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TETRACARPIDIUM CONOPHORUM EXTRACT EXHIBITS ANTI-FATIGUE ACTIVITY IN RATS VIA REDUCED PROTEIN CATABOLISM, INCREASED ANTIOXIDANT STATUS AND DELAYED LACTATE ELEVATION

[1]Ugochukwu Vincent Igbokwe, [2*]Ejike Daniel Eze, [3]Moses Dele Adams, [4]Karimah Mohammed Rabiu, [5]Iliya Ezekiel, [6]Prisca Ojochogu Ajeka, [7]Adam Moyosore Afodun

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[2] Department of Physiology, School of Medicine, Kabale University, Uganda.
[3] Department of Biochemistry, Faculty of Computing and Applied Sciences, Baze University, Abuja, Nigeria.
[4]Department of Biological Sciences, Faculty of Science, Yobe State University, Damaturu, Yobe, Nigeria.
[5]Department of Biological Sciences, Faculty of Pure and Applied Sciences, Federal University, Wukari, Nigeria.
[6]Department of Human Kinetics and Sports Science, Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.
[7]Department of Anatomy, Busitema University, Uganda.

[*]Corresponding Author's Email: edeze@kab.ac.ug

ABSTRACT
Thirty rats of both sexes were assigned into 5 categories of six animals apiece. Animals in the unadministered (control) group were placed on distilled water. Group 1M and Group 1F animals were administered 500 mg/kg body weight (b.w) of *T. conophorum* aqueous nut extract whereas animals in Group 2M and Group 2F were administered 750 mg/kg dosage of the extricate (0.5 ml) orally once daily for 32 days. Phytoconstituents present in the extract include: saponins, flavonoids, tannins, phenols and alkaloids. The extract at 750mg/kg b.w notably ($p<0.05$) raised extracellular glucose in masculine rats when matched with males that received 500 mg/kg b.w. The 500 mg/kg dose of the extract appreciably ($p<0.05$) elevated BUN in both sexes, but with reduction in both groups at 750 mg/kg b.w when juxtaposed with their respective untreated animals. The extract at 500 mg/kg b.w increased LDH activity in male group when compared with male rats that received 750 mg/kg dose. The 750-extract dosage did not statistically ($p>0.05$) alter LDH activity in both sexes. The extract at 500 and 750mg/kg b.w increased the 3rd–6th swim in male rats. Substantive ($p<0.05$) rise in swimming endurance time was first noticed at the 2nd swim when matched up with the control and group treated 500 mg/kg b.w, in female rats. Sequel to these research findings, it is hypothesized that the anti-weakness effect of *T. conophorum* might be adduced to delayed increase in lactate and reduction in protein catabolism.

Keywords: Fatigue; *Tetracarpidium conophorum*; Euphorbiaceae; Swim endurance time; Lactate.

INTRODUCTION

Fatigue is a complex phenomenon characterized by inability to sustain required muscular status, leading to reduced work output during prolonged physical activity (Fang *et al*., 2011). It describes a physical and/or mental state of being weak or tired (Gandevia, 2001). It is evident by reduced muscular output and exercise endurance owing to accumulated biomolecules (Bogdanis, 2012; Finsterer, 2012; Chae *et al*., 2015). Currently, Stress from work and life generally, are on the increase following continuous and unending needs and/or demands of life. Fatigue may therefore appreciably reduce daily work output. Because available treatment regime for fatigue cases in modern medicine are inadequate, coupled with its attendant shortcomings, the antifatigue effect of medicinal plants that can prevent and/or manage the disease is imperative (Tharakan *et al*., 2005; Ren *et al*., 2011; Shao *et al*., 2013). One potential plant is *Tetracarpidium conophorum*.

