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Antifatigue properties of an aqueous extract of *Mimosa pudica* Linn. (Fabaceae) in mice subjected to weight loaded force swimming test

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Abstract

Ethnopharmacological relevance: Traditionally, *Mimosa pudica* Linn. (Fabaceae) is used for its anti-inflammatory, sedative, anxiolytic, antioxidant and antifatigue properties.

Aim: To determine the antifatigue effects of *Mimosa pudica* aqueous extracts in experimental model of weight loaded force swimming test.

Materials and methods: Mice were divided into seven groups and treated for 28 consecutive days as follows: groups one and two received orally distilled water (10 mL/kg) and served as normal group and negative control group, respectively. Groups three to six, (test groups) received orally graded doses of *Mimosa pudica* (20, 40, 80 and 160 mg/kg) and group seven (positive control) received vitamin C (50 mg/kg), respectively. One hour after the treatment, mice were subjected to the weight loaded force swimming test with tail load, except for the normal group; and the swimming duration, body weight, food and water intake were measured. Twenty-four hours after the last treatment the serum level of noradrenaline, dopamine and serotonin, and the relative organs weight were measured.

Results: *Mimosa pudica* aqueous extracts significantly and dose-dependently increased the swimming duration and the weight of heart and lungs. The extracts did not induce a significant variation in the level of food and water intake, body weight, and serum of noradrenaline, dopamine and serotonin.

Conclusion: Pretreatment of mice with *Mimosa pudica* aqueous extracts was observed to have better antifatigue properties mediated via amelioration of swimming capacity and physical aptitude in the weight loaded force swimming model.

Keywords: Mimosa pudica; Antifatigue; Swimming; Noradrenaline; Dopamine and serotonin

1. Introduction

Training program is designed to ameliorate the performances of sports performers and to reduce the duration of the recovery processes thus minimize fatigue [1, 2]. Depending on the intensity, duration and initial training status, exercise

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improves aerobic and anaerobic capacities as such as increase performance [3]. However, the increased effort in sustained training program and decreased recovery period trigger fatigue and limit performances. The new determinant of sports performance relied mostly on dietary supplements or energetic drink from plant extract to enhance exercise capacity and improve performance [4, 5]. Interestingly, sports performers are orientated toward plant extracts, fatty and high carbohydrate diets as source of energy supply in replacement of identified substances that trigger excess release of catecholamine [6, 7].

A recent study has shown an upsurge in the use of natural products in African countries to ameliorate sport performance and improve on the threshold of fatigue [5]. Fatigue is the complexity to commence and maintain a voluntary effort, characterise by the sensation of physical or mental tiredness and impotence, occurring after a prolong period of stress, sustained physical and mental activities [8, 9]. The complications of fatigue are generally alteration of functions and deterioration of performances [10]. Fatigue can be central when it is cause by an inadequate activation of motor neurons in relation with reduced excitability of the motor complex or peripheral, when it involves a shortage of energetic substrate stock (creatine phosphate, ATP, glycogen and glucose), formation of free radicals, reduce calcium release during the excitation contraction coupling [11, 12]. Accumulation of metabolic waist (lactic acid and amino acid) causing acidosis and inhibits enzymatic reactions and modification of the ionic equilibrium of membrane; deterioration of muscles fibres and weakness of neurotransmitters [13, 14]. However, these two types are seen to be strongly linked [12, 15].

Mimosa pudica Linn. (Fabaceae) a medicinal plant, is known as a sensitive plant due to the rapid movement of its leaves [16, 17]. The plant is commonly used due to its numerous nutritive, medicinal, and industrial potentials. The traditional use of *Mimosa pudica* aqueous extracts is orientated toward the treatment of hypertension, hyperglycaemia, depression, insomnia, intestinal parasites, inflammations, pain, malaria, asthma, leukoderma, bronchitis, diarrheal, wound healing, general weakness, impotence and fatigue [18, 19]. Previous studies demonstrated antihyperglycemic, antivenom, antihelminthes, anti-inflammatory, analgesic, antinociceptive, wound healing, anti-diarrhoeal, antiulcer, immunomodulatory, antihepatotoxic, diuretic, neuroprotective, anxiolytic antimalarial activity and save in the acute toxicity test [20-29]. Phytochemical screening of *Mimosa pudica* showed the presence of bioactive components such as terpenoids, flavonoids (Quercetin-7-rhamnoside, Luteolin 3, Leutolin-3xyloside, Acacetin-7-rutinoside and Quercetin-3glucoside-7-), glycosides, alkaloids, quinines, phenols, tannins, flavonoids, saponins, coumarins, d-xylose d-glucuronic acid, mimosine, crocetin dimethyl easter, yellow fatty, green oil, vitamin A, B, C, E and adrenalin [22, 30, 31]. Considering the rich active constituents of *Mimosa pudica*, there is thus an urgent need for the evaluation of its pharmacological activities in experimental models of weight loaded force swimming to ascertain and/or facilitate the procedure of use. Therefore, this study aims to ascertain the effects of *Mimosa pudica* aqueous extracts on swimming capacity, physical aptitude and serum biochemical parameters in mice model of weight loaded force swimming test.

