening of the aqueous extract of the leaves of *Senna siamea* (FS.S).

Active principles	FS.S

Alkaloids		+
Polyphenolic compound	Catechetical Tannin	+
	Gallic Tannin	+
	Anthocyan	+
	Flavonoids	+
	Leucoanthocyan	+
Coumarins		+
Anthracene C-heterosides		+
Quinone compound		+
Saponosides		+
Terpenes		+
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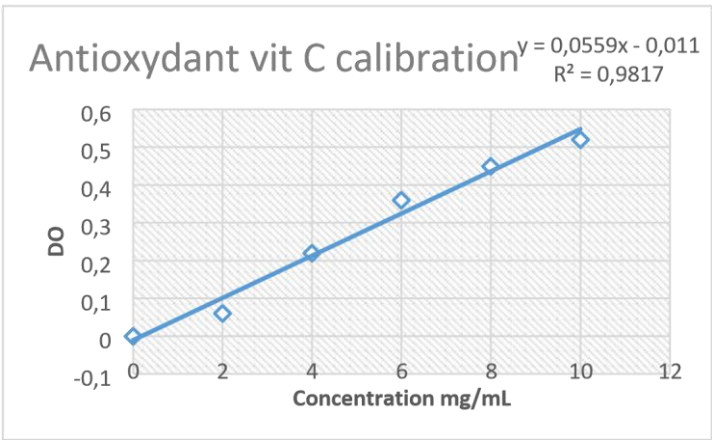

Figure 3 : Calibration curve of the standard reducing ascorbic acid of the radical.

Table 2 : Antioxidant activity of F.S.S and R.S.L.

Extracts	Activity in mmol EAA/mg		m ± s
	Essai 1	Essai 2	
F. S. S	342,51	364,72	353,61 ± 11,10

F. S. S = leaves of *Senna siamea* ; m ± s : Mean ± standard deviation

Table 3 : Effects of training on the performance of rats subjected to extracts.

	Grp_{EDSE} n=5 (m ± s)	**$Grp_{ED+Entr}$ n=5 (m ± s)**	**$Grp_{SS+Entr}$ n=5 (m ± s)**	**P**
Weight (g)	161.98 ± 5.806	163.580 ± 9.99	166.44 ± 4.091	
Time (mn)	56.308 ± 6.246	205.826 ± 21.654**	293.452 ± 18.319**	0.0019

Legend: n: number of participants; m ± s: mean plus or minus standard deviation; g: gram; min: minutes; Grp_{EDSE}: group treated with distilled water and not trained; $Grp_{ED+Entr}$: group treated with distilled water and trained; $Grp_{SS+Entr}$: *Senna siamea* treated group then subjected to training; p: probable value; **: significance level p less than 0.01

Table 4 : Effects of *Senna siamea* leaves on performance.

	$Grp_{ED+Entr}$ n=5 (m ± s)	**$Grp_{SS+Entr}$ n=5 (m ± s)**	**p**
Weight (g)	163.580 ± 9.99	166.44 ± 4.091	
Time (min)	205.826 ± 21.654	293.452 ± 18.319**	0.009

Legend: n: number of participants; m ± s: mean plus or minus standard deviation; g: gram; min: minutes; $Grp_{SS+Entr}$: group treated with *Senna siamea* and then trained; $Grp_{ED+Entr}$: group treated with distilled water and trained ; p: probability value; **: significance level p less than 0.01.

DISCUSSION

The objective of the present study was to evaluate the antioxidant properties and then the effects of *Senna siamea* on physical performance in rats. This plant was chosen because it is used in the traditional pharmacopoeia of the West African zone and particularly in Benin to treat several pathologies. Also, this study is part of a strategy of valorization of this plant which would be very useful for physical activities and this by securing the athletes to doping. Two fundamental aspects were addressed in this study: the aspect related to the antioxidant activity and the one related to the physical performance.

Regarding the first aspect, the yield obtained with the aqueous extract of *Senna siamea* leaves was 17%. This result is undoubtedly evident as it reflects a high content of polar compounds in the plant. Moreover, since these leaves are exposed to the sun, several authors have shown that the photosynthetic phenomenon promotes the biosynthesis and storage of secondary metabolites responsible for the biological properties of the plant (Olivier et al., 2012). This demonstrates why the use of leaves is more important in the population because its harvesting is easy (Olivier et al., 2012). These

results obtained are similar to those of Agbodjogbé et al. (2013) who obtained 18.3% for the aqueous extracts of *Senna siamea leaves*.

