EFFECT OF ADANSONIA DIGITATA L. ON PHYSICAL PERFORMANCE AND HAEMATOLOGICAL PARAMETERS IN RATS.

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Abstract
The improvement of physical performance remain important for the sportsmen but controls related to the doping substances are intensifying. To improve this physical performance without doping it would be indicated to have resort to the natural substances from food or medicinal plants. Adansonia digitata is widely used as vegetable in Togo and traditional healers advise currently young wrestlers in Kabyé land to eat diet from this plant many days before the competitions. This study aims to investigate the effects of fruit pulp and leaves of A. digitata on swimming performance and hematological parameters in rats. Male Wistar rats were orally administrated with A. digitata. Leaves or fruit pulp (1000 mg/kg/day) and submitted to swimming for two weeks (6 days of exercise a week without any load). On the 16th day, rats were made to swim till exhaustion with a load of 5% body weight attached to their tails. At the exhaustion point the duration of the effort is recorded. Hematological parameters before and after effort, serum biochemical parameters and serum TNF-α were measured. Antioxidant capacities of A. digitata were also investigated. The results showed that A. digitata leaf and fruit pulp significantly improve swimming time in treated rats (1000 mg/kg/day) compared to untreated rats. This performance could be related to the ameliorative effects of A. Digitata on hematological parameters and its capacity to enhance serum antioxidant potential. A. digitata leaf and fruit pulp could use as food complement to improve physical performance.

Keywords: Adansonia digitata L., antioxidant, Physical performance, Hemoglobin, Rat.

INTRODUCTION
Physical exercise is accepted as a vital component in the maintenance of good health (Ferrari, 2007; Fisher-Wellman and Bloomer, 2009), well-being and body weight control; but the beneficial effects of exercise are lost with exhaustion (Booth and Roberts, 2008). It is well known that exhaustive exercise can lead to oxidative stress and cellular injury (Fisher-Wellman and Bloomer, 2009; Ristow et al., 2009; Rousseau et al., 2006), and also causes structural damage or inflammatory reactions within the muscles (Gomez-Cabrera et al., 2008). Therefore, the muscle needs greater antioxidant protection against potential oxidative damage occurring during and/or after exercise. Antioxidant supplementation may provide protection against the negative health consequences caused by exercise (Goldfarb et al., 2005; Ristow et al., 2009). However, contradictory results have been reported where antioxidant effects have not been observed after supplementation with vitamin E (Viitala and Newhouse, 2004), vitamin C (Huang et al., 2000), vitamins E plus C (Huang et al., 2000) or vitamin E in combination with other antioxidants (Urso and Clarkson, 2003). Whilst some reports suggest a clear benefit, particularly after high doses of antioxidant vitamin supplements (Fisher-Wellman and Bloomer, 2009; Goldfarb et al., 2005), certain vitamins, when used alone or in high dosages, may in fact act as prooxidants (Bloomer et al., 2006; Urso and Clarkson, 2003). Thus it is necessary to find natural antioxidant in the current food plants.

Adansonia digitata L. (Bombacaceae) called Baobab in French (Sidibe and Williams, 2002) and known as “Telou” (Kabyé) or “Adidoti” (Ewe) in Togo, Benin and Ghana is among the main food plants in our communities. Different organs of the plant, especially leaves, fruit pulp, seeds and bark fibers, are used traditionally for medicinal and nutritional purposes (Chadare et al., 2009; Obizoba and Anyika 1994; Lunven and Adrian, 1960). The pulp is therapeutically employed as febrifuge, analgesic, anti-diarrhea, anti-dysentery and for treatment of smallpox and measles (Kerharo and Adam, 1974). The nutritional and medicinal applications of leaves and pulp of A. digitata L. including treatments for intestinal problems, of skin and various uses as agents anti-inflammatory drugs, antipyretic and analgesic (Karumi et al., 2008), antibacterial, antiviral and anti-trypanosome activities were reported (Anani et al., 2000; Hudson et al., 2000; Atawodi et al., 2003). Other previous investigations confirmed the high contents in vitamins, fibers food, mineral and essential amino acids in the leaves and fruits of A. digitata L. (Favier et al., 1993; Soloviev et al., 2004).

