ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR FOOD SCIENCE AND NUTRITION

Exercise Performance Improvement and Anti-Fatigue Effect of Hot Water Extract of Koroso fish (*Oreochromis niloticus*)

By: Anley Teferra

Advisor: Paulos Getachew (PhD)

Co-Advisor: Kaleab Baye (PhD)

A Thesis Submitted to the School of Graduate Studies College of Natural and Computational Sciences in Partial Fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition

Addis Ababa, Ethiopia

March 2017

Addis Ababa University

College of Natural and Computational Sciences

Center for Food Science and Nutrition

RESEARCH THESIS APPROVAL

This is to certify that the thesis prepared by Anley Teferra, entitled: *Exercise Performance Improvement and Anti-Fatigue Effect of Hot Water Extract of Koroso fish (Oreochromis niloticus)* and submitted in partial fulfillment of requirements for the degree of Master of Science in Food Science and Nutrition complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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The Thesis has been approved for submission by:

Name of Supervisor Signature Date

Paulos Getachew (PhD) (Advisor) _____ _____

Kaleab Baye (PhD) (Co-Advisor) _____ _____

Abstract

Traditionally, the hot water extract of Koroso fish (*Oreochromis niloticus*) has been used as a nourishing tonic soup and as an aid for recovery from physical fatigue. In this study, we investigated the antifatigue effects and exercise performance endurance of mice orally administered with hot water extracts of Koroso fish. Accordingly, physical parameters including swimming endurance, forelimb grip strength, muscle density and body weight were assessed following oral administration of the extracts at a dose of 10 µL/g body weight (once per day for 7 days). After 7 days of oral administration, the blood chemistry of the mice was investigated. Mice given the Koroso fish extracts from Hawassa (HF), Bahir Dar (BF) and the positive control Octacosanol (OC) had significantly greater forelimb grip strength (130.07%, 133.93 %), (129.32%, 136.52%) and (136.6%, 155.4%) on day 3rd and 7th day respectively compared with zero day's performance. On similar days, HF, BF and OC fed mice had increased swimming endurance by (171.52%, 152.4%), (172.19%, 133.43%) and (177.4%, 144.8%) than 0 day's performance. Also, HF, BF and OC fed mice were found with increased forelimb and hindlimb muscle density by (131.5%, 137.5%), (135.9%, 134.8%) and (125.2%, 122.2%) than normal saline fed mice. After exercising, on the 7th day HF, BF and OC fed mice were found with higher blood glucose level by (161.34%, 168.54%, 136.74%), lactate dehydrogenase by (118.74%, 108.7%, 121.87%) and HDL by (154.77%, 142.79%, 179.92%) than the saline fed control mice respectively. In contrast, the levels of urea by (90.85%, 90.91%, 83.32%), creatine kinase by (79.21%, 74.69%, 13.1%), LDL by (96.41%, 71.6%, 93.1%) triglyceride by (68.26%, 66.2%, 59.37%) and total cholesterol (92.57%, 91.92%, 93.26%) were significantly lower than the saline group mice. These results

suggest that hot water extract of Koroso fish can improve physical exercise performance and prevent fatigue caused by exhaustive physical workouts.

Key Words*: Oreochromis niloticus,* hot water extract, anti-fatigue, exercise performance

Acknowledgements

I would like to thank my beloved and respected sister Helen Teferra for everything she did for me. I also would like to thank my advisor Dr. Paulos Getachew and my co-advisor Dr. Kaleab Baye for their great effort to support and guide me throughout this research with patience and thoughtfulness.

I would like to thank Ethiopian public Health Institute (EPHI) for allowing me to utilize their laboratory and Mr. Bikila Negassa for devoting his time and energy to perform the biochemical analysis.

I wish to extend my heartiest appreciation and profound regards to all staffs at Center for Food Science and Nutrition, Addis Ababa University and to all the members of my thesis evaluation committee for managing their valuable time in reviewing my thesis and valuable suggestions.

I greatly appreciate all my fellow class mates, lab mates and colleagues Center for Food Science of Addis Ababa University for their kind cooperation and consistent help.

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Abbreviations and Acronyms

NANDA	North American Nursing Diagnosis Association
LDH	Lactate Dehydrogenase
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
CK	Creatine Kinase
TG	Triglyceride
EPHI	Ethiopian Public Health Institution
AMP	Adenosine Mono Phosphate
ADP	Adenosine Di Phosphate
ATP	Adenosine Tri Phosphate
NS	Negative control group taking normal saline
HF	Hawassa Fish Hot Water Extract
BF	Bahir Dar Fish Hot Water Extract
OC	Octacosanol

1. Introduction

1.1. Background

Fatigue is a very great tiredness or exhaustion. It can be emotional or physical. Fatigue is one of the negative effects and challenges of a person who is sick, elderly, labor servant and doing physical exercise. But fatigue is seen on healthy individuals too[1-3]. A few researchers have also explored the phenomenon of fatigue during child bearing experience. Patients and elderly who lack strength in antigravity muscles have difficulty to stand up, fatigue easily when walking, and have difficulty maintaining their balance after perturbation. These symptoms are reported by at least 20% of patients seeking medical care[4-8]. Fatigue can cause various disorders of the bio regulatory, autonomic nervous system, endocrine, and immune systems[9]. Therefore, it is necessary to investigate natural anti-fatigue compounds without adverse effects to improve physical activity and athletic ability, postpone fatigue and accelerate the elimination of fatigue in human beings[10].

The community around Hawassa are rich in traditional knowledge about some available food sources in their surrounding and their nutritional value for health. There is tradition in Hawassa (38.4767^0E longitude, 7.0602^0N latitude) and Arba Minch (37.5500^0E longitude, 6.0333^0N latitude), in which people use Koroso fish (*O. niloticus*) soup for medicinal purposes including to get relieve from cough, cold and also to gain physical strength to work actively (Fig 1). There are small tent shops around Hawassa lake and medium class restaurants in Arba Minch, which primarily serve fish soup. In South East Asian countries like Korea also there is a tradition producing tonic soup from aquatic animals like soft shell turtle (*Trionyx sinensis*). People used this tonic soup to get the advantage of reducing

fatigue[11,76] which is proven scientifically by different studies. Therefore, in the present study, we intended to prepare a hot water extract from Nile Tilapia/Koroso fish collected from Hawassa and Bahir Dar area and investigated its effect on fatigue relieve based on physical and biochemical parameters after orally administrating the extracts to mice for 7 days.

Figure 1. Customer drinking fish soup in small tent beside Lake Hawassa, Hawassa, Ethiopia.

1.2. General Objective

To investigate the anti-fatigue activity of hot water extracts of Koroso fish (*Oreochromis niloticus*) and its effect on physical activity and exercise performance improvement of mice.

1.3.Specific objectives

- To investigate the antifatigue effect of hot water extracts of Koroso fish (*O. niloticus*) from Lake Tana on mice.
- To investigate the antifatigue effect of hot water extracts of Koroso fish (*O. niloticus*) from Lake Hawassa on mice.
- To investigate the exercise performance improvement effect of hot water extracts of Koroso fish (*O. niloticus*) from Lake Tana on mice.
- To investigate the exercise performance improvement effect of hot water extracts of Koroso fish (*O. niloticus*) from Lake Hawassa on mice.

2. Literature Review

2.1. Physical Activity and Exercise

The beneficial effects of regular, non-exhaustive physical exercise have been known for a long time. Exercise is a part of the treatment of common diseases such as diabetes mellitus and coronary heart disease. It improves plasma lipid profile, increases bone density, and helps to maintain balanced weight. Recent investigations have revealed even greater reductions in the risk of death from any cause and from cardiovascular disease through exercising. For instance, being fit or active was associated with a >50% reduction in risk of diseases [12]. Both aerobic and resistance types of exercise have been shown to be associated with a decreased risk of type 2 diabetes[13-18]. Routine physical activity can improve musculoskeletal fitness. There is increasing evidence that enhanced musculoskeletal fitness is associated with an improvement in overall health status and a reduction in the risk of chronic disease and disability[13,14]. In a large prospective study[16], each increase of 500 kcal in energy expenditure per week was associated with a decreased incidence of type 2 diabetes. However, the beneficial effects of exercise are lost with exhaustion and with lack of training. Indeed, it is well known that exhaustive exercise causes muscle damage, for instance evidenced as an increase in the plasma activity of cytosolic enzymes such as creatine kinase. The degree of oxidative stress and of muscle damage does not depend on the absolute intensity of exercise but on the degree of exhaustion of the person who performs exercise. Some of this damage is due to the production of free radicals and it may be prevented by optimizing nutrition[11].

