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Antioxidant Property of a Dietary Supplement of *Moringa oleifera* Leaves and *Pleurotus ostreatus* in Wistar Rats Subjected to Forced Swimming Endurance Test

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Abstract

The antioxidant property of *Moringa oleifera* leaves and *Pleurotus ostreatus* on swimming performance and related biochemical parameters were investigated in thirty adult male and female wistar rats. **Purpose:** The aim of the present study was to evaluate the antioxidant property of *M. oleifera* and *P. ostreatus* mixture in specific proportions in wistar rats. **Material and Methods:** The mushroom species and *M. oleifera* leaves were cultivated at the biotechnology laboratory of the University Cheikh Anta Diop de Dakar, Senegal. Oxidative stress in rats was induced by swimming. The dietary supplement was composed of a mixture of powders of *Moringa oleifera* and *Pleurotus ostreatus* in 2:1 ratio. Three groups received different doses of dietary supplements 500 mg/kg, 1000 mg/kg, and 1500 mg/kg, with one group being the stressed and the other the control group. Parameters in serum such as serum glutamyl oxaloacetate transaminase, serum glutamyl pyruvate transaminase, albumin, Testosterone, creatinine, and, oxidative stress parameters (catalase, reduced glutathione, and malondialdehyde) were measured. **Results:** Results showed that the dietary supplement has an effect on oxidative stress because it increases the activity of catalase and the level of cellular glutathione in rats. The 500 mg/kg dose would be the most appropriate dose for stressful conditions. The 1000 mg/kg dose would be the most appropriate dose for liver damage. **Conclusion:** This study shows that the antioxidant properties of *M. oleifera* leaves and *Pleurotus ostreatus* are demonstrated by

their ability to improve body energy stores and tissue antioxidant capacity. The dietary supplement of *M. oleifera* leaves and *P. ostreatus* powders mixture could be good in stressful conditions.

Keywords

Moringa oleifera, *Pleurotus ostreatus*, Dietary Supplement, Antioxidant, Oxidative Stress

1. Introduction

Fatigue is best defined as difficulty in initiating or sustaining voluntary activities [1]. Fatigue is accompanied by a feeling of extreme physical or mental tiredness, resulting from severe stress and hard physical or mental activity [2]. Physical fatigue is thought to be accompanied by deterioration in performance [3]. There are several theories about the mechanisms of physical fatigue. The radical theory suggests that intense exercise can produce an imbalance between the body's oxidation system and its antioxidation system. The accumulation of reactive-free radicals will put the body in a state of oxidative stress and bring injury to the body by attacking large molecules and cell organs. *Moringa oleifera* Lam. is a plant of Indian origin that is now widespread in Asia and Africa. It belongs to the Moringaceae family with about 13 species [4]. It is commonly referred to as the "tree of life", "miracle tree", or "divine plant" due to its numerous nutritive, medicinal, and industrial potentials [5]. The leaves are widely consumed as a legume and used in traditional medicine in Africa in general. The leaves are an excellent source of protein [6] and are rich in metabolisable energy [7], vitamins (A, B, C, and E), minerals, for example, iron, calcium, zinc, selenium, and β-carotene [8].

M. oleifera leaves are used in traditional medicine to treat malnutrition, fever, headaches, nerve pain, and diabetes [9]. In part one of a comprehensive review of the medical evidence for its nutritional, therapeutic, and prophylactic properties, *M. oleifera* has been cited for its numerous applications in disease treatment and prevention. These include antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis, and even reduction of Schistosoma cercariae titer.

Many studies have shown the health and nutritional interest of edible mushrooms. Mushrooms and some plants provide proteins, carbohydrates, minerals, fibers, vitamins, minerals, and fatty acids [10]. They have therapeutic properties and many of them have been used in medicine all over the world. Researchers have shown that *P. ostreatus* has antitumor effects, antioxidant properties, antihyperlipidemic effects, and antidiabetic effects [11]. However, according to current previsions, Africa will have the most increasing percentage of death because of non-communicable diseases. Among the risk factors, bad feeding and poor con-

sumption of fruits and vegetables could be the cause of 1.7 million of death (WHO, 2010). In order to fight hunger and diseases, many organisms and governments, encourage the use of vegetal species as minerals and protein sources [12].