During the initiation ceremonies in Kabyé land (Togo), wrestlers called locally Evala consume profusely leaves and fruits of A. digitata L. as food, sauces and natural refreshing drink in order to improve their physical performance. Based on the above-mentioned report, we have undertaken this study in order to investigate the anti-fatigue effects leaves and fruit pulp of A. digitata L. in male Wistar rats.

MATERIAL AND METHODS

Samples collection
Leaves and fruit pulp of A. digitata L. were bought at the market of Tcharé, village located in Kara area at 400 km in the North of Lomé, and identified by the Department of Botany, Faculty of Sciences/University of Lomé. Leaves were powder, after drying under air-conditioner.

Animals
A total of 36 mature male Wistar rats, provided by animals' facilities of the Laboratory of Physiology/Pharmacology, Faculty of Sciences, University of Lomé, weighing 157.2 ± 5.06 g were used. The rats were housed at ambient temperature and humidity, with a 12:12 light-dark cycle. Body weight of the animals was maintained among the groups by diet and water ad libitum. All animals were fed and their body weight was recorded daily. The principles of laboratory animal care were followed throughout the experiment. The procedures used in this study were in accordance with the Animal Ethics Committee of the University of Lomé.

Chemicals
Sodium acetate, acetic acid, Ferric sulfate, Ferric chloride, Quercetin, Folin-Ciocalteau reagent, Sodium carbonate and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-
Aldrich Chemical (St. Louis, MO, United States); 2,4,6-tripyridyl-S-triazine (TPTZ) is purchased from Avocado Research Chemicals (Lancashire, England).

**Evaluation of the Anti-fatigue activity of leaf and fruit pulp of A. digitata**

The animals were divided into four groups (n = 5) and received oral administration once daily via a stomach tube during 16 days, as follow:
- Group A (Untrained rats) and Group B (Trained rats) given only vehicle;
- Group C (Trained rats with the leaves of A. digitata L.) and Group D (Trained rats with the fruit pulp of A. digitata L.) at the dose of 1000 mg/kg/d.

The exercise groups (B, C and D) were subjected to swimming training without any load for two weeks (6 days of exercise a week) following the modified protocol of Saxena et al. (2010). Swimming was performed in groups of five in plastic containers (120 cm × 50 cm × 80 cm) filled with water up to 100 cm and maintained at a temperature between 35 and 36°C. During the exercise, the untrained control animals (group A) were kept in the similar plastic cage containing about 3 cm of water maintained at same temperature to exclude potential stress and other potential confounding effects. On the 16th day, all the rats were made to swim till exhaustion with a load of 5% body weight attached to their tails. Weights have been attached to the tail to standardize the workload and reduce the swimming time in the container (Matsumoto et al., 1996). The uncoordinated movements and staying under the water for 10 seconds without swimming at the surface were accepted as the exhaustion criteria of the rats. At this point, rats were rescued; swimming time was recorded as minute for each rat. Then whole blood was obtained after anesthetization and leaver of rats weighed.

**Blood analysis**

Five ml blood samples were taken into tubes with EDTA and hematological parameters such as red blood cell count (CBC), hemoglobin (HGB), hematocrit value (HCT), mean corpuscular volume (MCH), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), were analyzed with "architect" brand blood count device in laboratory.

Blood serum was prepared by centrifugation at 3000 rpm and the concentrations of Aspartate aminotransferase (AsT), Alkaline Phosphatase (AlP), creatinine and total bilirubin were measured in the serums using commercial kits purchased from Human GmbH (Wiesbaden, Germany).

TNF-α in serum were determined using ELISA kits (Ray Bio® Rat TNF-alpha ELISA Kit Protocol, Aachen, Germany) according to manufacturer protocol.