T. conophorum (*Euphorbiaceae*) (Mull. Arg.) Hutch. & Dalziel is commonly called African walnut or Nigerian walnut or Black walnut or Conophor (Gbile and Adesina, 1981; GRIN, 2010). In Nigerian dialects, the plant is mostly called "*Aduruku*" or "*Gawudi bairi*" in Hausa; "*Ukpa*'' in Igbo; "*Okhue*" or "*Okwe*" in Edo; "*Epkoro*" by the Efiks as well as Ibibios; "*Asala*" or "*Awusa*" in Yoruba language. In Africa, as "*Kaso*" or *Ngak*" in the coastal and westward Cameroun (Dalziel, 1937; Ogunsua and Adebona, 1983, Ojobor *et al*., 2015). As a climbing shrub, the plant completes its life cycle in three years. It is 10 – 20 ft in height, and easily seen in sub-Saharan Africa (forest zones). It enjoys geographical spread from southern and eastern part of Nigeria (Ayoola *et. al*., 2011), where the consumption of this seeds is ubiquitous. It is an unending climber located both in

disinfectant, mostly lethal to bacteria and pathogens and moisture-laden forest regions of sub-Sahara Africa (Adebona *et al*., 1988). As a core protein source, it is chiefly ingested as nuts that are prepared by exposing their innermost peptide bond easily accessible to chewing, and often employed to denature substances (Ajaiyeoba *et al*., 2006; Edem *et al*., 2009). Walnuts have been found useful as antiulcer agent (Ezealisiji *et al*., 2014b; Anosike *et al*., 2015), safe edible snack (Edet *et al*., 2009; Udedi *et al*., 2013; Nwabunnia and Otogbor, 2015), male fertility agent (Gadekar *et al*., 2010; Obianime and Uche, 2010; Akpan and Anietie, 2014; Akomolafe *et al*., 2015), pharmaceutical formulation (Chikezie, 2017), antimicrobial activity (Ajaiyeoba and Fadare, 2006; Ogbolu and Alli, 2012; Bello *et al*., 2013), antioxidant status (Amaeze *et al*., 2011), non-toxic effect (Akomolafe *et al*., 2017), antihyperglycaemic agent (Onwuli *et al*., 2014; Nwaichi *et al*., 2017), nutritional agent (Kanu *et al*., 2015; Ojobor *et al*., 2015), injury recovery action (Ezealisiji *et al*., 2014a), anti-hypercholesterolemic effect (Ezealisiji *et al*., 2016; Oriakhi and Uadia, 2016), antiplasmodial activity (Dada and Ogundolie, 2016), functional protein (Gbadamosi *et al*., 2012).

Scientific confirmation of the anti-fatigue effect of *Tetracarpidium conophorum* nuts included Kim and Kim (2013) who evaluated the anti-fatigue action at 300, 600 and 900 mg/kg body weight, Fang *et al* (2018) of fractions at 200, 400 and 800 mg/kg body weight, Willis *et al* (2009) of diet at 2, 6 and 9% and Chang *et al* (2013) on current input of 500 Hz, 1024 Hz and 2048 Hz on pulse status. Despite these studies, there is little or no available data, as far as we know, in the open online journals on the anti-fatigue nature of the flora extricate at the ethnobotanically derived dosage levels of 500 and 750 mg/kg body weight. This investigation was therefore carried out to supply details on the likely anti-fatigue mode of action of aqueous extricate of *Tetracarpidium conophorum* nuts in experimental models.

MATERIALS AND METHODS

Materials

Plant Materials and Authentication

Freshly boiled nuts derived from *Tetracarpidium conophorum* were acquired amid Nkwo Nnewi market at Otolo region in Anambra State, Nigeria. The nuts were identified and confirmed botanically by Mr. A. Odewo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, with an identification number (FHI107515) designated to it at their botanical garden.

Animal Husbandry

Thirty (30) healthy albino rats comprising 15 males (157 162g) and females (142-168g) respectively were collected from an animal farm at Nnamdi Azikiwe University Nnewi campus, Anambra State, Nigeria. The experimental animals were kept at (a temperature of 25 ^{0}C; light/dark cycle of 12h) enjoying proximity to pellet as well as drinking water, for a period of fourteen days before the onset of the investigation, to get used to the new environment. The swimming times of all rats were measured before walnut extract administration. The animals were well positioned in rectangular plastic cages with holes followed by maintenance of good environmental hygiene.