2.2. Metabolic changes in working muscle

A feature of fast muscle is that it can consume ATP, producing ADP and P_i, faster than it generates it. Because the creatine kinase (PCr + ADP ⟷ Cr + ATP) and the adenylate kinase (2ADP ⟷ AMP + ATP) reactions are close to equilibrium, the net consumption of ATP leads to relatively stereotyped changes in the concentrations of ATP, ADP, P_i, phosphocreatine (PCr), creatine (Cr), and AMP which can be calculated from the equilibrium constants[19,20]. The pathways that resynthesize ATP include anaerobic glycogenolysis and the aerobic breakdown either of glycogen, glucose or fat. Anaerobic glycolysis is of central importance in muscle fatigue because it is turned on rapidly during activity, and the net reaction is breakdown of glucose units to lactate ions and protons causing the early acidosis associated with rapid-onset fatigue. Typically, this can lead to an acidosis of ~0.5 pH units [21]. It is well known that energy consumption and deficiency will result in physical fatigue during exercise, and the endurance capacity of the body is markedly decreased if the energy is exhausted. Energy for exercise is derived initially from the breakdown of glycogen[22]. After strenuous exercise, muscle glycogen will be exhausted. Because of this, lactic acid reconstitution is important. Reconstitution of lactic acid system means mainly the removal of the excess lactic acid that has accumulated in all the fluids of the body. This is especially important because lactic acid causes extreme fatigue. When adequate amounts of energy available from oxidative metabolism, removal of lactic acid is achieved in two ways: (1) A small portion of it is converted back into pyruvic acid with the help of lactate dehydrogenase enzyme and then metabolized oxidatively by all the body tissues. (2) The remaining lactic acid is reconverted into glucose mainly in the liver, and the glucose in turn is used to replenish the glycogen stores of the muscles.

2.3. Fatigue

The classification of nursing diagnosis (NANDA, 1900) definition of fatigue is: “An overwhelming sustained sense of exhaustion and decreased capacity for physical and mental work”. Fatigue is usually defined as the reversible decline of performance during activity, and most recovery occurs within the first hour. However, there is also a slowly reversible component that can take several days to reverse[24]. During fatigue, body faces difficulty in initiating or sustaining voluntary activities. It induces alteration in performance leading to decrease in muscular power and endurance, decreased motor skill performance and diminished physical as well as mental functions[25]. The etiology of muscle fatigue is important, as it can lead to serious limitations in muscle and whole body performance[23, 26], and in clinical situations to respiratory failure and death[27]. While the exact causes of muscle fatigue and the relative importance of particular factors remains controversial[23,28], it is clear that an individual’s state of fitness, dietary status, muscle fiber type composition, and intensity duration of the exercise all affect the process. Accumulation of metabolic byproducts like magnesium, inorganic phosphates, lactate, reactive oxygen species etc. can potentially contribute to fatigue during exercise and limit the performance. Physiological fatigue may also result due to metabolic disorders like diabetes, hyperthyroidism, anemia, high body mass index, liver disease etc.[29]. Patients with chronic fatigue syndrome often complain of exercise intolerance. Many patients report that even minor efforts at physical activity lead to significant worsening of fatigue. Additionally, some evidences suggested that many chronic fatigue syndrome patients cope with their illness by resting or avoiding physical activity[30-32]. Several studies have focused on chronic fatigue syndrome patients’ strength, level of conditioning, and physiological response to exercise. A number of studies

have provided evidence for a model in which physical deconditioning helps to maintain physical disability[33]. An increase in lactic acid can be a response to intensive exercise[34], reductions in capacity for oxygen transport[35], small number of muscle mitochondria[36], low physical fitness and exercise capacity[37-39]. Other studies, however, have found normal or near-normal aerobic capacity[40,41] and muscle function[42,43] and post-exercise lactate concentrations comparable to those in sedentary comparison subjects[41].

The energy consumption of skeletal muscle cells may increase up to 100-fold when going from rest to high intensity exercise. This high energy demand exceeds the aerobic capacity of the muscle cells, and a large fraction of ATP required will come from anaerobic metabolism. High intensity exercise also leads to a rapid decline in contractile function known as skeletal muscle fatigue. It therefore seems logical that there is causal relationship between anaerobic metabolisms and muscle fatigue. Anaerobic metabolism in skeletal muscle also involves hydrolysis of creatine phosphate (CrP) to creatine and inorganic phosphate (P_i). Creatine has little effect on contractile function, whereas there are several mechanisms by which increased P_i may depress contractile function. Thus, on the basis of recent findings[44-49], increased P_i appears to be the most important cause of fatigue during high intensity exercise.

To prevent fatigue accumulation and improve exercise endurance, traditionally in South East Asian countries like Korea, consumption of tonic soup from aquatic animals like soft shell turtle (*Trionyx sinensis*) is popular. People used this tonic soup to get the advantage of reducing fatigue [11, 76] which is proven scientifically by different studies. Similar tradition has been observed near Hawassa lake, people consuming hot water extract of fish for

medicinal purposes. Commercially, a supplement named Octacosanol is available as anti-fatigue aid. However, as a general fact it is much better to use natural extracts as good sources of nutrients. Hence, the main objective of this study was focusing on investigating potential antifatigue activities from our fish species extract using mice.

2.4. Fish

Ethiopia is endowed with 7400 Km^2 of lakes and a total river length of about 7000 Km which harbor various fish species that are ecologically and economically important to the country[50]. Some of these species include Koroso/Nile Tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), Barb fish (*Barbus Sp*), Nile perch (*Lates niloticus*) and others. Particularly the Nile Tilapias are the most widely spread in the tropical and subtropical waters. They can tolerate a wide range of temperature, salinity and oxygen profiles[51]. In Ethiopia, Koroso fish (*Oreochromis niloticus*) is found in most rivers and lakes. The inland capture fishery comprises: Rift Valley lakes (lakes Chamo, Abaya, Hawassa, Ziway and the northern part of Lake Turkana) and Lake Tana; rivers; and small water bodies (reservoirs and natural ponds). There is fishing on all these water bodies (Table 1). There are 180 different species of fish in Ethiopia and 30 of those are endemic to the country[52].

Table 1. Summary of Ethiopian water bodies and their fisheries[75]

Water bodies type	Extent (km^2)	Fishery potential (ton/year)	Catch (ton/year)
Major lakes	6,477	23,342	10,598
Major reservoirs and dams	857	4,399	1,366
Small water bodies	275	1,952	303
Rivers	7,185	21,788	3,121
Total	-	51,481	15,389

- : data cannot be summed up

Fish as a source of human food has a long history in Ethiopia. Traditionally, people consume large amount of fish in fasting season. Meanwhile, in big cities around production areas and towns, especially in Ziway, Arba Minch, Hawassa, Bahir Dar and Addis Ababa, relatively the consumption rate is higher even in non-fasting seasons also[75].

Fresh fish is produced in the Great Rift Valley lakes and in some other northern parts of the country. Price wise too, fish is relatively expensive compared with the local prices of vegetables and grains on a unit weight basis, but it is frequently less costly than alternative animal protein sources. With increased marketing efforts and increase in supply, the demand for the fish product could be tremendously increased from the current level[75].

The demand for fish is higher than supply especially, in Ethiopian fasting season and if it not fasting season supply is higher. This is because of religious influences on consumption

pattern; the demand for fish is only seasonal. During lent, Christians who abstain from eating meat, milk, and eggs consume fish, since fish is the substitute of meat[75].

Even if the available stocks of these fishery waters will be fully exploited in the near future, both current and future demand for fish by the population cannot be met. For instance, total demand for fish in 2003 is about 67 thousand tonnes, which is envisaged to grow nearly to 95 thousand tonnes in 2015 and 118 thousand tonnes in 2025. To fill this gap, therefore, new alternative fish supply source must be found[75].

Fish is one of the known Aquatic animals in general do contain a high level of protein (17-29%) with an amino acid profile, similar to that of meat from terrestrial sources. The flesh of fish is also readily digestible and immediately utilizable by the human body, which makes it suitable for complementing high carbohydrate diets. Compared with land animals, aquatic animals have a high percentage of edible flesh, with little wastage[75].

Aquatic animals are a source of minerals such as calcium, iron and phosphorus as well as trace elements and vitamins. Marine species are particularly rich in iodine. The lipid content is high in polyunsaturated fatty acids and particularly those which are attributed to reduce blood cholesterol. Some of these fatty acids in fish may provide protection against renal and cardiac diseases. Increasing the percapita consumption of fish in any country can benefit the society with its mentioned health benefits standards[75].

Fish properly preserved, prepared and presented in the right form is popular in most households, particularly in big towns. The appeal of an otherwise tasteless diet is greatly improved, and much use is made of fish and shellfish as soups and condiments, especially when smoked or dried. Among some religious groups, such as the Coptic Orthodox Church in Ethiopia, fish plays an important role in fasting days when the eating meat product is forbidden[75].

The fish consumption per head per year of the country is very low. However the rapid growth of population and the progressive shortage of livestock products had changed the situation to a growing demand of fish[75].

2.5. Octacosanol

Nutritional supplements have become increasingly popular among the general public worldwide, and many supplements are taken as ergogenic aids, a term used for substances that enhance athletic performance and increase stamina and exercise capacity. Ergogenic aids are believed to increase athletic performance by either renewing or increasing energy stores in the body, modulating the biochemical reactions contributing to fatigue, or maintaining optimal body weight[53]. Octacosanol[$HO\text{-}CH_2\text{-}(CH_2)_{26}CH_3$], a high molecular weight compound is one of the predominant commercially available Ergogenic aids. It is the main component of policosanols (fatty alcohol mixture), found in plant waxes common in fruits, leaves, surface of plants and whole seeds[54,55]. Long-term clinical studies have demonstrated that, octacosanol was well tolerated and safe[56]. Sugar cane is one of the major crops in the world, which is an ideal source of octacosanol. The bagasse from sugar

cane contains a higher amount of policosanol than sugar cane leaves and other materials, and has a high and stable content of octacosanol[56,57]. More recently, studies[58,59] have suggested beneficial effects of octacosanol on physical performance in different systems. Therefore, in our study octacosanol was used as a positive control being orally administered to mice.