**In vitro and in vivo antioxidant activity**

Free radical scavenging activities of the leaves and pulp of A. digitata L. was determined using the DPPH assay (Mccune and Johns, 2002; Agbonon et al., 2010). Antioxidant potential of serum from control and treated rats was performed using FRAP (Ferric reducing activity of plasma) assay (Agbonon et al., 2010). Total phenolic and flavonoid content in the plant materials were quantified respectively with Folin-Ciocalteu’s reagent using the method described by Singleton et al. (1999) and colorimetric assay (Kim et al., 2003). For the inhibition of malondialdehyde assay, the liver of rat was homogenized in 10 mL KCl solution (1.5%). The homogenate was centrifuged for 10 min at 3000 rpm. Lipid peroxidation was determined by measuring malondialdehyde (MDA) concentration in the supernatant of liver homogenate of rat, as described by Agbonon et al. (2009).

**Statistical analysis**

Results are expressed as mean ± SD (n = 5). Statistical analysis was performed using ANOVA one- way followed by Tukey’s multiple comparison. p-values < 0.05 were considered statistically significant.

**RESULTS**

**Effects of A. digitata L. on swimming time**

The swimming time of all trained groups increased significantly compared with that of the control group (Tables 1). Among the four groups, the group treated with Pulp (D) was found to possess the highest swimming time. The swimming time decreased in the order: D (323.20 ± 46.5 min) > C (289.83 ± 40.3 min) > B (201.83 ± 50.0 min) > A (50.83 ± 23.4 min) (Tables 1).

**Table(1) Effect of A. digitata L. pulp and leaves orally administered on the swimming time and TNF-α concentration**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Times (mn)</th>
<th>TNF-α (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained rat (A)</td>
<td>50.83± 23.4</td>
<td>3938,82±721.96</td>
</tr>
<tr>
<td>trained rat (B)</td>
<td>201.83±50.0*</td>
<td>2631,20±200,05</td>
</tr>
<tr>
<td>Pulp of A. digitata (D)</td>
<td>323.20±46.5**</td>
<td>3384,75±312,5</td>
</tr>
<tr>
<td>Leaves of A. digitata (C)</td>
<td>289.83±40.3**</td>
<td>3200,79±1113,64</td>
</tr>
</tbody>
</table>

Data represents the mean ± SD for five animals per group; with *p < 0.05 and **p < 0.01 when compared to Control group (ANOVA followed by Tukey’s multiple comparison).

**Effects of A. digitata L. on bloods parameters**

- **Effects of A. digitata L. on hematological parameters and body weight**

Hematological parameters such as RBC, HGB, HCT value increased significantly (P < 0.001) in administrated groups as compare to the control group (Tables 2). But in contrary, body weight of trained rats was not significantly different from that of untrained rats (A = 158.3 ± 7.0 g, B = 170.3 ± 5.9 g, D = 175.5 ± 3.2 g, C = 171.7 ± 9.5 g).

**Table 2: Bloods parameters of rats before and after intense exercise and complementation with A. digitata L.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Hematological parameters</th>
<th>Body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>158.3 ± 7.0 g</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>170.3 ± 5.9 g</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>175.5 ± 3.2 g</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>171.7 ± 9.5 g</td>
<td></td>
</tr>
</tbody>
</table>

A= untrained rats; B= trained rats; L= Leaves (1000mg/kg/d); P= Pulp (1000mg/kg/d); C= Leaves + Exercise (1000mg/kg/d); D= Pulp + Exercise (1000mg/kg/d)
Effects of *A. digitata* on serum biochemical parameters

Following supplementation of rats with *A. digitata* L. extract on some serum biochemical parameters, the results were shown on Table 3. The activity of ALAT, ASAT, Creatinine, Total Bilirubin and ALP show no significant (p>0.05) difference between the control and the supplemented rats. Table 3 Blood biochemical parameters after exercise and supplementation of *A. digitata*