Nutrient supplementation to improve exercise performance has involved the use of high-fat diets, carbohydrate supplements, and various dietary supplements or "tonics" of plant origin to enhance exercise capacity. In the fight against fatigue, more and more sports professionals and athletes are turning to plant extracts as sources of energy in replacement for banned doping substances. Studies have revealed the widespread use of plant extracts in many African countries for performance enhancement [13]. It is therefore important to develop efficient and safe plant-based antifatigue products that can enhance exercise performance without deleterious effects on the health of the users.

M. oleifera has multiple therapeutic properties. It has been widely claimed that "ounce-for-ounce", Moringa leaves contain more Vitamin A than carrots, more calcium than milk, more iron than spinach, more Vitamin C than oranges, and more potassium than bananas" [14]. Also, *Moringa oleifera* leaves extract has antimicrobial activity and profound antioxidant activity. So the plants may be considered as good sources of natural antioxidants for medicinal uses such as against aging and other diseases related to radicals [15]. Another study focused on the Antioxidant Capacity in Extracts from *Moringa oleifera* Plants Grown in Jamaica, has shown that extracts prepared using polar solvents had significantly higher antioxidant capacities than others and may have clinical applications in any disease characterized by a chronic state of oxidative stress, such as sickle cell anemia [16].

Given the rich nutrient, phytochemical, antioxidant capacity, and organoleptic potential of *M. oleifera* and *P. ostreatus*, we designed the present experiment to study the antioxidant potency of a dietary supplement of *Moringa oleifera* and *Pleurotus ostreatus* in rats subjected to the forced swimming test. In this paper, we are going to code the dietary supplement by FMP16.

2. Materials and Methods

2.1. Experimental Design

Thirty (30) adult male and female wistar rats weighing 150 to 200 g were divided into 5 groups of 6: 1 control group who was not stressed and received only the normal diet (water and food ab-libitum); 1 control group who was stressed and received the normal diet (water and food ab-libitum) and the vehicle starch paste; three others groups that received respectively 500 mg/kg, 1000 mg/kg, 1500 mg/kg of the dietary supplement, one hour before being subjected to 2 hours of swimming. The dietary supplement was composed of a mixture of powders of *M. oleifera* and *P. ostreatus* in a 2:1 ratio. The mixture was dissolved in 0.01% starch paste. Each group was subjected to intense physical activity, which is swimming. Each animal was supplied with a load (corresponding to 5% of the bodyweight) tagged to the tail and placed individually in a swimming pool, filled

with water to a depth of 35 cm and maintained at 25˚C ± 1˚C. The pool was adjusted daily according to the change in weight in order to increase the intensity of the exercise. The rats were placed in a light cycle of 12 hours per day (dark 12 h - 12 h light), with unlimited access to water and food. They were weighed daily and force-fed with FMP16 using a gastroesophageal catheter and they swam 2 hours a day for 21 days with a rate of 5 days of swims per week. Exhaustion was determined by observing the loss of coordinated movements and failure to return to the surface within 10 seconds and swimming time was recorded immediately. The day before the sacrifice of animals, they fasted overnight. After the 23rd day, the animals were anesthetized and sacrificed by decapitation. Blood and organs such as the liver, kidneys, brain, and testes were removed.

The fresh leaves of *M. oleifera* were harvested at the botanical garden of the University Cheikh Anta Diop de Dakar, Senegal and identified at the botanical department (UCAD) The leaves were cleaned immediately after harvest, cut into small pieces, and dried in the shade for about 2 weeks. The dried material was ground into a powder using a manual homogenizer. *P. ostreatus* were obtained by cultivation at the biotechnological laboratory of the University Cheikh Anta Diop de Dakar. The dietary supplement was composed of the mixture of powders of *Moringa oleifera* and *Pleurotus ostreatus* in 2:1 ratio. The mixture was prepared as described by [6]. For feeding the rats, the mixture was dissolved in 0.01% starch paste.

Each animal was supplied with a constant load (corresponding to 5 % of the body weight) tagged to the tail and placed individually in a swimming pool (150 cm × 50 cm), filled with water to a depth of 35 - 40 cm and maintained at 25˚C ± 1˚C. Exhaustion was determined by observing loss of coordinated movements and failure to return to the surface within 10 sec and swimming time was recorded immediately. At the 23rd day, the rats were rested for a night and then sacrificed (under ether anesthesia) by cutting through the jugular vein. The blood was collected and organs (liver, kidneys, brain and testicles) of the rats were dissected and kept at −20˚C until analysis.