3. Materials and Methods

3.1. Fish Extract Preparation

Hot water extraction of Koroso fish collected from Hawassa and Bahir Dar area (Fig 2) has been done at Addis Ababa University, College of Natural and Computational Sciences, Center for Food Science and Nutrition laboratory. Two kilograms (2Kg) fillet of Koroso fish/obtained from 4.8 Kg Lake Tana whole fish and 5 Kg Lake Hawassa whole fish was extracted using 20 L boiled water through gradual addition for 25 hours at 92 ^{0}C[76]. The extracts have been filtered through 2mm and then by 0.425mm mesh size sieves respectively. The extract was centrifuged for 15 minutes at 3000g centrifugal force (China, 800D model). After removing the residue (lower layer) and oily (upper layer) by centrifugation, the middle layer has been collected and centrifuged again for 15 minutes at 3000 g force for 15 minutes. Then the residue and oily layer has been removed again, the middle layer became clear and collected. The extract in 15 ml falcon tube was kept at -20^{O}C in refrigerator until further analysis (Fig 3).

Figure 2. Collecting fish from local market, Bahir Dar, Ethiopia

1. 

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8. 

Figure 3. Preparation of hot water fish extract

3.2. Determination of Crude Protein

Crude protein content of the extracts was determined by Kjeldhal method according to AOAC (2000) protocol (Distiller: China, K9840 model, Hanon Instruments; Digester: China, SH220N/220F model, Hanon Instruments).

Addition of reagents: 5mL and 10mL hot water Hawassa fish extract; and 5mL and 10mL hot water Bahir Dar fish extract were weighed in a tectar tubes and placed in the tectar rack (in triplicate). Then 6ml of sulfuric acid mixture was added carefully by using a pipette and mixed with the samples immediately. Then 3.5ml of 30% hydrogen peroxide was added step by step till the reaction stopped. As soon as the reaction ceased, the tubes were hand-shaken for a few minutes and were put back into the rack. Then, 3g of catalyst (K_2SO_4 mixed with copper sulphate) was added and the mixtures were allowed to stand for 5-15 minutes before digestion.

Digestion: the tubes in the rack were lowered into the digester in the fume hood at 370^{o}C. The exhaust manifold was located on top of tubes and the digestion was continued until clear solution appeared for 4 hours in the fume hood. After digestion was completed, the content in the flasks was diluted by water and concentrated sodium hydroxide 40% was added to neutralize the acid and to make the solution slightly alkaline.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + 2H_2O + NaSO_4$$

The ammonia was then distilled into receiving flasks which contained a solution of excess boric acid. The borate ion was formed as a result of the reaction of the boric acid and the ammonia and this was titrated with standard acid (0.1 HCl) until the green color changed to pink.

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$$

Total Nitrogen (%) = [(V-Vb)xNx14]/ W

Crude protein (%) = Total Nitrogen(%)x 6.25

Where: V = Volume of HCl acid consumed to neutralize the sample

Vb = Volume of HCl consumed to neutralize the blank

N x 14 = Normality of the acid x equivalent weight of nitrogen

6.25 = conversion factor from total nitrogen to crude protein

W = Weight of the extract on wet basis

3.3. Determination of the Extracts Total Concentration

According to previous studies [11,76], used concentration of hot water fish extracts on dry weigh basis (88 and 50) mg/ml and in terms of protein (50 and 30) mg/ml. Therefore, in this study after crude protein determination, the concentration of the extracts was adjusted to average values of the previous studies. Hence, the dry weight and protein concentration of the Hawassa and Bahir Dar fish extracts were (63.5 and 40) mg/ml respectively (Fig 4). Adjusting the concentration of the extracts in terms of protein was done using rotary

evaporator (Fig 4) (England, STUART, RE300B model). Meanwhile, the dry weight concentration was conducted using moisture analyzer (Japan, AND, ML-50 model)

Figure 4. Adjusting concentration of hot water fish extracts by using rotary evaporator

3.4. Normal Saline and Octacosanol Preparation

Normal saline (0.9%) was bought from pharmacy in Addis Ababa, Ethiopia and octacosanol tablet obtained from Ferngrove Pharmaceuticals Pty, Ltd., Sydney Australia. 200mg octacosanol recommended for 60Kg body weight. After crushing the 200mg tablet, it has

been dissolved in a 600 ml normal saline with 5% tween80 surfactant. The final concentration of octacosanol was adjusted to 3.33µg/10µl pergram of body weight.

3.5. Feeding

Forty-eight white albino mice (6-8 weeks old) which were approximately 27-35 gram were obtained from Ethiopian Public Health Institute (EPHI). Twelve mice per group and they were kept in a controlled environment at room temperature in a cage at Traditional and Modern Medicine Research Directorate Laboratory, EPHI. Mice were orally administered with the fish extracts (HF and BF), normal saline (NS), octacosanol (OC). The mice were treated by obeying current laws and guiding principles of animal ethics committee. The 1st group mice Hawassa Fish extract (HF) and 2nd group mice, Bahir Dar Fish extract (BF) were orally administered with the extracts at (0.4mg/10µL protein concentration for 1g of body weight) once a day for 7 days. The 3rd group mice were orally administered Octacosanol (OC) at (3.33µg/10µL for 1g of body weight) once a day for 7 days. Similarly, 0.9% physiological saline (10µL/g of body weight) was orally administered for the 4thcontrol group (NS) (Fig 5). All groups underwent body weight, grip strength measurements and swimming tests on zero day before the feeding started. Also on the 3rd and 7th day 1 hour after oral administration of the extracts, the same tests were conducted[11,76].

Figure 5. Oral administration of extracts to the mouse

3.6. Forelimb Grip Strength Test

A low force analogue force guage testing equipment (China, NK-10 model, Yueqing Handpi instruments Co. Ltd) was used to measure the forelimb grip strength of the mice. The mice grasped at the base of the tail and pulled slightly backward by the tail while the two forelimbs grip the metal, which causes counter-pull (Fig 6). The grip strength meter record the grasping force in Newton (N). Before the experiment the mice in each group were trained to perform this procedure for 3 days. Grip strength (N) and body weight (g) measured 3 times on day 0 before treatment, and 1 hour after treatment on the 3rd and 7th days. Each mouse was

subjected to 3 grip trials with at least 1 minutes rest between trials. The maximum force exerted by the mouse counter-pull was recorded as the forelimb grip strength[11,76].

Figure 6. Testing forelimb grip strength of the mouse

3.7. Swimming Endurance Test

Swimming test was done on day 0 before treatment, on day 3 and day 7 after treatment by submerging the mice in transparent plastic cylinder (13 cm diameter and 22 cm depth) filled with water (25^0C). The mice were allowed to swim for a total of 6 minutes, in which 2 minutes was acclimitation /adaptation time. The remaining 4 minutes swimming was video recorded and total time of active swimming is calculated accordingly[11,76].

Figure 7. Swimming endurance test of the mice

3.8. Biochemical Assay

Biochemical assay has been done at the Clinical Chemistry Laboratory, EPHI. One hour after the last (7^{th} day) grip strength trial and swimming test, blood samples were collected from mice. To collect the blood, the mice were euthanized humanly by using diethyl ether and then blood was directly drawn from the heart by dissecting each mouse (Fig 8). The blood samples were collected in a serum separator, kept for 30 minutes until it coagulates and centrifuged for 7 minutes to obtain the serum by using centrifuge (Rotanta, 96R model). The serum levels of glucose, high density lipoprotein, low density lipoprotein, urea, creatine

kinase, triglyceride and total cholesterol, content were determined by spectroscopy (Cobas 6000/Cobas C 501, Tokyo Japan). Lactate dehydrogenase level was determined by spectroscopy (BT 2000 plus, biotechnical instruments, Germany).

Figure 8. Blood collecting directly from the heart of the mice

3.9. Reagents

Assay kits used to determine blood chemistry were triglyceride (TRIGL Kit), urea (UREAL Kit), blood glucose (GLUC2 Kit), cholesterol (CHOL2 Kit), high density lipoprotein (HDLC3 Kit), low density lipoprotein (LDL-C plus 2nd generation Kit), lactate dehydrogenase (LDH-L Kit) and creatine kinase (CKL Kit).

3.10. Muscle Density Measurement

Mice (n =12 per group test) were orally administered hot water fish extracts (HF and BF), octacosanol (positive control), and normal saline (negative control) as described above. One hour after the last administration, mice were humanely euthanized and the skin was peeled off. Then, forelimb and hindlimb were disarticulated from scapula to carpus and from ilium to medial malleolus, respectively (Fig 9). They were quickly weighed and the volume was measured in 10 ml syringe. After boiling for 1 minute to enable muscles to be cleaned easily, only the bone was weighed. The volume was taken for both the muscle and bone (Fig 10)[76]. Then, the forelimb and hindlimb muscle density were expressed in g/cm^3.