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>A (g/day)</th>
<th>B (g/day)</th>
<th>C (g/day)</th>
<th>D (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>56.33±10.07</td>
<td>43.91±11.15</td>
<td>47.41±15.71</td>
<td>66.83±12.38</td>
</tr>
<tr>
<td>AST</td>
<td>90.16±21.17</td>
<td>76.58±18.33</td>
<td>85.83±11.5</td>
<td>79±14.19</td>
</tr>
<tr>
<td>Creatinine</td>
<td>6.25±0.41</td>
<td>5.83±0.75</td>
<td>6.33±0.81</td>
<td>5.25±0.41</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>1.06±0.42</td>
<td>0.88±0.67</td>
<td>0.96±0.69</td>
<td>1.15±0.24</td>
</tr>
<tr>
<td>ALP</td>
<td>183.33±62.64</td>
<td>157.16±81.39</td>
<td>168.83±58.46</td>
<td>170.83±18.10</td>
</tr>
</tbody>
</table>

A = Untrained rats; B = trained rats; C = Leaves (1000mg/kg/d) + training; D = Pulp (1000mg/kg/d) + training.

Effects of *A. digitata* on TNF-α and malondialdehyde (MDA) concentration

Immediately after swimming, the following finding was observed: TNF-α of non-stress (A) increase more than others (trained group) but not significantly (Tables 1); the levels of MDA rat liver did not have a significant effect among supplemented groups and controls groups (Figure 1).

In vitro and in vivo antioxidant activity

In DPPH radical scavenging assay, IC50 (concentration required to scavenge 50% of free radical) values of leaves and pulp were 1.79 ± 0.04 mg/mL and 8.57 ± 0.24 mg/mL respectively. Total phenolic content of leaves and pulp of this plant were respectively 1.2 ± 0.5 mg/g and 63.56 ± 0.79 mg/g gallic acid equivalent. Total flavonoid contents of leaves and pulp of the same plant were 7.75 ± 1.56 mg/g and 1.72±0.59 mg/g quercetin equivalent. Table 4, it indicated that concentration of Fe2+ was significantly increased when animals were complemented with leaves and pulp of *A. digitata* L. at 1000 mg/kg compared to the control animals. Results indicated that pulp of *A. digitata* L showed a higher antioxidant property in comparison with leaves.

Table(4) Fe2+ concentration in the serum of the rats complemented with leaves and pulp of *A. digitata* L. compared to control rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>258.00±69.8</td>
<td>271.83±81.4</td>
</tr>
<tr>
<td>Leaves at 1000mg/kg</td>
<td>277.05±17.8</td>
<td>425.10±49.6*</td>
</tr>
<tr>
<td>Pulp at 1000mg/kg</td>
<td>266.78±155.6</td>
<td>909.08±210.6**</td>
</tr>
</tbody>
</table>

Data represents the mean ± SD for five animals per group; with *p < 0.05* and **p < 0.001 when compared to Control group (ANOVA followed by Tukey’s multiple comparison).

DISCUSSIONS

The objective of the present investigation was to determine the effects of fruit pulp and leaves of *A. digitata* L. on swimming performance and hematological parameters in rats. Loaded swimming exercise of rats has been used as an endurance test and also to examine whether certain agents have anti-fatigue effects (An et al., 2006). Dawson and Horvath (1970) pointed out that swimming has advantages over other forms of exercise, including the treadmill. Group swimming was used because it promotes more vigorous exercise than when rats are allowed to swim alone (Ueno et al., 1997). In this study, the swimming time of each test group increased significantly compared with that of the control group after 15 days. Farlinge and Beamish (1978), show elevated critical swim speeds after swimming training; that can explain why the swimming time of train control group, in this study increased. Our result suggested that *A. digitata* L. could evidently extend exhaustive swimming time of rats, suggesting that *A. digitata* L. had anti-fatigue activity. The present study demonstrates that oral supplementation of *A. digitata* L. pulp and leaves enhance swimming endurance capacity in rats. Supplementing oral effective dose for 15 days, significantly improves time of exhaustion irrespective of exercise regime. These effects are related to the enhancement of the immunity (Salman et al., 2000).