Prior to biochemical analysis, each liver sample was homogenized using a protector placed on ice and 10% homogenates were prepared using the KCL buffer solution (1.15%). The homogenates were then centrifuged at 3000 rpm for 30 min at 4˚C to collect the supernatant used for analysis. The supernatant of each sample was aliquoted in 1.5 ml eppendorf tubes for an estimation of the activity of antioxidant parameters (peroxidized lipids LPO, glutathione cellular GSH, catalase CAT). All liver parameters were expressed as activity per mg proteins. The proteins concentration in each fraction was determined by the method of [17].

2.2. Determination of Lipid Peroxidation

The mean malondialdehyde (MDA) content (μmol/mg protein) a measure of lipid peroxidation was assayed in the form of thiobarbituric acid-reacting substances by the method of [18].

2.3. Quantitative Analysis of Enzyme Activities

- Catalase: The mean catalase activity was assayed by the method of Sinha, (1972).
- Glutathione cellular: The mean Glutathione cellular activity was evaluated by the method of [19].

2.4. Determination of Parameters in Serum

- Serum glutamyl oxaloacetate transaminase (SGOT) and serum glutamyl pyruvate transaminase (SGPT) activities were assayed by the method of Karmen *et al.* (1955) [20] and measured by standard essay kits SGM Italia Rome, Via Eschilo, 10139, 2012).
- The concentration was assayed by the method of Ferreria et Price (1974) [21] and measured by standard essay kits Hospitex diagnostics, Via Arno, 4001010L (2013).
- The concentration was assayed by the method of [22] and measured by standard essay kits Hospitex diagnostics, Via Arno, 4001621L (2014).
- The concentration was assayed by the method of [23] and measured by Kit ELISA (DRG Diagnostics, Germany, EIA-1559, 2009).

2.5. Statistical Analysis

IBM SPSS Statistics 20 software was used for statistical analysis and data processing. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons and *P*-values less than 0.05 were considered significant. The results are expressed as mean ± standard deviaton (SD).

3. Results and Discussion

The forced swimming test represents a valid animal model for screening antifatigue potency of various bioactive compounds [24] [25]. In this study, we did not observe significant differences in final body weights (155 - 173 g) (**Table 1**). Body weight gain ranged between 0.8 and 19 g for the four treatment groups. A decrease of 10% in the weight of D3N group was observed. These results corroborate those of [26] who found that a diet enriched with *Pleurotus ostreatus* decreases the body weight of animals. On the other hand, [27] have shown that it has no effect on the weight as well as [28] who worked on humans with a daily dose of 30 g of dried oyster mushrooms, found that this had no effect on anthropometric data. [29] showed that the daily intake of 30 g of *Moringa oleifera* improves nutritional recovery in children suffering from malnutrition. [30] showed that a dose of 600 mg/kg of *Moringa oleifera* lowers the body mass index in obese subjects. Furthermore, the combination of these species at a high dose of 1500 mg/kg, would explain the weight loss. This result was in contrast with results obtained by Osman *et al.* who reported up to 14% changes in body weight of rats given *M. oleifera* extract for 21 days, attributing these changes to the rich

nutrient quality of the extract.

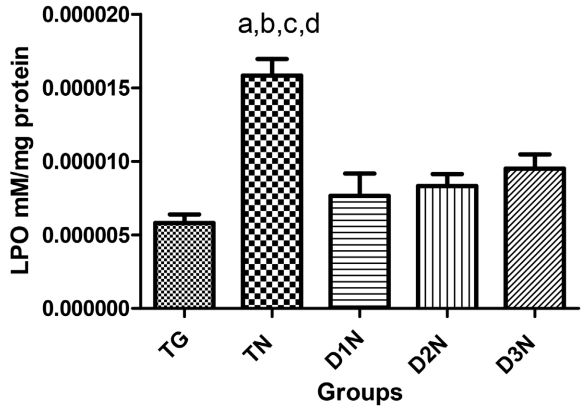
Intense physical exercise also causes oxidative stress in the body due to excessive generation of oxygen-derived free radicals. These radicals, in turn, oxidatively degrade biomolecules such as lipids, proteins, and nucleic acids and therefore affect the homeostatic environment of cells. A vast amount of evidence indicates that reactive oxygen species (ROS) are responsible for exercise-induced protein oxidation and contribute strongly to muscle fatigue [31]. Peroxidation is an important indicator of oxidative stress that results from degradation of cell membrane by free radicals.