METHODS

Ethical Approval

Ethical approval was requested and obtained from the Investigation Ethical Appraisal Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria (NAUTH/CS/66/Vol. 2/149) in a letter dated March 17, 2017.

Preparation of Aqueous Extract of *Tetracarpidium conophorum* Nut

The fresh nuts which were cracked open, sliced were dried under room temperature and milled. Fifty gram measured from the dried walnut material was macerated into 250 ml of luke warm ionized water. The mixture was shaken using a machine-like shaker (Uniscope SM 101) for 24hrs. Thereafter, the solution was filtered using Whatman No. 1 filter paper, and filtrate concentrated at 45 ^{0}C using a steam bath (Model: TT-9023A, Switzerland).

Qualitative Screening of Secondary Metabolites

Qualitative test for alkaloids, phenols, flavonoids, anthraquinones, cardiac glycosides, saponins, tannins, phlobatannins, [terpenoids and steroids] were carried out as per standard protocols (Harborne, 1989; Trease and Evans, 1983; Walls *et al*., 1996; Usman and Osuji, 2007; Sofowora, 1993; Awe and Sodipo, 2001; Odebiyi and Sofowora, 1978; Mainasara *et al*., 2012; [Yadav and Agarwala, 2011]) respectively.

Quantitative Screening of Secondary Metabolites

Quantitative determination of alkaloids (Harborne, 1989), flavonoids (Oyedemi *et al*., 2010), saponins (Brunner, 1984; Obadoni and Ochuko; 2001), tannins (Swain, 1979) and phenols (Makkar *et al*., 1997) were carried out as per standard protocols.

Animal Arrangement and Extract Treatment

After acclimatization, the models were re-arranged in a group of five of six rats each. Animals in the control were given 0.5 ml of distilled water and basal diet. Group 1M and Group 1F were administered 500 mg/kg body weight of aqueous extract of *T. conophorum*. Group 2M and Group 2F were administered 750 mg/kg body weight of the extract. The animals took the extract by oral means one a day between the hours of 9 a.m and 10 a.m for 32 days using oral cannula. It was done with extreme care to avoid hurting the animals or spilling the extract. Gender wise analysis of pharmacological effect of the extract was carried out for possible comparison.

Getting Blood and Preparing Serum

The rats were brought into unconsciousness when subjected to a glass tank soacked with diethyl ether cotton wool material. Blood (5 ml), which was collected via retro-orbital method, in plain bottles was centrifuged (using High Speed Centrifugal Machine, Model: YGH-4, Manchester, England) at 1500 g for 7 minutes and serum used for the biochemical analyses.

Pressure Induced Swimming Investigation

The pressure influenced swimming evaluation were carried out twice per week by employing the previously described methods with some modifications (Ikarashi *et al*., 2013). In this study, the rats were neither subjected to any form of preliminary swim nor rest. There was no current generated in the pool. The rats were freely allowed to remain in swimming position until the scheduled end duration of the swimming scheduled test, which was often held to indicate the period at which the rats are unable to rise to the top platform of the water to take in oxygen within 7 seconds and begin to gulp in water. Swim tests was conducted in a cylindrical pool (50×80×60 cm).

Determination of Weight of Animals

The weight (in grams) of the experiment models was measured before the start time of administration and at the end of whole study period. It was done using an animal weighing balance (Freitag *et al*., 2010).

Determination of Selected Anti-Fatigue Parameters

Blood glucose level, serum blood urea nitrogen (BUN) and lactate dehydrogenase (LDH) activity were estimated by employing the assay protocol of (Wroblewski and La-Due, 1955; Tietz *et al*., 1994; Jakobsen, 2009) respectively.

Statistical Analysis

Finding from the study were presented as the average of five discoveries ± SEM. Substantive variation was estimated via determinative method of differences (ANOVA) at precisely 5% trust degree with SPSS 26.0 Version Package.