Body weight result is in accordance with those found by Gamperl et al. (1988) who have shown that endurance-trained in swimming don’t alter body composition.

Erythrocytes (RBC) are synthesized in bone marrow and they contain hemoglobin (HGB) and their main function is oxygen transport. The proportion of the blood marked by RBC is called as hematocrit. Leucocytes (WBC) are part of immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents and foreign substances.

Pulp and leaves of *A. digitata* L. increased RBC, HGB and HCT. So they can be used in stress situation like strenuous exercise which decrease RBC, HGB, HCT (Ercan et al., 1986) and increase the number of leucocyte (Ricci et al., 1988). In sports people who perform intense exercise programs, hemoglobin and hematocrit values decrease and this is recognized as sportsman anemia (Londeann, 1978). It is a general finding that exercises performed regularly for a long duration of time have an effect on organism (Fox et al., 1999; Güney et al., 2006) and hematology that is included within the blood biochemistry which reflects all the changes the organism is also affected by this positive result (Koçet al., 2010). So, pulp and leaves of *A. digitata* L. supplementation can help to resolve this problem.

It is being reported that exercise exerts physiological “stress” on the body and results number of chemical (hormonal) and cellular changes, beside physical change as raised blood pressure, body
temperature and oxygen intake (Bahman, 1997; Iloimcx et al., 1983; Hedfors, 1976). This depends on number of factors as the type and duration of exercise, climate, physical body status, and nutrition etc. Exercise also induces immune like response, results leukocytosis that is quantitatively similar to the response against physiological insults to immune system. As exercise induced in percentage of reactive leukocytosis is considered to be dependent on various factors, we have found that after strenuous exercise the number of leukocyte become almost double, hemoglobin (HGB) decrease. The exercise induced leukocytosis has been often compared to an inflammation like reaction (Northoff and Berg, 1992; Northoff and WeinStock, 1994).

Different kinds of exercise may have various effects on immune parameters based on the nature, intensity and time delay between exercise bouts and immune parameter. It has been reported that moderate or intermittent exercise enhances immune function but prolonged and sever exercise cause numerous changes in immunity, which possibly reflects physiological stress and suppression (Kazeem et al., 2012; Zhao et al., 2012). Ours results indicated that immediately after exercise TNFα concentrations increased but not significantly in control groups compared to others. The control group result (A) is in accordance with Ostrowski et al. (1998), who reported that “the plasma level of, TNF alpha, peaked in the first hour after exercise. It is proposed that an acute bout of physical activity may cause physiological responses which are very similar in many aspects to those induced by infection, sepsis or trauma (Northoff et al., 1998). Therefore, our findings showed that after supplementation of A. digitata and prolonged strenuous exercise there is a chronic low systemic inflammation due to TNF alpha and IL-6 elevation as an anti-inflammatory cytokine.

After regular exercise with adequate intensity and duration the body has the capability to cope with the exercise (stressor) and as a result adaptation takes place. That can explain why the TNFα of exercise group control (B) decreased. The results of the present study showed that prolonged and overtraining exercise causes numerous changes in immunity that possibly reflect physiological stress and immune suppression, which is in agreement with hormesis theory. But orally supplementation of leaves and pulp of A. digitata, enhanced immunity on the supplemented group who have swim long time than control group.

The results indicated that exhaustive exercise, such as swimming, affects the level of MDA in the livers of both trained and untrained rats. The levels of MDA in control rat increased but not significantly than stress control group. This result is in accordance with many studies who have reported that regular aerobic exercise can reduce the oxidative damage caused by acute exhaustive exercise (Aksoy et al., 2006; Miyazaki et al., 2001). The result also showed that the levels of MDA decreased in leaf complemented group(C), so we can conclude that A. digitata L. have antifatigue effect. But after a long time of swimming, levels of MDA increased in pulp complemented group (D), but not significantly. The leaves and pulp of A. digitata have no significant effect on the MDA formation induced by hydrogen peroxide on the supplemented group and control rats livers.