2. Material and methods

2.1. Plant collection and authentication

The leaves and stems of *Mimosa pudica* used in our study were harvested in Buea, Fako division (South West Region of Cameroon harvesting coordinates 4°15′06″ N and 9°29′03″ E). The field studies did not involve endangered or protected species. The plant sample was authenticated by the National Herbarium of Yaoundé (Cameroon), where a voucher was deposited (Sample Number 54102/HNC).

2.2. Experimental animals and ethical consideration

Adult male mice, *Mus musculus* Swiss, 25 ± 2 g, 2-3 months old, were used in this experiment. Animals were housed in standard cages at 25°C, on a 12/12-hour light-dark cycle. They were supplied with food and water ad libitum. All experiments were performed according to the Guide for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and the Cameroon National Ethical Committee (Reg No. FW-IRB00001954).

2.3. Chemicals

Vitamin C was obtained from Rossmann, Altapharma Germany and administered at a dose of 50 mg/kg. All other chemicals and reagents used in the biochemical estimation were obtained from Sigma, St. Louis, MO, USA.

2.4. Methods

2.4.1. Preparation of the aqueous extracts of Mimosa pudica

The leaves and stems of *Mimosa pudica* were cut into pieces and allowed to dry (air dry) at room temperature (25°C). The dried leaves and stems were then reduced to fine particles. The doses of the extracts used in this study were obtained from that which is normally used by traditional practitioner. The powder (500 g) was boiled in 5000 mL of distilled water for 20 min. After it cooled, the supernatant (concoction) was collected and filtered with Whatman No. 1 filter paper and dried using an oven. The yield of the extraction was 10.20% (w/w). The initial concentration 16 mg/mL was prepared by introduction of 80 mg of crude extract in 5 mL distilled water. The respective concentrations, 8, 4 and 2 mg/mL were obtained by dilution of the initial concentration at $\frac{1}{2}$, $\frac{1}{4}$, and $\frac{1}{8}$, and administered in a volume of 10 mL/kg.

2.4.2. Pharmacological analysis

Weight loaded force swimming test

Mice were randomly divided into seven groups of six mice. They were supplied with an additional load corresponding to 10% of the body weight gripped to the tail for the swimming test, excepted the normal group. The swimming apparatus consists of a rectangular swimming pool (90 cm length ×45 cm width × 45 cm height) made with a transparent plexiglas. The water level was 35 cm from the bottom and marked on the tank to ensure that the volume of water was consistent across mice, and maintained at $25 \pm 1^{\circ}$ C (32). Mice were divided into seven groups and treated for 28 consecutive days. Briefly, animals in groups one and two received distilled water orally (10 mL/kg) and served as normal group and negative control group, respectively. Groups three to six, (test groups) received orally graded doses of *Mimosa pudica* (20, 40, 80 and 160 mg/kg) and those in group seven (positive control) received vitamin C (50 mg/kg), respectively. One hour after the treatment, mice were subjected to the weight loaded force swimming test with tail load, except of normal group; and the swimming duration was recorded immediately at the exhaustion. Fatigue was determined by failure of coordinated movements and incapacity to return to the surface of the water in 10 seconds. The mice were then removed from the swimming pool, dried with a paper towel, and returned to their original cages. In addition, body weight, food and water intake were measured daily. At the end of 28 days training sessions, mice were sacrificed, blood and organs were collected for biochemical studies [33].

Biochemical analyses

Blood was centrifuged at 3000 rpm for 5 min to obtain serum, which was used to determine the level of serotonin, dopamine and noradrenalin. Immediately after blood collection, the mice were decapitated under ether anaesthesia and vital body organs (brain, liver, heart, kidney, testis, spleen, lungs) were dissected. The organs were quickly removed, cleaned with ice-cold saline and then weighed using a sensitive electronic balance.