Our results also showed that there isn't significant difference in the protein's concentrations in the liver, in stressed groups by swimming. There was a significant increase ($p < 0.05$) in lipid peroxidation in the control group (TN) which was stressed and didn't receive the dietary supplement (**Figure 1**). There was a decrease in the concentration of peroxidized lipids by 63% in the unstressed control group (TG), by 60% in the dose group 1 (D1N), by 47% in the dose group 2 (D2N) and 39% in the dose 3 group (D3N) compared to the stressed and untreated group

Table 1. Effect of the dietary supplement on rat weights.

GROUPES	Starting body weight (g)	Final body weight (g)	P-value*
TG	154 ± 3.34	173.67 ± 9.16[a]	0.02
TN	154.33 ± 3.44	173 ± 9.94[b]	0.03
D1N	154.40 ± 3.29	158.80 ± 8.65	0.2
D2N	153.20 ± 2.28	168.40 ± 7.12	0.06
D3N	154.80 ± 3.03	155.60 ± 5.38	0.8

*ANOVA test; TG: control group; TN: stressed group; D1N: stressed and treated group 500 mg/kg: D2N: stressed and treated group 1000 mg/kg; D3N: stressed and treated group 1500 mg/kg; a, b: mean statistically significant to D3N, $p < 0.05$ (Bonferroni test); a, d, e: mean statistically significant to D3P at $p < 0.05$ (Bonferroni test).



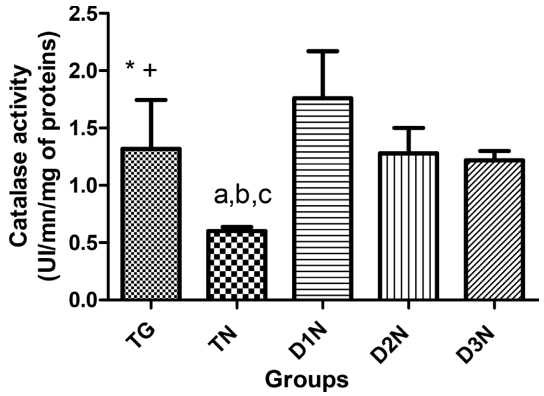
The values are expressed as mean ± SD. TG: Control group rats with food and water ad libitum, TN: stressed rats without treatment, D1N: dose of 500 mg/kg, D2N: dose of 1000 mg/kg, D3N: dose of 1500 mg/kg.

Figure 1. Effect of the dietary supplement on concentrations of peroxidized lipids (LPO).

(TN). Our results might indicate that intense physical activity would cause a stress in rats. These results are in accordance with the findings by [32].

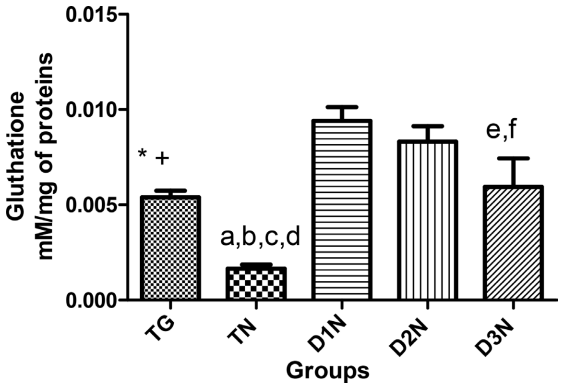
The administration of the FMP16 lowered the concentration of peroxidized lipids in stressed groups who received 3 different doses (500, 1000, 1500 mg/kg). There was no significant difference between the different group but also with the control group (TG) which was not stressed, which would mean that the dietary supplement maintains the concentration of peroxidized lipids at a normal level when stress is induced by swimming. These results are in accordance with those of [33].

Enzymatic antioxidant systems such as catalase, and cellular glutathione, are important in scavenging free radicals and their metabolites [34]. This antioxidant defense mechanism becomes weaker during chronic fatigue and other disease conditions. Our results indicated that there was a decrease in the concentration of catalase and cellular glutathione with the stressed rats and an increase with treated rats (**Figure 2**, **Figure 3**). These results are in accordance with the findings by [33] who found the same results with *Pleurotus ostreatus* extract and



The values are expressed as mean ± SD. *, + mean statistically different with D1N and D2N, $p < 0.05$ (Bonferroni test); a, b, c mean statistically different with D1N, D2N and D3N, $p < 0.05$ (Bonferroni test).