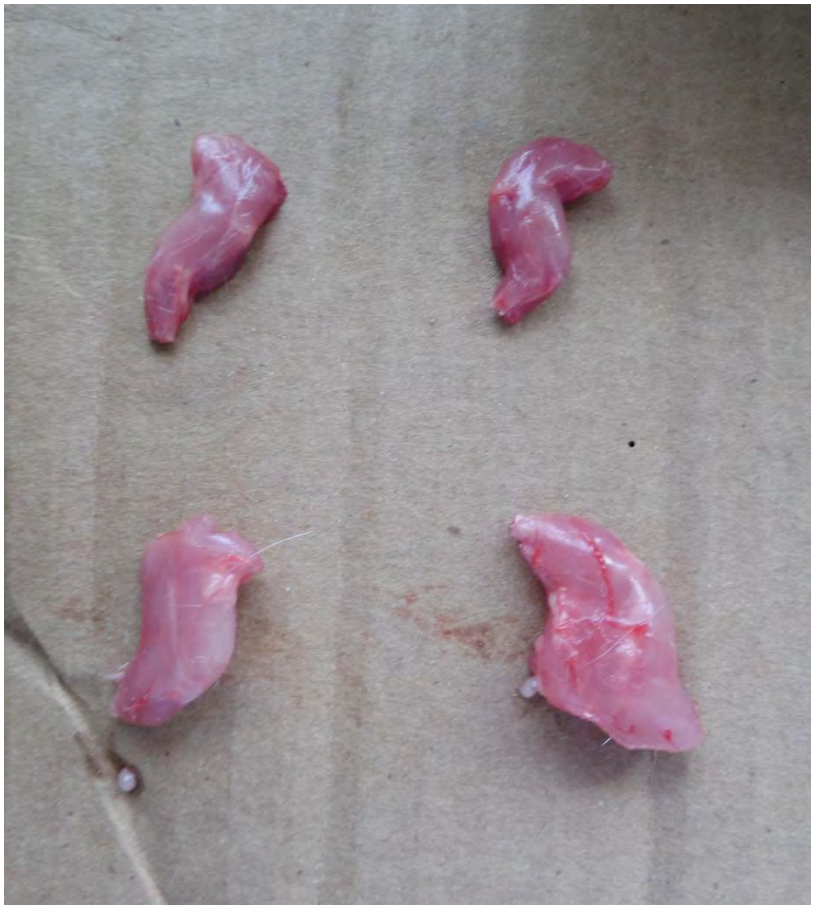

Figure 9. Forelimb (upper-left and right) and hindlimb (lower-left and right) muscle of the mouse

$$\text{Muscle density } (g/cm^3) = \frac{\text{Attached muscle \& bone mass (g)- bone mass (g)}}{\text{Attached muscle \& bone volume } (cm^3) - \text{bone volume } (cm^3)}$$

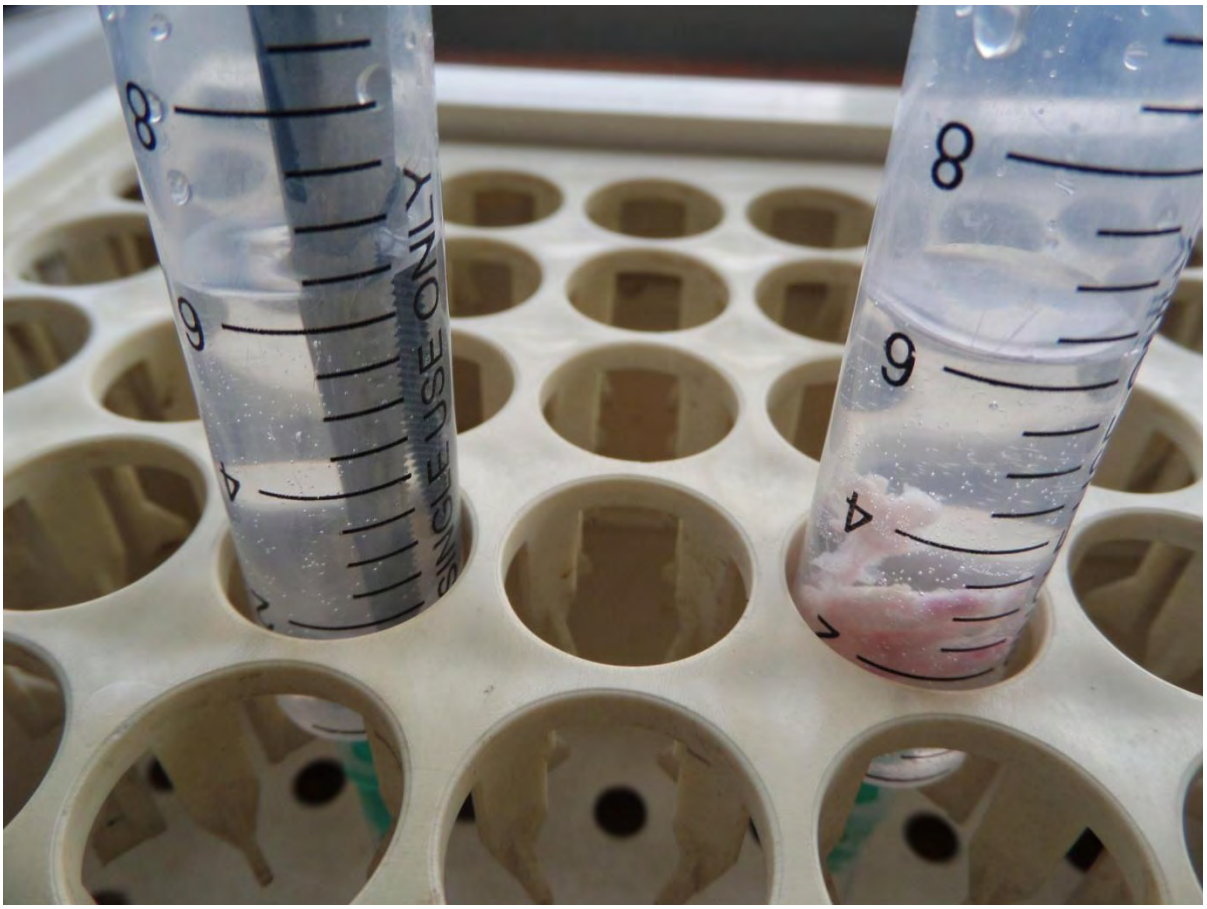

Figure 10. Forelimb and hindlimb muscle and bone volume measurement in a water filled syringe

3.11. Statistical Analysis

Statistical analysis was performed using student's t-test. All of the animal experiments were done with a minimum of 12 mice per group. Values are reported as mean $\pm$ standard error (SE).

4. Result

a) Body weight

Mice fed with the Koroso fish extracts (HF and BF) and Octacosanol (OC) had no significant difference on their body weight when compared with saline group (NS) in all the experimental days. Also there was no significant difference in body weight within HF, BF and OC at the 3rd and 7th day compared with day 0 (Fig 11 and Fig 12).

b) Grip strength

Mice fed with the Koroso fish extracts (HF and BF) and Octacosanol (OC) had significantly greater forelimb grip strength HF 1.038 ± 0.03 N (130.07%), BF 1.032 ± 0.05 (129.32%) N and OC 1.09 ± 0.04 (136.6%) N at ($P<0.001$) on day 3 compared with their performance with normal saline administered mice (Fig 14). Similarly, on the 7th day the forelimb grip strength of HF, BF and OC treatments was 1.192 ± 0.03 N (133.93 %), 1.215 ± 0.05N (136.52%) and 1.383 ± 0.05 N (155.4%) respectively, which is significantly different from the grip performance by saline fed mice (Fig 14) at ($P<0.001$) (0.798 ± 0.04 N on day 3 and 0.890 ± 0.03 N on day 7).

Similarly, day 3 and 7 grip strengths for HF, BF and OC were (1.038 ± 0.03, 1.192 ± 0.03)N, (1.032 ± 0.05, 1.215 ± 0.05)N and (1.090 ± 0.04, 1.383 ± 0.05)N respectively. These values are significantly different by (HF 148.71%, 170.77%), (BF 143.73%, 169.225%) and OC (155.05%, 196.73%) on day 3 and day 7 respectively at $P<0.001$ compared with the grip strength recorded at day 0 (HF 0.698 ± 0.03) N, (BF 0.718 ± 0.03) N and OC (0.703 ± 0.03) N (Fig 13).

c) Swimming Endurance

Following oral administration of hot water extracts of fish in mice, swimming time averaged for HF (154.8 ± 17.10 (*P<0.001*), 187.6 ± 13.63 (*P<0.001*)) sec, BF (155.4 ± 12.07 (*P<0.001*), 164.25 ± 17.49 (*P<0.05*)) sec and OC (160.1 ± 14.45 (*P<0.001*), 178.25 ± 13.14 (*P<0.001*)) sec on day 3 and 7 respectively. The averaged time recorded in the extract group and positive control was significantly higher than the values recorded for the saline group mice at 3rd and 7th day (Fig16). The percentage increment of the swimming time for (HF, BF and OC was (171.52%, 152.4%), (172.19%, 133.43%), (177.4%, 144.8%) on day 3 and 7 respectively compared with the averaged time recorded for the normal saline group, i.e. (90.25 ± 13.01, and 123.1 ± 13.19) sec on day 3 and 7 respectively (Fig 16).