Quantification of noradrenalin level

The level of noradrenaline was quantified as described by Al-Delymi and Al-Ghabsha [34]. Serum (0.4 mL) collected was introduced into a tube (0.625 mL), and mixed with acetic acid (0.2 N) and n-butanol solution (1.25 mL) for amine extraction. The mixture was centrifuged at 3000 rpm for 5 min and the aqueous phase was collected for the determination of norepinephrine. The determination of norepinephrine is based on the interaction of peroxidation, which is caused by a decrease in the rate of reaction between p-chlorophenol with aminoantipyrine in the medium. The alizarin sulfonate sodium (0.001 M) was prepared by introducing 0.0856 mg of the compound into 250 mL absolute ethanol. The aqueous phase of the previous homogenate (1.5 mL) was taken and mixed with 2 mL of alizarin sulfonate sodium, and then 1 mL distilled water was added to the mixture which was heated at 50°C for 5 min. The concentration of norepinephrine in the samples was determined by the fluorescence resulting from the reaction in the medium using a spectrophotometer (530 nm).

Quantification of serotonin level

The level of serotonin was evaluated by Schlumpf et al. method [35]. The serum (2.5 mL) was mixed with 5 mL n-butanol, 0.4 mL HCl, 0.1 N and 1% L-cysteine, and then vortexed for 5 min. The mixture was centrifuged and 5 mL of the aqueous phase was removed. Furthermore, 0.6 mL of 0.004% o-phthalaldehyde in 10 N HCl was added to 0.1 mL of aqueous phase, mixed and boiled in a bath at 60°C for 15 min. After cooling the optical density of each solution was determined by the fluorescence in the medium using a spectrofluorometer (360/470 nm).

Quantification of dopamine level

The level of dopamine was estimated by a colorimetric enzyme assay with reference to standard methyl dopamine [36]. The working reagent was prepared by mixture of 0.125% n-butanol, 1.5 mL of serum, 1 mL distilled water, 1 mL sodium acetate (pH 8) and 1 mL 2,6-dichloroquinone-4-chlorimide solution. After incubation, the total volume of the solution was made up to 10 mL with distilled water. The concentration of methyl dopamine contained in the homogenate samples was determined by the fluorescence resulting from the reaction between 2,6-dichloroquinone-4-chlorimide and methyl dopamine in the basic medium using a spectrophotometer (400 nm).

2.5. Statistical analysis

Statistical analyses were performed using Graphpad prism (GraphPad Software, San Diego, CA, USA) and Microsoft excel. Statistical differences between control and treated groups were tested by a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. The differences were considered significant at P<0.05.

3. Results

3.1. Effects of *Mimosa pudica* aqueous extracts on swimming duration in mice subjected to weight loaded force swimming test

The results indicated a significant difference in the delay of fatigue after four weeks of swimming test [F(6, 35) = 959.5; P<0.01] in the different groups of mice (Figure 1). The swimming duration in *Mimosa pudica* extracts-treated mice was significantly increased to 3149.33 ± 53.11 seconds (P<0.05) and 3123.67 ± 43.66 seconds (P<0.05) at the respective doses of 80 and 160 mg/kg when compared to the negative control group where the swimming duration was 2497.66 ± 92.66 seconds (P<0.05). These results were comparable to that of vitamin C (50 mg/kg) where the swimming duration was 3258.83 ± 90.83 seconds (P<0.05).

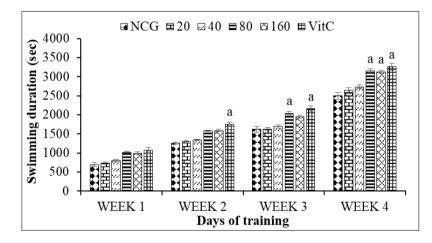


Figure 1 Effects of Mimosa pudica aqueous extracts on swimming duration in mice

Results are expressed as mean \pm S.E.M. n = 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Tukey's multiple comparison test, the differences were considered significant at ^aP<0.05 when compare to the negative control group. NCG: negative control group treated with distilled water; Vit C: positive control group treated with vitamin C; 20, 40, 80 and 160 different groups of mice treated with the respective doses 20, 40, 80 and 160 mg/kg of *Mimosa pudica* aqueous extracts; sec: seconds.