RESULTS AND DISCUSSION

Results

The check on secondary constituents of aqueous extract of *T. conophorum* nut indicated it has saponins (5.03±0.2mg/ml), flavonoids (2.70±0.10mg/ml), tannins (0.45±0.10mg/ml), phenols (1.51±0.10mg/ml) and alkaloids (0.35±0.10mg/ml) (Table 1).

Table 1: Phytocomponents of *T. conophorum* Aqueous Nut Extract

Phytoconstituents	Amount (Quantitative) (mg/ml)	Representation (Qualitative)
Saponins	5.03±0.20	+++
Cardiac glycosides	-	-
Flavonoids	2.70±0.10	++
Phlobatannins	-	-
Tannins	0.45±0.10	+
Phenols	1.51±0.10	+
Terpenoids	-	-
Anthraquinone	-	-
Alkaloids	0.35±0.10	+
Steroids	-	-

+++ Appreciable amount
++ Moderate amount
+ Minute amount

Blood glucose showed remarkable ($p<0.05$) increase in all albino rat (both male and female) administered with *T. conophorum* aqueous nut extract when liken with the normal untreated rats. There was an appreciable ($p<0.05$) increase in the level of blood sugar for male rats that received 750 mg/kg dosage of the extract when matched up with male rats that got 500 mg/kg dose of the extricate (Figure 1).

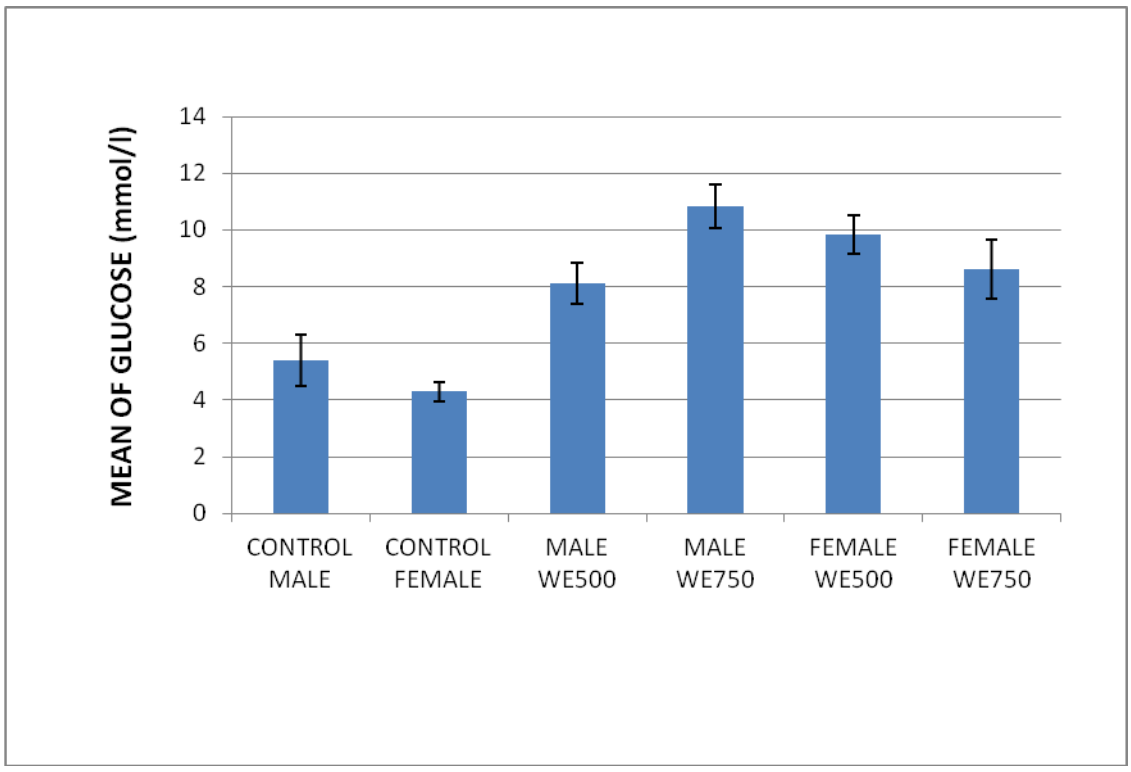

Figure 1: Blood glucose (sugar) level of male and female albino rats administered aqueous extract of *T. conophorum* nut