Regarding phytochemical screening, this technique revealed the presence of polyphenols, saponosides, coumarins, mucilages, steroids, terpenes, reducing compounds, alkaloids, tannins, quinone derivatives and free anthracene derivatives. These results corroborate on the one hand the work of Ahonsou (2011) and on the other hand that performed by Agbodjogbé et al. (2013) with thin layer chromatography. The polyphenolic compounds mainly identified in the leaves of *Senna siamea* are in agreement with the antiradical activity. These compounds are widely distributed in plant tissues among which many antiradical and antioxidant molecules are found (N'Guessan et al., 2009). The functional groups present in phenolic compounds in general can easily give up an electron or a proton to neutralize free radicals (Koné, 2009). Thus, the antioxidant activity of the aqueous extract of *Senna siamea* leaves would be related to a high content of phenols. As a result, flavonoid plants are highly sought after in the treatment of digestive disorders which are the most recurrent ailments by the population (Ngene et al., 2015). They are indeed powerful antioxidants as they allow direct scavenging of ROS (Reactive Oxygen Species), inhibition of enzymes and chelation of metal traces responsible for the production of ROS (Reactive Oxygen Species). In addition, they inhibit the oxidation of cholesterol by free radicals, abolish the tendency of small blood cells or platelets to clump and form blood clots. In addition to these properties mentioned above, flavonoids contribute significantly to digestion, reduce cardiovascular risks by acting as free radical scavengers by preventing and repairing damage caused by ROS (Bruneton, 2009). Finally, they are able to modulate the activity of certain enzymes and modify the behavior of several cellular systems, which gives them a multitude of biological activities, including antioxidant and anti-ulcer activities (Pincemail et al., 2001). As for tannins, they waterproof the outermost layers of the skin and mucous membranes, protecting the underlying layers and promoting tissue regeneration in case of superficial injury or burns (Bruneton, 2009). Like coumarins, reducing compounds, all water extractable, can, alone or in synergy of action, be responsible for antioxidant and antihyperglycemic activities. Indeed, polyphenols are of interest to nutritionists, food manufacturers and consumers because of their antioxidant properties and their involvement in the prevention of various pathologies associated with oxidative stress and in the prevention of degenerative diseases such as cancer, cardiovascular diseases, osteoporosis (Rock, 2003). Studies on the purification of molecules on *Senna siamea* had isolated triterpenes, sterols (Edward et al, 2016), which confirms our results.

Evaluating the antioxidant activity of this plant in the present study, the results show that all tested *Senna siamea* leaf extracts significantly reduce DPPH. This reducing power is determined by a decrease in absorbance induced by the substance (Knez et al., 2007). The reducing activity of food plant extracts is explained by the fact that these extracts give up a hydrogen atom or an electron to DPPH (Yesufu et al., 2010). This plant is therefore endowed with a free radical reducing activity. It could thus prevent the initiation and/or propagation of radical reactions. It is important to note that flavonoids are secondary plant metabolites that belong to the polyphenol family. They possess remarkable biological and pharmacological activities that are antioxidant activities (Edward et al., 2016) and protect plants from UV radiation both by absorbing this radiation and by neutralizing the reactive oxygen species formed (Mata et al., 2007). The polyphenols and particularly the flavonoids contained in the extracts of the two plants studied are probably responsible for the antioxidant activity of these extracts. These results are in agreement with the work done on Byrsocarpus coccineus schum et thonn

(Connaraceae), a species rich in phenolic compounds that are responsible for many biological activities, including antioxidant and anti-inflammatory activities (Zhao, 2015).

The presence of coumarimes and polyphenolic compounds could thus account for antioxidant (Gandonou et al., 2018), antiinflammatory, antimicrobial, and anticoagulant properties in this study (Dosseh et al., 2002). These results were expected as pharmacological tests have confirmed the antioxidant activities of *Senna siamea leaves* (Pietta, 2000) and in the prevention of free radical (FR) damage (Lenoir, 2011).

Given that the extract of this plant has antioxidant and anti-inflammatory properties, could it not have effects on athletic performance?

To answer this question, rats were subjected to swimming in the present study. Swimming was chosen because swimming, when performed in a group, promotes more vigorous exercise than when rats are allowed to swim alone or run alone on a treadmill (Trayhurn, 2017). In addition, the weight of the rats used did not vary. Therefore, it can be said that the weight of the rats did not influence the performance of the rats as studies have shown a decrease in performance following an increase in body weight on the performance of athletes, during running, swimming or vertical jumps (Al-Hashem et al., 2012 ; Ferauti and Remmert, 2003).

In this study, the performances obtained after 15 days proved that training alone without extract supplementation had a positive impact (increase in swimming time) on the trained rats compared to the control group without training. Furthermore, the results showed that the group of rats that received the extract and trained for two weeks (Table 4) significantly improved swimming performance. This relatively longer swimming time than that observed in the second group suggests that the extracts used demonstrated greater resistance to fatigue. These results are consistent with the experimental study conducted by Kpatcha et al (2016b) which showed that oral supplementation with *Adansonia Digitata* leaf or pulp improved not only endurance capacity, but also time to exhaustion independent of exercise regime.

However, other complementary studies are essential in order to evaluate on the one hand the dose to be taken according to the age categories and on the other hand the hematological and inflammatory modifications related to these two extracts following a chronic physical activity practice.

Conclusion

The aim of this study was to evaluate the extracts of *Senna siamea* leaves on the physical performances on the animal model after analysis of the antioxidant properties. The results showed that these used in traditional medicine in Africa in general and particularly in Benin contain active principles such as alkaloids, polyphenols, present an antiradical activity and are of capital importance in the therapeutic potential. This could allow to consider the development of antiradical, anti fatigue phytomedicines standardized à from these extracts. The results also showed an antioxidant activity. In addition, administration of aqueous extract of *Senna siamea* leaves at the dose of 300 mg/kg significantly improved the physical performance of rats.

All these results constitute elements of appreciation for scientific validation of the use of the leaves of *Senna siamea* in preventive medicine as well as in the optimization of the performance without doping risk.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors actively participated in the work since the project phase, then the setting up of the experimental protocol and its realization and finally the writing of the article.

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