3.2. Effects of Mimosa pudica aqueous extracts on food and water intake in mice

Oral administration of *Mimosa pudica* aqueous extracts did not bring about significant differences in food intake [F(6, 35) = 1.393; P=0.24] and water intake [F(6, 35) = 1.95; P<0.09]. The results showed that the food intake of the negative control group was 6.81 ± 0.32 g/group/day. There was no significant modification of food intake in mice treated with the plant extracts at the doses of 80 and 160 mg/kg compare to the negative control group, where the food intakes were 7.66 ± 0.57 and 7.40 ± 0.50 g/group/day, respectively (Figure 2 a). The water intake was not significantly different in the mice treated with distilled water (6.10 ± 0.40 mL/group/day) compared to the mice treated respectively with *Mimosa pudica* at the doses of 80 mg/kg (6.03 ± 0.40 mL/group/day) and 160 mg/kg (6.15 ± 0.10 mL/group/day)

(Figure 2 b). These results were comparable to that of vitamin C (50 mg/kg) where the water intake was ($6.51 \pm 0.00 \text{ mL/group/day}$).

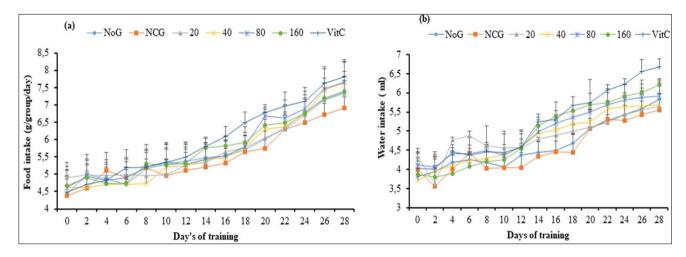


Figure 2 Effects of Mimosa pudica aqueous extracts on food (a) and water (b) intake in mice

Results are expressed as mean \pm S.E.M. n = 6 animals. No significant difference in any food and water intake was observed between the groups of mice. NoG: normal group untrained treated with distilled water; NCG: negative control group treated with distilled water; Vit C: positive control group treated with vitamin C; 20, 40, 80 and 160 different groups of mice treated with the respective doses 20, 40, 80 and 160 mg/kg of *Mimosa pudica* aqueous extracts.

3.3. Effects of Mimosa pudica aqueous extracts on body weight in mice

Following 28 days of *Mimosa pudica* aqueous extracts administration, the body weight gain was not significant [F(6, 35) = 0.98; P=0.45] (Table 1). The results obtained indicated that the body weight gain after four weeks of training varies from 25.72 ± 0.35 g in the negative control group to 25.91 ± 0.41 and 26.21 ± 0.15 g at the doses of 80 and 160 mg/kg, respectively. Interestingly, Vitamin C at a dose of 50 mg/kg (26.56 ± 0.37 g) did not bring about any significant difference in body weight. However, when comparing the body weight in week 1 and week 4 both positive control group of mice and animals treated with *Mimosa pudica* aqueous extracts presented constant increase in body weight.

Treatments	Dose	Body weight (g)					
	(mg/kg)	Week 1	Week 2	Week 3	Week 4		
Normal group	-	24.33 ± 0.18	24.45 ± 0.26	24.73 ± 0.14	25.88 ± 0.31		
Negative group	-	24.41 ± 0.25	24.00 ± 0.10	24.53 ± 0.17	25.72 ± 0.35		
Mimosa pudica	20	24.88 ± 0.12	24.78 ± 0.05	25.10 ± 0.23	25.81 ± 0.38		
Mimosa pudica	40	24.23 ± 0.13	24.95 ± 0.35	25.23 ± 0.28	25.85 ± 0.31		
Mimosa pudica	80	24.40 ± 0.23	24.98 ± 0.25	25.50 ± 0.06	25.91 ± 0.41		
Mimosa pudica	160	24.08 ± 0.08	25.13 ± 0.23	25.68 ± 0.31	26.21 ± 0.15		
Vitamin C	50	24.50 ± 0.13	25.21 ± 0.24	25.80 ± 0.26	26.56 ± 0.37		

Table 1 Effects of Mimosa pudica aqueous extracts on body weight in mice

Results are expressed as mean ± S.E.M. n = 6 animals. No significant difference in any body weights was observed between the groups of mice.