Figure 2. Effect of the dietary supplement on catalase activity (UI/mn/mg proteins).



Values are means ± SD. a, b, c d mean statistically different with TG, D1N, D2N et D3N, $p < 0.05$ (Bonferroni test); e, f, *, + mean statistically different with D1N et D2N à $p < 0.05$ (Bonferroni test).

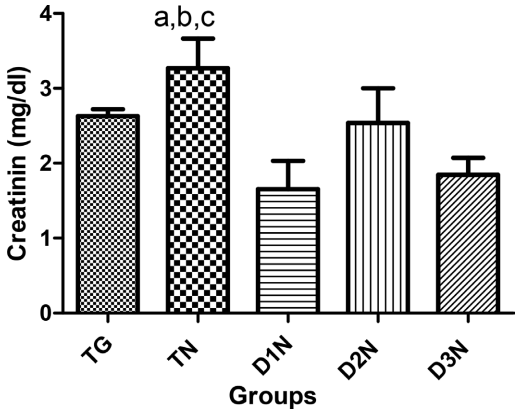
Figure 3. Effect of the dietary supplement on glutathione cellular activity (mM/mg proteins).

Moringa oleifera extracts in stressed rats.

The results shown that there was no significant difference in SGPT concentrations with rats treated with 500 mg/kg (D1N) compared to stressed rats (TN) and control group (TG). In treated rats (D2N) 1000 mg/kg, SGPT concentrations increased by 30% compared to control group (TG) and by 69% compared to stressed group (TN). Also, in treated rats 1500 mg/kg (D3P), it increased by 28% compared to control group (TG) and by 39% compared to stressed group (TN). Compared to the results of [26], who found that a dose of 1600 mg/kg of *Moringa oleifera* leaves increased SGOT and SGPT activity and a supplemented diet of 5% of *Pleurotus* decreased activities of transaminases, we might think that dietary supplement tends to regulate their activities due to the antagonistic effect of these species.

In this study, there was a significant difference in the concentration of creatinine. There was a decrease in creatinine's concentration by 46%, 35%, and 46% respectively with D1N, D2N, and D3N compared to TN (**Figure 4**). The results are in accordance with those of Adedapo *et al.* 2009 and Sirag, 2009. On the other hand, [26] found that *Pleurotus* didn't have any effects on creatinine concentrations.

Albumin and testosterone concentrations are presented in **Table 2**. There was no significant difference in rats. Other studies such as, [6] found that M. oleifera and Pleurotus increase sexual performance and could be used as a potential



Values are means ± SM. a, b, c mean statistically different with D1N, D2N and D3N at $p < 0.05$ (Bonferroni test).

Figure 4. Concentrations of creatinine (mg/dl) in rat serum.

Table 2. Concentrations of SGOT, SGPT, Albumin and Testosterone in rat serum.

GROUPES	ALAT (U/I)	ASAT (U/I)	ALBUMINE (g/dl)	TESTOS. (ng/dl)
TG	32.74 ± 7.09	160.92 ± 30.02	1.53 ± 0.20	0.31 ± 0.06
TN	23.68 ± 7.29	136.41 ± 13.87	1.77 ± 0.33	0.42 ± 0.03
D1N	26.54 ± 5.36	187.38 ± 25.93	1.83 ± 0.35	0.30 ± 0.005
D2N	31.60 ± 2.63	231.08 ± 45.70	1.70 ± 0.12	0.41 ± 0.013
D3N	20.26 ± 3.14	224.36 ± 34.38	1.80 ± 0.38	0.33 ± 0.03

aphrodisiac.

In conclusion, dietary supplement possesses antioxidant properties. It improved the antioxidant enzyme activities and decreased the peroxidized lipids. Also, it has an effect on creatinine and SGPT and SGOT concentrations. The antioxidant effect potential may be expressed through mechanisms that involve the antioxidant activity of *M. oleifera* and *P. ostreatus*. Further studies are needed to determine the effect of the dietary supplement on stress.

4. Conclusion

A dietary supplement of *Moringa oleifera* leaves and *Pleurotus ostreatus* in wistar rats shows that the powders of *M. oleifera* leaves and *P. ostreatus* mixture could be good for stressful conditions. The powders are demonstrated by their ability to improve body energy stores and tissue antioxidant capacity. This dietary supplement has an effect on oxidative stress.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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