Similarly, mice orally administered with fish extracts and octacosanol showed higher averaged swimming time (HF 154.8 ± 17.10, 187.6 ± 13.63) sec, (BF 155.4 ± 12.01, 164.25 ± 17.49) sec and (OC 160.1 ± 14.45, 178.25 ± 13.14) sec on day 3 and 7 respectively than day zero at *P<0.001*. These values represented a higher percentage increments of swimming time for HF, BF and OC (160.41%, 194.4%), (190.67%, 201.53%) and (165.48%, 184.24%) on day 3 and 7 respectively compared with the recorded time at day 0 (HF 96.5 ± 14.76 sec, BF 81.5 ± 14.41 sec and OC 96.75 ± 13.63 sec)(Fig 15).

d) Muscle density

Mice orally administered with HF, BF and OC were found with increased forelimb muscle densities of (1.354 ± 0.05, 1.4 ± 0.05, 1.29 ± 0.03) g/cm^3 at (*P<0.001*) than normal saline fed mice (Fig 17). These represented a percentage increment of (131.5%, 135.9%, 125.2%) from the saline fed mice respectively (Fig 17). Similarly, hindlimb muscle density for the HF, BF

and octacosanol administered mice was (1.53 ± 0.04, 1.5 ± 0.05and 1.36 ± 0.03)g/cm^3. These represented (137.5%, 134.8%, 122.2%) significant increment at *P<0.001* than saline administered group (forelimb and hindlimb muscle densities;1.03 ± 0.03 g/cm^3, 1.113 ± 0.02 g/cm^3 respectively)(Fig 17).

e) Biochemical tests

To investigate the antifatigue or fatigue recovery effects of Koroso fish hot water extracts, blood samples for biochemical analysis were collected at 30 min after physical trials on day 7. Accordingly, one of the parameters tested, blood glucose level in HF, BF and OC fed mice were averaged (207.48 ± 20.63, 216.75 ± 23.71, 175.85 ± 8.84)mg/dL (P<0.05) which were (161.34% (*P<0.001*), 168.54% (*P<0.001*), 136.74% (*P<0.05*)) significantly higher than saline fed mice group (128.6 ± 22.98 mg/dL)(Table 5 and 6) respectively. Though not statistically significant, lactate dehydrogenase level in HF, BF and OC fed mice were averaged (1553.27 ± 96.4, 1421.86 ± 139.59, 1594.12 ± 103.28) U/L respectively. These values showed a percentage increment by (118.74%, 108.7%, 121.87%) compared with the level in normal saline administered mice (1308.2 ± 160.9) U/L (Table 5 and 6). With similar fashion, High Density Lipoprotein (HDL) level in HF, BF and OC (77.62 ± 11.73, 71.61 ± 9.17, 90.23 ± 4.43) mg/dL respectively. This marked a significant increment by (154.77% (*P<0.05*), 142.79% (*P<0.001*), 179.92% (*P<0.001*)) compared with the level recorded with normal saline fed group (50.15 ± 12.12) mg/dL (Table 5 and 6).

In contrast, urea level in HF, BF and OC were averaged with lower values (57.40 ± 6.31, 57.44 ± 3.48, 52.64 ± 2.29)mg/dL, which represented 90.85%, 90.91% and 83.32% decrement from the value found in the normal saline administered mice group (63.18 ±

6.76)mg/dL (Table 5 and 6). Similarly, creatine kinase level in HF, BF and OC orally administered mice were (938.7 ± 150.7, 885.2 ± 358.14, 155.3 ± 22.03)U/L which represented HF 79.21%, BF 74.69% and OC 13.1% significantly lower value than the level found in saline fed mice (1185.1 ± 272.36) U/L at (*P<0.001*) (Table 5 and 6). Low Density Lipoprotein level in HF, BF and OC was (35.24 ± 4.09, 26.17 ± 3.96 and 34.03 ± 2.93) mg/dL respectively. Interestingly, these values represented a 96.41%, 71.6%, 93.1% lower value than in normal saline fed mice (36.55 ± 4.63) (Table 5 and 6). A significantly decreased triglyceride level was averaged in HF, BF and OC administered mice (97.24 ± 6.38, 94.31 ± 9.33, 84.57 ± 3.8)mg/dL at *P<0.001* respectively. These lower values represented 68.26%, 66.2%, 59.37% decreased levels compared with the average values in the saline fed mice group (142.45 ± 11.2) mg/dL (Table 5 and 6) . Similarly, total cholesterol in HF, BF and OC was averaged (109.83 ± 10.2, 109.06 ± 8, 110.66 ± 4.33)mg/dL; which represented 92.57%, 91.92%, 93.26% lower values than recorded in saline group (118.65 ± 5.11 mg/dL) (Table 5 and 6)

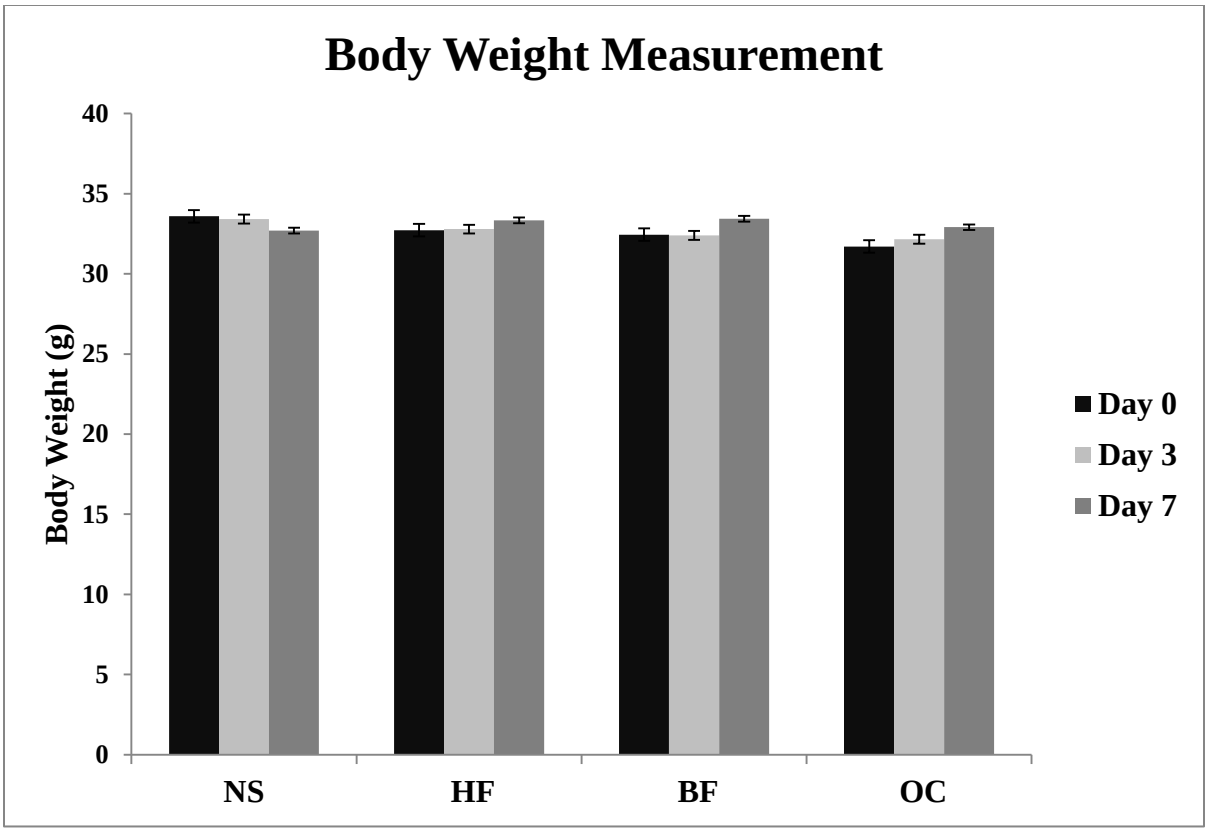

Figure 11. Effect of Koroso fish extracts (HF and BF) on body weight. Mice underwent body weight measurement, and orally administered the extracts once per day for 7 days at a dose of 0.4mg/10µl protein concentration for 1g of body weight. Octacosanol the positive control was given at a dose of 3.33µg/10µl for 1g of body weight. Data are presented as the mean $\pm$ SE (n =12) compared with day 0 (within a group).