3.4. Effects of Mimosa pudica aqueous extracts on mice serum neurotransmitters

Mimosa pudica aqueous extracts administered orally to mice, did not induce a significant modification in the serum level of serotonin [F (6, 35) = 0.21; P=0.97], dopamine [F (6, 35) = 0.59; P=0.73] and noradrenalin [F(6, 35) = 0.87; P=0.52] (Figure 3)

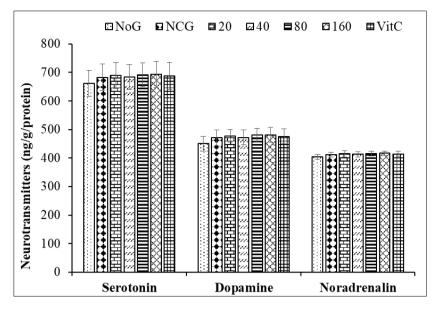


Figure 3 Effects of Mimosa pudica aqueous extracts on mice serum neurotransmitters

Results are expressed as mean \pm S.E.M. n = 6 animals. No significant difference in any biochemical parameters was observed between the groups of mice. NoG: normal group, untrained treated with distilled water; NCG: negative control group treated with distilled water; Vit C: positive control group treated with vitamin C; 20, 40, 80 and 160 different groups of mice treated with the respective doses 20, 40, 80 and 160 mg/kg of *Mimosa pudica* aqueous extracts.

3.5. Effects of Mimosa pudica aqueous extracts on relative organs weight in mice

Table 2 Effects of Mimosa pudica aqueous extracts on relative organs weight in mice

Organs	NoG	NCG	Mimosa pud	Vit C (mg/kg)			
			20	40	80	160	50
Brain	2.03 ± 0.03	2.06 ± 0.07	2.03 ± 0.03	2.04 ± 0.01	2.03 ± 0.03	2.01 ± 0.03	2.03 ± 0.07
Heart	0.51 ± 0.02^{a}	0.55 ± 0.01	0.58 ± 0.01	0.58 ± 0.01	0.59 ± 0.01 ^a	0.59 ± 0.01^{a}	0.59 ± 0.02^{a}
Spleen	0.40 ± 0.02	0.43 ± 0.00	0.42 ± 0.00	0.42 ± 0.00	0.42 ± 0.00	0.41 ± 0.02	0.43 ± 0.00
Liver	5.45 ± 0.10	5.71 ± 0.29	5.76 ± 0.13	5.76 ± 0.14	5.70 ± 0.07	5.63 ± 0.06	5.56 ± 0.03
Lungs	0.85 ± 0.02 ^c	0.93 ± 0.02	0.95 ± 0.02	0.98 ± 0.02^{a}	0.98 ± 0.02^{a}	0.99 ± 0.01 ^b	1.07 ± 0.02 ^c
Right kidney	2.54 ± 0.05	2.58 ± 0.05	2.55 ± 0.05	2.55 ± 0.02	2.55 ± 0.03	2.56 ± 0.02	2.55 ± 0.04
Left kidney	2.07 ± 0.09	2.04 ± 0.02	2.11 ± 0.09	2.12 ± 0.15	2.06 ± 0.13	2.12 ± 0.19	2.08 ± 0.00
Right testis	3.49 ± 0.05	3.54 ± 0.23	3.53 ± 0.09	3.46 ± 0.28	3.50 ± 0.41	3.49 ± 0.24	3.56 ± 0.23
Left testis	3.47 ± 0.07	3.08 ± 0.27	3.16 ± 0.10	3.41 ± 0.28	3.92 ± 0.04	3.34 ± 0.11	3.65 ± 0.05

Results are expressed as mean ± S.E.M. n = 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Tukey's multiple comparison test, the differences were considered significant at ^aP<0.05, ^bP<0.01, ^cP<0.001, when compare to the negative control group. NoG: normal group; untrained treated with distilled water; NCG: negative control group treated with distilled water; Vit C: positive control group treated with vitamin C

As depicted in Table 2, no significant differences were observed in the relative weight of the brain [F(6, 35) = 0.42; P=0.86], spleen [F(6, 35) = 2.25; P=0.06], liver [F(6, 35) = 2.19; P=0.07], right kidney [F(6, 35) = 0.40; P=0.87], left kidney [F(6, 35) = 0.21; P=0.97], right testis [F(6, 35) = 0.075; P=0.99] and left testis [F(6, 35) = 0.56; P=0.76] following the physiological adaptation's after the 28 consecutive days of treatment by the oral route and training. However, the recorded value of the heart presented a significant disparity among the groups of animals [F(6, 35) = 19.52; P<0.001], revealing that *Mimosa pudica* administered at the doses of 80 and 160 mg/kg significantly increased the heart relative weight to 0.59 ± 0.01 (P<0.05) and to 0.59 ± 0.01 (P<0.05) respectively, when compare to 0.55 ± 0.01 in the control group of mice. Similarly, the results for the vitamin C treated group showed 0.59 ± 0.02 (P<0.05), a comparable value to

that of the dose treated group with 80 mg/kg aqueous extract. Also, there was a statistical difference in the relative lungs weight between groups [F(6, 35) = 50.84; P<0.001]. The aqueous extracts of *Mimosa pudica* significantly increased the lungs relative weight from 0.93 ± 0.02 in the negative control group of mice to 0.99 ± 0.01 (P<0.01) for a dose of 160 mg/kg. Compared with distilled water, vitamin C administered at a dose of 50 mg/kg resulted in a significant increase in the relative lungs weight to 1.07 ± 0.02 (P<0.001).