A positive correlation between increased lipid peroxidation and biochemical parameters like AST, ALT, was found in previous animal studies during high-intensity exercise (Hinchcliff et al., 2000), indicating oxidative stress. The biochemical parameters results obtained with untrained rats are in accordance with those studies. However, administration of A. digitata L. leaves and pulp decreased but not significantly, the levels of AST, ALT, creatinine, total bilirubin and ALP in trained and complemented rats. From these results, it can be concluded that this plant has antifatigue and antistress effects. The results obtained from this study indicated that rats orally administered with A. digitata leaves or pulp for two weeks swim longer than the untreated rats. The results also showed that both the leaves and pulp of A. digitata L. strengthened serum antioxidant capacity contain total phenolic compounds and are capable of scavenging DPPH radical. The scavenging ability may be a reflection of the total activities of various components present in these extracts (Wang et al., 2008; Rached et al., 2010). Indeed, several studies have reported that the antioxidant activity of most plants with therapeutic properties may be due to the presence of natural substances mainly phenolic compounds (Atrooz, 2009; Wang et al., 2008).

The concentration of Fe2+ was significantly increased when animals were complemented with leaves and pulp of A. digitata L., at 1000 mg/kg compared to the control animals. The results indicated that pulp of A. digitata L. showed a higher antioxidant property in comparison with leaves. According to Powers and Jackson (2008), antioxidant can be used as the indication of prevention from increased oxidative stress and recovery from fatigue, so leaves and pulp of A. digitata L. supplementation may provide protection against the negative health consequences caused by exercise.

In general, total phenolic and flavonoid content results for pulp and leaves extracts of A. digitata L. showed that the pulp contained higher phenolic content than the leaves. Several studies have demonstrated that the antioxidant activity is strongly correlated with the total content of phenolic compounds (Lim et al., 2007; Nurliyana et al., 2010; Sim and Khing, 2011). Natural polyphenols from plant vegetables have been found to exert their beneficial effect by removing free radicals, chelating metal catalyst, activating antioxidant enzymes, preventing lipid peroxidation (Rached et al., 2010; Oboh et al., 2009; Lopez-Velez et al., 2003). A linear correlation was observed between inhibition of lipid peroxidation and the total phenolic and flavonoid contents. This suggests, therefore, that the phenolic compounds in the extracts contributed significantly to the inhibition of lipid peroxidation.

Free radicals such as hydroxyl radical and superoxide radical are generated from sequential reduction of oxygen during the normal course of aerobic metabolism. Over abundant radicals cause oxidative stress, which can lead to cell injury and tissue damage (Yu et al., 2008). Moreover, the generation of oxidative stress may play an important role in the etiology of many pathological conditions (Karuna et al., 2009). Antioxidant can be used as the indication of prevention from increased oxidative stress and recovery from fatigue (Morihara et al., 2006; Powers and Jackson, 2008). In this study, the data indicated that A. digitata L. has in vitro antioxidant activities, which possessed superoxide and hydroxyl radical scavenging activity. Therefore, leaves and pulp of this plant may have the potential to reduce the oxidative stress in vivo and to fight fatigue.

CONCLUSION

Pulps and leaves of A. digitata L. exhibited antioxidant capacity. They also have antifatigue and antistress effects. These results confirm the local used of this plant. So it may be considered as potential sources of natural antioxidants for therapeutic or industrial purpose and as alternative for the synthetic products, which are known for their multiple disadvantages. To evaluate the physiological or pharmaceutical effects of leaves and pulp of A. digitata in vivo, we need a more detailed understanding of the factors that enable them to exert antifatigue and antistress effects.
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