There was a great statistical ($p<0.05$) difference in the serum blood urea nitrogen (BUN) level between the untreated rats and those that took 750 mg/kg dosage of *T. conophorum* nut extract in both sexes of white rats. It also showed sufficient ($p<0.05$) drop in BUN amount between the groups that received 500 mg/kg and 750 mg/kg doses of the extricate in both sexes of the animals. There was equally a noticeably ($p<0.05$) low level of BUN in both male and female rats that took 750 mg/kg dose of the extract when juxtaposed with the untreated set of rats (Figure 2).

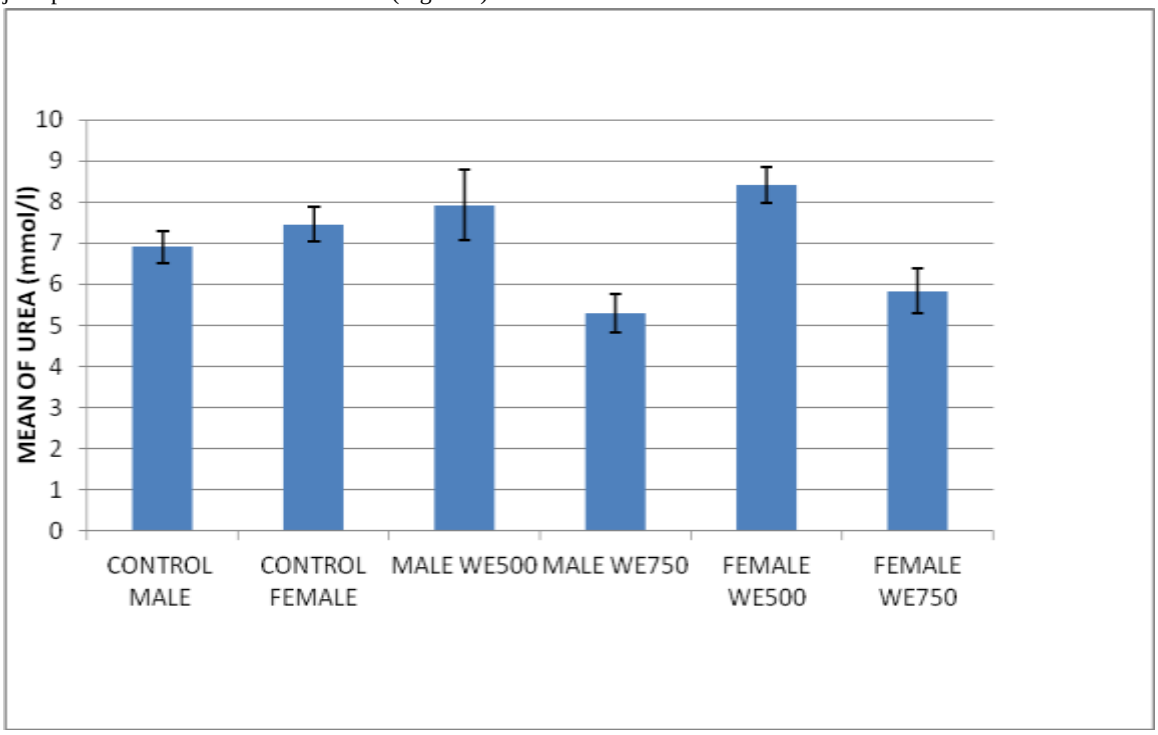

Figure 2: Effect of *T. conophorum* aqueous nut extract on extracellular urea nitrogen (BUN) level in both sexes of animals

Administration of *T. conophorum* aqueous nut extract did not substantively ($p>0.05$) affect the major action of lactate dehydrogenase (LDH) in the blood of both male and female rats when matched up with the untreated rats. However, the blood LDH capacity was elevated in masculine rats that took 500 mg/kg dosage of the extract when matched up with the normal untreated animal group and male rats treated with 750 mg/kg dose of the extricate. The dose independent action, by the extricate, on blood LDH activity in most of the groups, was ascertained (Figure 3).