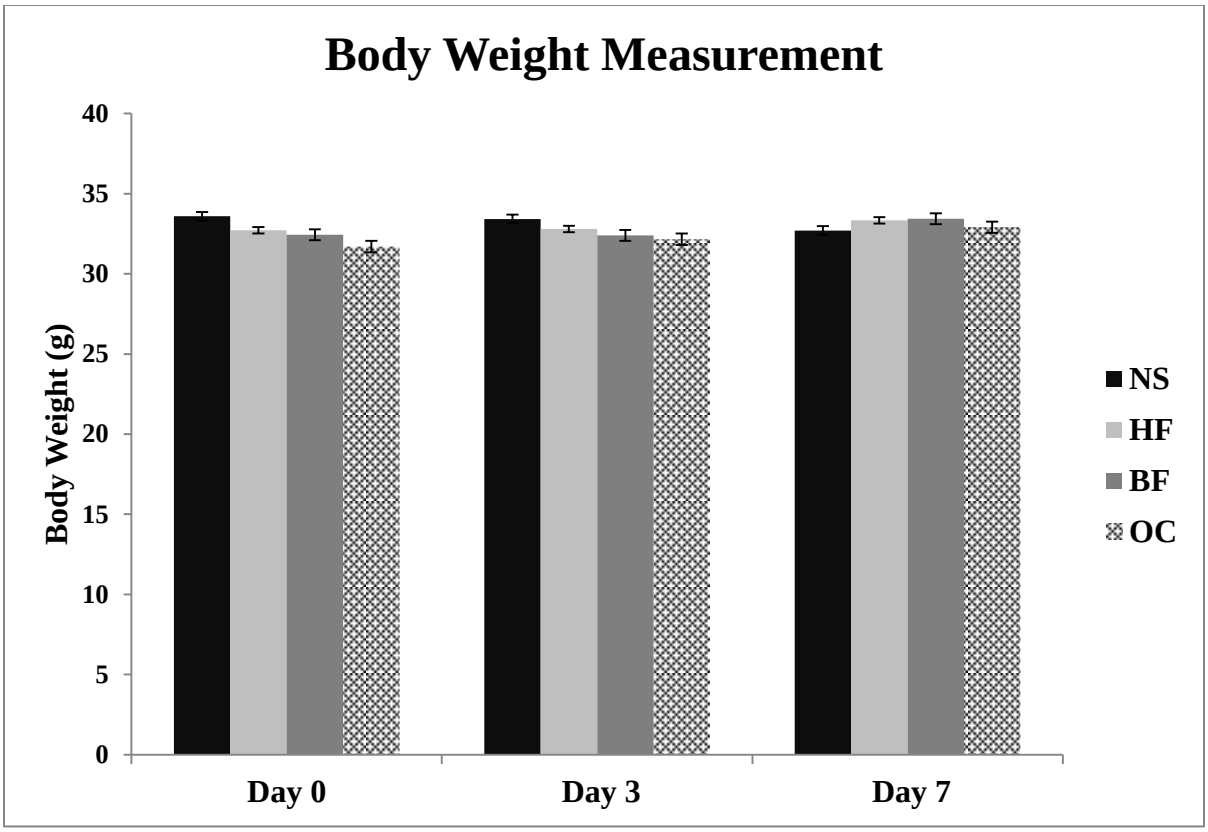

Figure 12. Effect of Koroso fish extracts (HF and BF) on body weight. Mice underwent body weight measurement, and orally administered the extracts once per day for 7 days at a dose of 0.4mg/10µl protein concentration for 1g of body weight. Octacosanol the positive control was given at a dose of 3.33µg/10µl for 1g of body weight. Data are presented as the mean $\pm$ SE (n =12) compared with NS (between the groups).

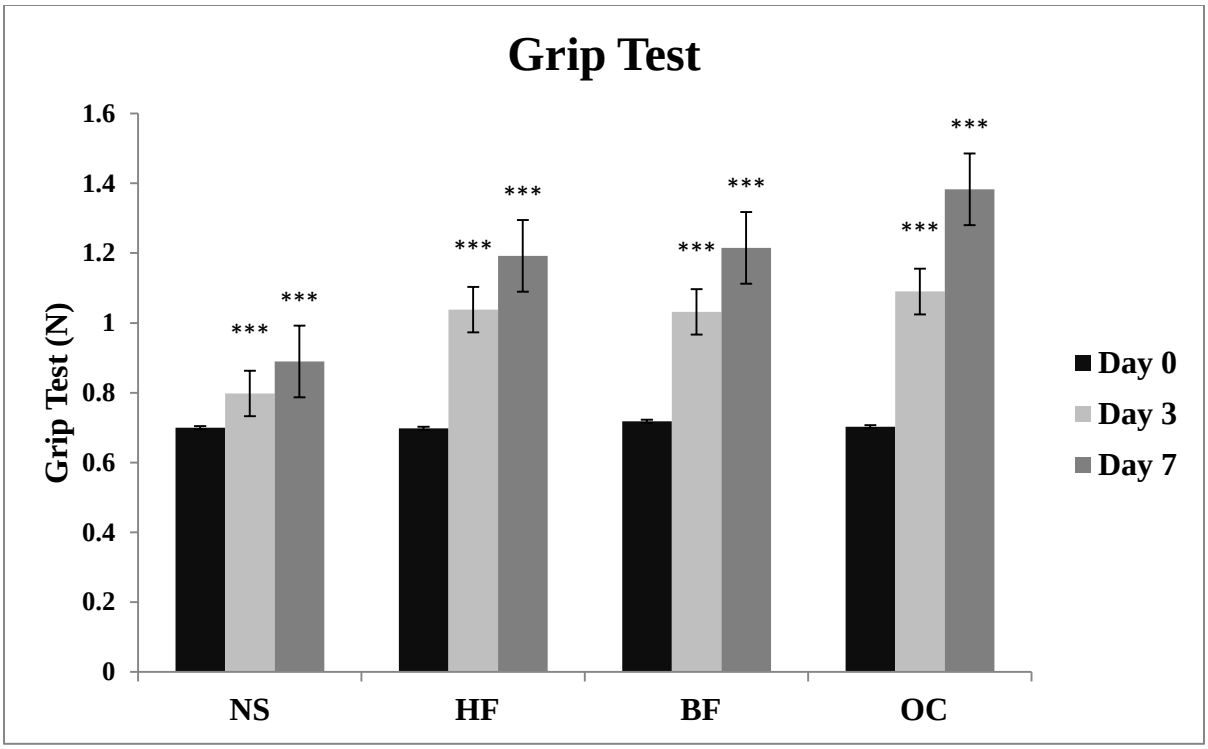

Figure 13. Effect of Koroso fish extracts (HF and BF) on forelimb grip strength. Mice were orally administered the extracts once per day for 7 days at a dose of 0.4mg/10µl protein concentration for 1g of body weight, and underwent the forelimb grip test 1 hour after administration. Octacosanol the positive control, was given at a dose of 3.33µg/10µl for 1g of body weight. Data are presented as the mean $\pm$ SE (n =12) ***$p<0.001$ compared with 0 days (within a group).

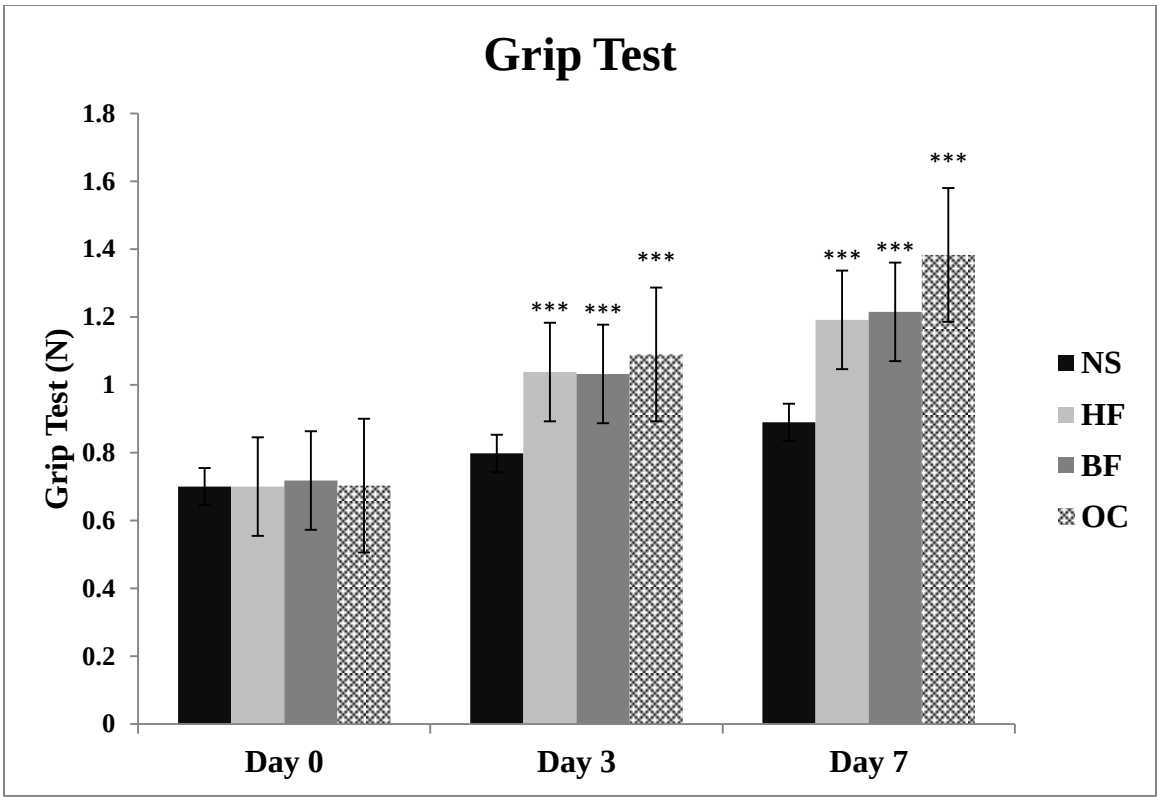

Figure 14. Effect of Koroso fish extracts (HF and BF) on forelimb grip strength. Mice were orally administered the extracts once per day for 7 days at a dose of 0.4mg/10µl protein concentration for 1g of body weight, and underwent the forelimb grip test 1 hour after administration. Octacosanol the positive control, was given at a dose of 3.33µg/10µl for 1g of body weight. Data are presented as the mean $\pm$ SE (n =12) $^{***}p<0.001$ compared with NS (between the groups).