4. Discussion

The present study was undertaken to evaluate the antifatigue properties of *Mimosa pudica* aqueous extracts in mice subjected to weight loaded force swimming test. Plants are recognised as source of secondary metabolites such as polyphenols, glycosides and vitamins. Medicinal plants are highly valorised for their preventive and therapeutic functions. For the screening of antifatigue property of various bioactive compounds, the weight loaded swimming test represents valid animal model and was employed to assess the swimming capacity [10, 37]. In this orientation we initially investigated the swimming duration, the feeding behaviours and the biochemical parameters of mice pretreated with *Mimosa pudica* aqueous extracts and subjected to weight loaded force swimming test.

From the obtained results, the swimming duration to exhaustion of each extract-treated group was significantly longer than that recorded to the negative control group. The extracts induced 26.09% increase in the swimming duration of mice. Therefore, it is obvious that the extracts of *Mimosa pudica* enhanced the swimming capacity by delaying the onset of physical fatigue in mice. This could be due to the presence of glycoside in the extracts that help to supply energetic substrates [38, 39]. The results obtained by Nworgu and Egbunike [40] revealed that *Mimosa pudica* aqueous extracts is rich in minerals such as potassium (0.45 - 1.85%) and calcium (0.60 - 1.73%). It is supposed that *Mimosa pudica* aqueous extracts enhanced the swimming capacity by lessening fatigue in mice. This could justify the prolonged and improved effort after administration of *Mimosa pudica* aqueous extracts, knowing the key function of potassium and calcium in the excitation contraction coupling during muscles contraction.

Administration of *Mimosa pudica* aqueous extracts did not significantly affect mice food and water intake. However, we observed a significant difference in the final body weight gain (2.13 g; 8.90%) following 28 days of *Mimosa pudica* aqueous extracts administration. These results are in accordance with previous research where the body weight of animals treated with *Mimosa pudica* significantly increased up to 10%, attributing these changes to the rich nutrient quality of the extract such as crude protein (21.36-23.34%), glycosides, D-xylose D-glucuronic acid, yellow fatty, green oil, and vitamins. These changes were also being attributed to the growth process in mice [40].

Many substances that affect sports performances are considered doping when their concentration in the blood is above the normal range [41, 42]. Dopamine, noradrenaline, and serotonin are the three major monoamine neurotransmitters that are known to be modulated by exercise. Regulations of the secretion of neurotrophic factors, vasculotropic factors, inflammatory mediators, and neurotransmitters are also involved in exercise's influence on brain function [43, 44]. Among these effects, secretion of neurotransmitters, especially monoamines, have been linked to the exercise-induced neuronal adaptation. The results obtained from this study depicted that *Mimosa pudica* aqueous extracts (20 – 160 mg/kg) did not induce a significant increase in serum levels of serotonin, dopamine and noradrenalin demonstrating that the extract doesn't increase the swimming capacity through the modulation of brain functions and neuronal activation [43, 45, 46].

The results of this study indicated that the relative organs weight did not increase significantly among the different groups of animals except the significant increases in the relative heart and lungs weights compared to the negative control groups of mice following 28 days of extracts administration. This result is in accordance to that obtained by Nghonjuyi et al. who reported a significant increase in organ weights of animal given *Mimosa pudica* extracts, attributing these changes to the active constituents of the extracts [47].

5. Conclusion

In summary, the leaves and stems of aqueous extracts of *Mimosa pudica* possesses antifatigue properties. It improved the swimming ability of mice by maintaining the regulation of serum dopamine, noradrenaline, and serotonin concentrations at the normal level, and by increasing the relative heart and lungs weights. Study results suggest that *Mimosa pudica* aqueous extract has significant health benefits due to its antifatigue activity thus, providing scientific evidence for further development of natural products for prevention and treatment of diseases related to central and peripheral fatigue conditions.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996).

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