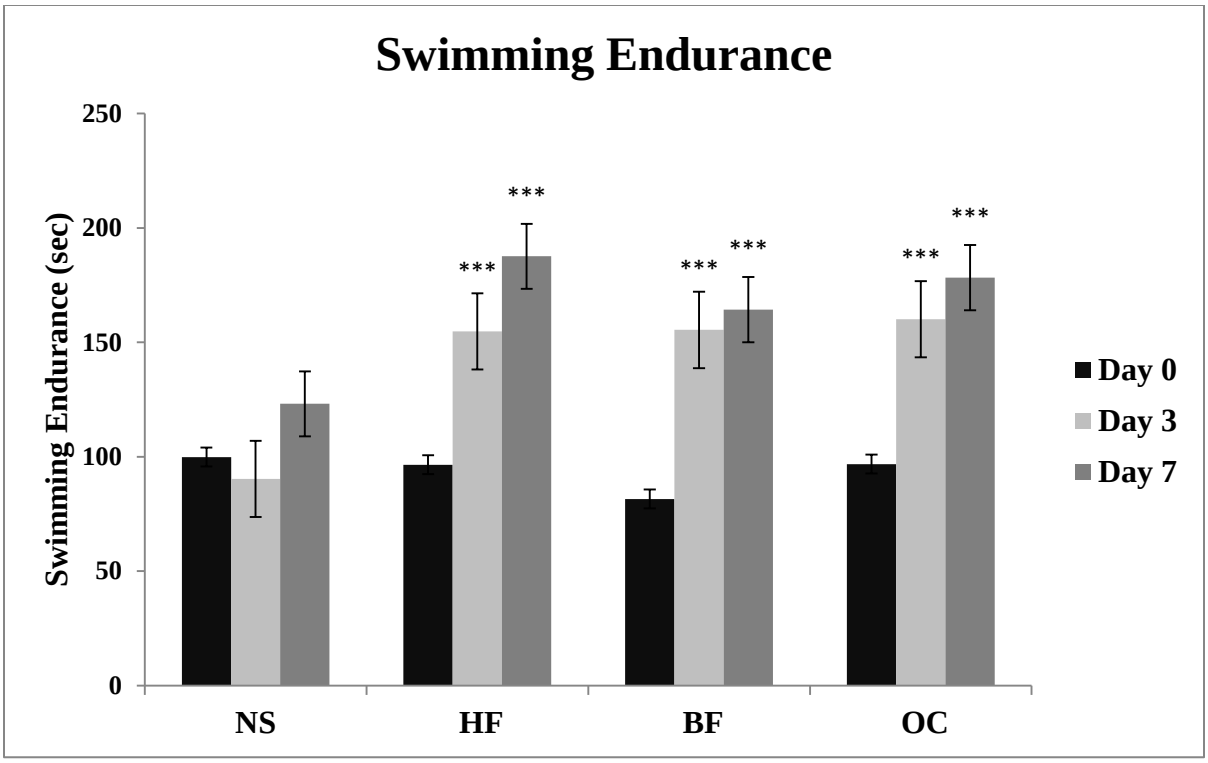

Figure 15. Effect of Koroso fish extracts (HF and BF) on swimming endurance. Mice were orally administered the extracts once per day for 7 days at a dose of 0.4mg/10µl protein concentration for 1g of body weight, and underwent the swimming endurance test after grip test. Octacosanol the positive control was given at a dose of 3.33µg/10µl for 1g of body weight. Data are presented as the mean ± SE (n =12) **p<0.05, ***p<0.001* compared with 0 days (within a group).

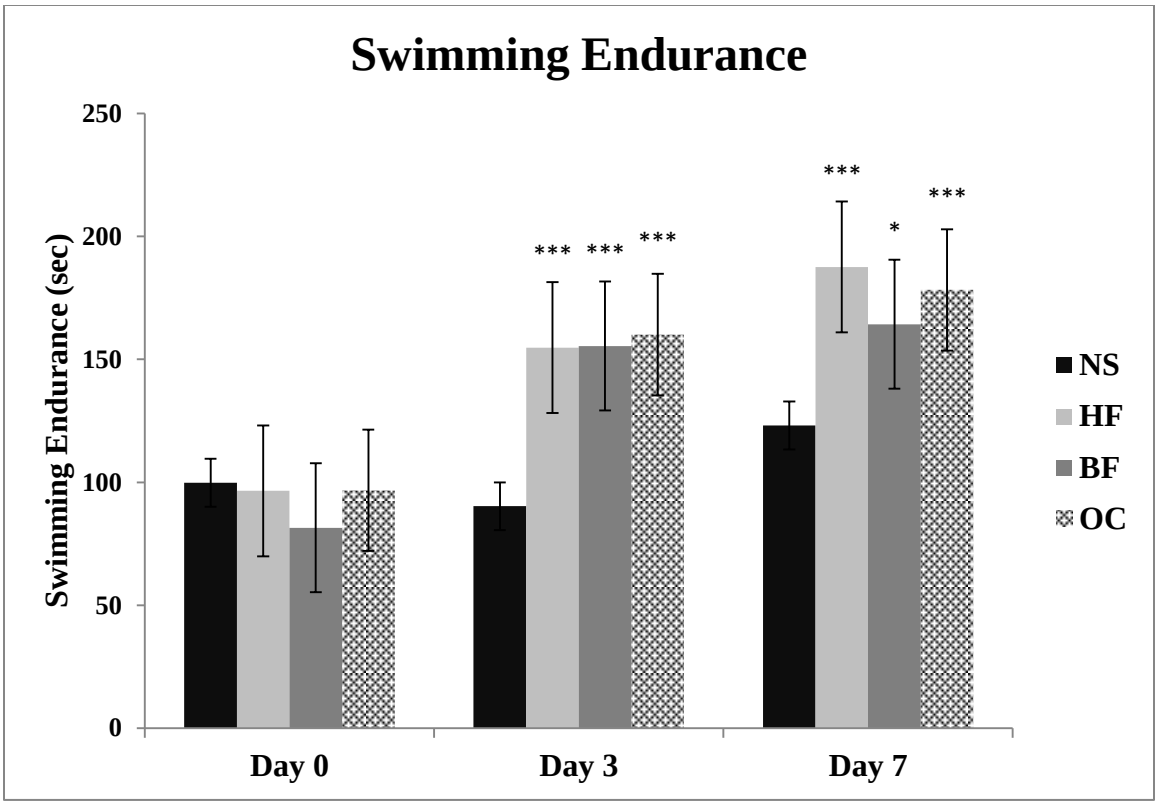

Figure 16. Effect of Koroso fish extracts (HF and BF) on swimming endurance. Mice were orally administered the extracts once per day for 7 days at a dose of 0.4mg/10µl protein concentration for 1g of body weight, and underwent the swimming endurance test after grip test. Octacosanol the positive control was given at a dose of 3.33µg/10µl for 1g of body weight. Data are presented as the mean $\pm$ SE (n =12) *$p<0.05$, ***$p<0.001$ compared with NS (between the groups).

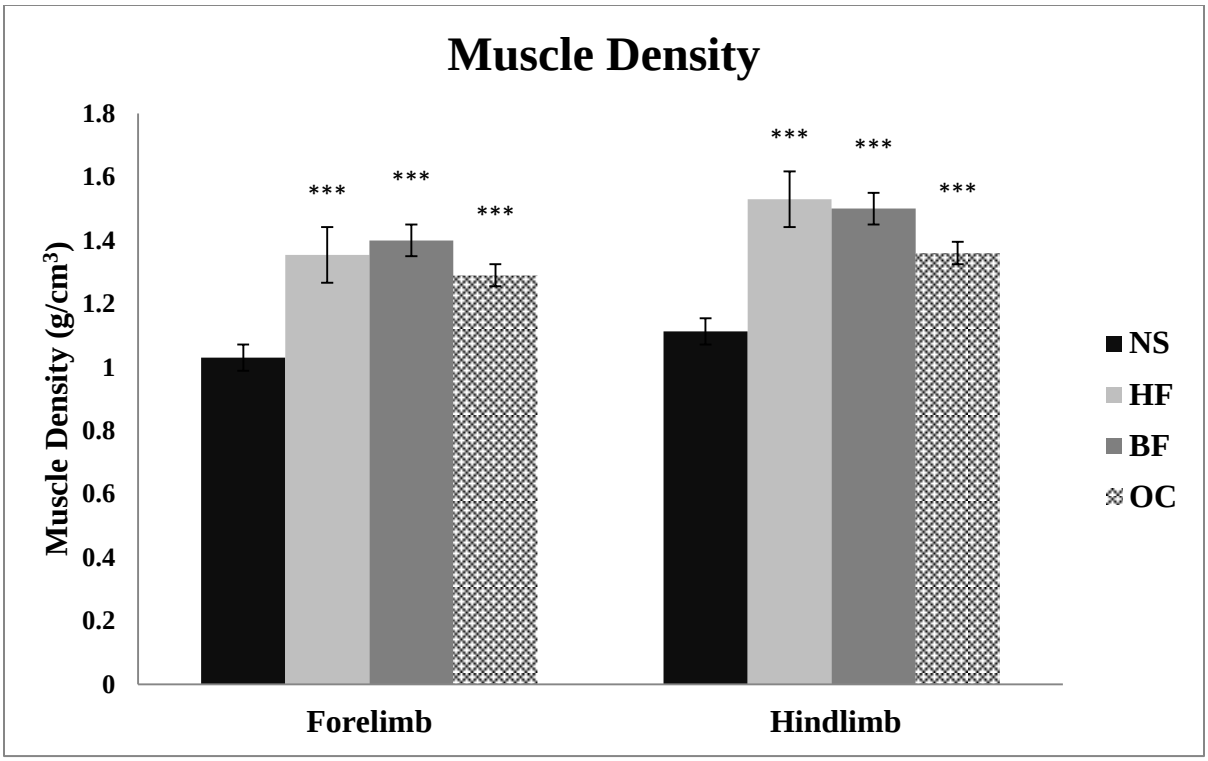

Figure 17. Effect of Koroso fish extracts (HF and BF) on Forelimb and Hindlimb muscle density. Mice were orally administered the extracts once per day for 7 days at a dose of 0.4mg/10µl protein concentration for 1g of body weight, and underwent the muscle density measurement after 1 hour ending all experiment. Octacosanol the positive control was given at a dose of 3.33µg/10µl for 1g of body weight. Data are presented as the mean $\pm$ SE (n =12) ****p<0.001* compared with NS (between the groups).

Table 2. Effect of Koroso fish extract (HF) on serum glucose, LDH, urea, triglyceride, HDL, LDL, cholesterol and creatine kinase 1 hour after the experiment.

	Glucose (mg/dL)	LDH (U/L)	Urea (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Cholesterol (mg/dL)	Creatine kinase (U/L)
Normal saline	128.60 ± 22.98	1308.08 ± 152.64	63.18 ± 6.76	142.45 ± 11.72	50.15 ± 12.12	36.55 ± 4.63	118.65 ± 5.11	1185.10 ± 272.37
Hawassa fish	207.48 ± 20.63***	1553.27 ± 96.40	57.40 ± 6.31	97.24 ± 6.38***	77.62 ± 11.73*	35.24 ± 4.09	109.83 ± 10.20	938.70 ± 150.71
Relative activity (%)	161	119	90	68	154	96	92	79
Octacosanol	175.85 ± 8.84*	1594.12 ± 103.29	52.64 ± 2.29	84.57 ± 3.80***	90.23 ± 4.43***	34.03 ± 2.93	110.66 ± 4.33	155.30 ± 22.03***
Relative activity (%)	137	122	83	59	180	93	93	13

Glucose, LDH, urea, triglyceride, HDL, LDL, cholesterol and creatine kinase. Values are the mean ± SE (n =12) *$p<0.05$ and ***$p<0.001$ compared NS control (between the groups).

Relative activities are expressed as a percentage of the values against the saline control group (NS=100%).

Table 3. Effect of Koroso fish extract (BF) on serum glucose, LDH, urea, triglyceride, HDL, LDL, cholesterol and creatine kinase 1 hour after the experiment.

	Glucose (mg/dL)	LDH (U/L)	Urea (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Cholesterol (mg/dL)	Creatine kinase (U/L)
Normal saline	128.60 ± 22.98	1308.08 ± 152.64	63.18 ± 6.76	142.45 ± 11.72	50.15 ± 12.12	36.55 ± 4.63	118.65 ± 5.11	1185.10 ± 272.37
Bahir Dar Fish	216.75 ± 23.71***	1421.86 ± 110.92	57.44 ± 3.49	94.31 ± 9.33***	71.61 ± 9.17	26.17 ± 3.96*	109.06 ± 7.99	885.20 ± 358.15
Relative activity (%)	169	109	90	66	143	72	92	75
Octacosanol	175.85 ± 8.84*	1594.12 ± 103.29	52.64 ± 2.29	84.57 ± 3.80***	90.23 ± 4.43***	34.03 ± 2.93	110.66 ± 4.33	155.30 ± 22.03***
Relative activity (%)	137	122	83	59	180	93	93	13

Glucose, LDH, urea, triglyceride, HDL, LDL, cholesterol and creatine kinase. Values are the mean ± SE (n =12) $*p<0.05$ and $^{***}p<0.001$ compared to NS control (between the groups).

Relative activities are expressed as a percentage of the values against the saline control group (NS=100%).

5. Discussion

In this study, mice were administered orally with hot water extracts of Koroso fish collected from Hawassa and Bahir Dar sites. The extracts significantly improved physical exercise performance, forelimb and hindlimb muscle density, swimming endurance time, forelimb grip strength and blood biochemical parameters in the mice. Hence, indicated potential reduction in fatigue development. Exercise induced physical fatigue can be evaluated by physical exercise performance or endurance. Forelimb grip strength and swimming endurance tests are common experimental models to evaluate physical fatigue and physical exercise improvement. In this study, results showed that grip strength increased and swimming times prolonged significantly in mice fed with Lake Hawassa (HF) and Lake Tana (BF) Koroso fish hot water extracts compared with saline control (NS) group. Similarly, HF, BF and octacosanol orally administered mice showed increased grip strength and prolonged swimming times significantly, on day 3 and day 7, compared with the values in day 0.

In contrast, there was no significant body weight gain in both extracts and octacosanol fed mice compared with normal saline fed mice. Also, there is a slight increment in body weight of both extracts fed and octacosanol groups on day 7, but no significant difference in body weight was observed in both extracts and octacosanol fed groups, on day 3 and day 7. Similarly, [11,76] reported no significant change in body weight in mice fed with hot water extracts of soft shell turtle and leather carp respectively.

The homeostasis of blood glucose, a breakdown product of skeletal and liver glycogen, plays an essential role during prolonged and strenuous exercise [60]. Exhaustive exercise usually

results in hypoglycemia, which can suppress brain activity. Hence the speed and degree of fatigue development can be explained by blood glucose levels [61]. Therefore, glucose is an acceptable index to evaluate fatigue level in animals. In the present study, those mice orally administered with Koroso fish extracts showed a significant increase in the blood glucose levels compared with the control (NS). This increase in blood glucose levels may be one pathway of extracts to improve physical exercise performance and mediated anti-fatigue effect.

The other blood parameter tested, lactic acid causes extreme fatigue and it has to be reconstituted. Lactic acid converted back into pyruvic acid with the help of lactate dehydrogenase enzyme and then metabolized oxidatively by all the body tissues. The HF and BF extracts and octacosanol increased the activity of Lactate Dehydrogenase (LDH), allowing the animals to regain energy after workout.

High intensity exercise could physically or chemically cause tissue damage. It can also cause sacromeric damage and muscular cell necrosis [62]. Then, cells release specific proteins such as Creatine Kinase (CK) and myoglobin into the blood as muscular damage indexes. Clinically, CK is assayed in the blood tests as a marker of myocardial infarction, rhabdomyolysis (severe muscle breakdown), muscular dystrophy, autoimmune myositides, and acute renal failure [61]. In this study, serum CK level was significantly higher in NS than HF, BF and OC administered mice. This indicated the potential of the extracts and octacosanol to improve physical exercise performance.

Blood urea nitrogen is another biochemical index associated with physical fatigue. It is formed in the liver as the end product of protein and amino acid metabolism. The urea level reflects kidney function, although many other factors affect its level, including protein breakdown, dehydration, stress and fatigue [63]. After prolonged physical activity, blood urea level normally will tend to increase. Upon giving mice HF and BF fish extracts, blood urea levels remained low compared to the control group (NS). This well-marked that the fish extracts potential to prevent protein catabolism.

Regarding blood lipid profile, our results showed that the fish extracts resulted in significant increase in HDL and significant reduction in LDL, TG and total cholesterol. The fish extracts enhanced the exercise capacity of mice, perhaps in part, by increasing fat utilization via decreasing triglyceride levels in the blood. Although the mechanism by which the extract decreased triglyceride and cholesterol levels are unclear, the effect might be beneficial during extended exercise, because better utilization of triglyceride might spare glycogen and glucose [64,65]. As a result the extracts improved the physical exercise performance and prevented the onset of fatigue.

In general, muscle weakness is clearly associated with lower muscle mass and muscle quality is an important indicator of muscle function [66]. Hormone supplementation, nutritional interventions, and strength and exercise training are most often used to increase muscle mass and strength [67-70]. Perhaps, these factors may decrease the amount of fat infiltration into the muscle [68-71]. Low muscle density has been associated with greater risk of functional limitation [72], poorer strength [73], and worsen metabolic function [74]. In this study, the

extract fed groups and the positive control group were found with greater muscle density than the control group (NS).

6. Conclusion

In this study, the extraction of Koroso fish from two different lakes has been investigated their exercise performance improvement and anti-fatigue effect. One is from Lake Hawassa and the other is from Lake Tana. And the results indicated that both extractions presented a positive effect on exercise performance improvement and the fatigue related parameters when compared to the negative control group.

This result may come from the presence of protein, terpenoids, polysaccharides and antioxidative property of the hot water extracts of Koroso fish.

7. Recommendation

However, further investigation is needed for more details on the components of the extracts, and the relationship between the components of the extract, exercise performance improvement ability and anti-fatigue activity. It is also important to see the glutathione peroxidase (GPx) and superoxide dismutase (SOD) antioxidant enzyme of mice blood. GPx and SOD were not available in Ethiopia to include in this study.

According to this research results indication, it is recommended to use hot water extract of Koroso fish as a tonic soup for individuals who are vulnerable to fatigue like patients, elders etc. It is also recommended to use as a tonic soup for professional athletes and physical exercise performer.

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Addis Ababa University

College of Natural Science

Center for Food Science and Nutrition

RESEARCH THESIS APPROVAL

This is to certify that the thesis prepared by Anley Teferra, entitled: *Exercise Performance Improvement and Anti-Fatigue Effect of Hot Water Extract of Koroso fish (Oreochromis niloticus)* and submitted in partial fulfillment of requirements for the degree of Master of Science in food science and nutrition complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the College of Graduate Studies (CGS) of the candidate's major department.

I hereby certify that I have read this thesis prepared under my direction and recommend that it is accepted as fulfilling the thesis requirement.

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