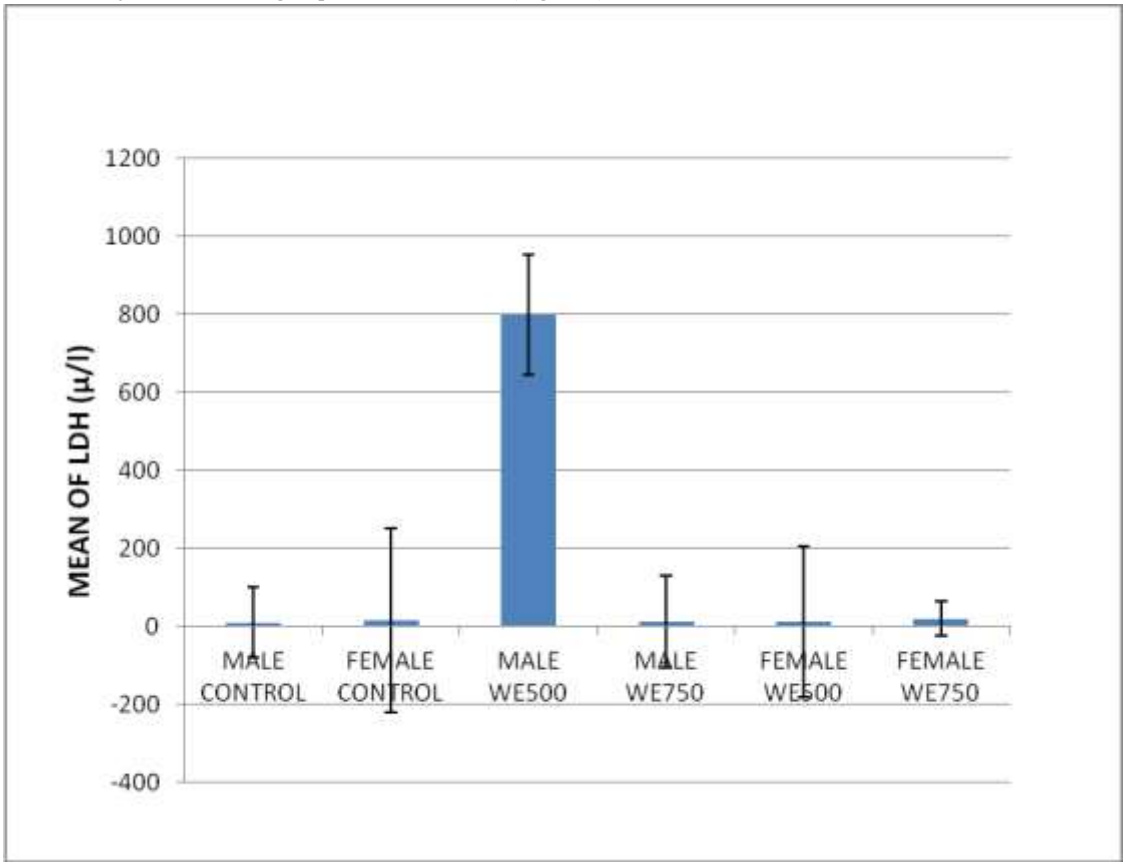

Figure 3: Serum lactate dehydrogenase (LDH) action in male and female albino rats after oral intake of *T. conophorum* aqueous nut extract

In the masculine models, there was substantive ($p<0.05$) rise in swimming scheduled period. This was first noticed at the 3rd swim comparable to the distilled water treated group and the group that took 500 mg/kg dose of *T. conophorum* aqueous nut extract. Appreciable ($p<0.05$) increase in swimming time was subsequently noticed in the 4th, 5th and 6th swim when juxtaposed with the untreated normal rats and the group that received 500 mg/kg dose of the extract. During the 3rd, 4th, 5th and 6th swim, a notable ($p<0.05$) increase was seen between groups treated with 500 and 750 mg/kg doses of the extract (Figure 4a).

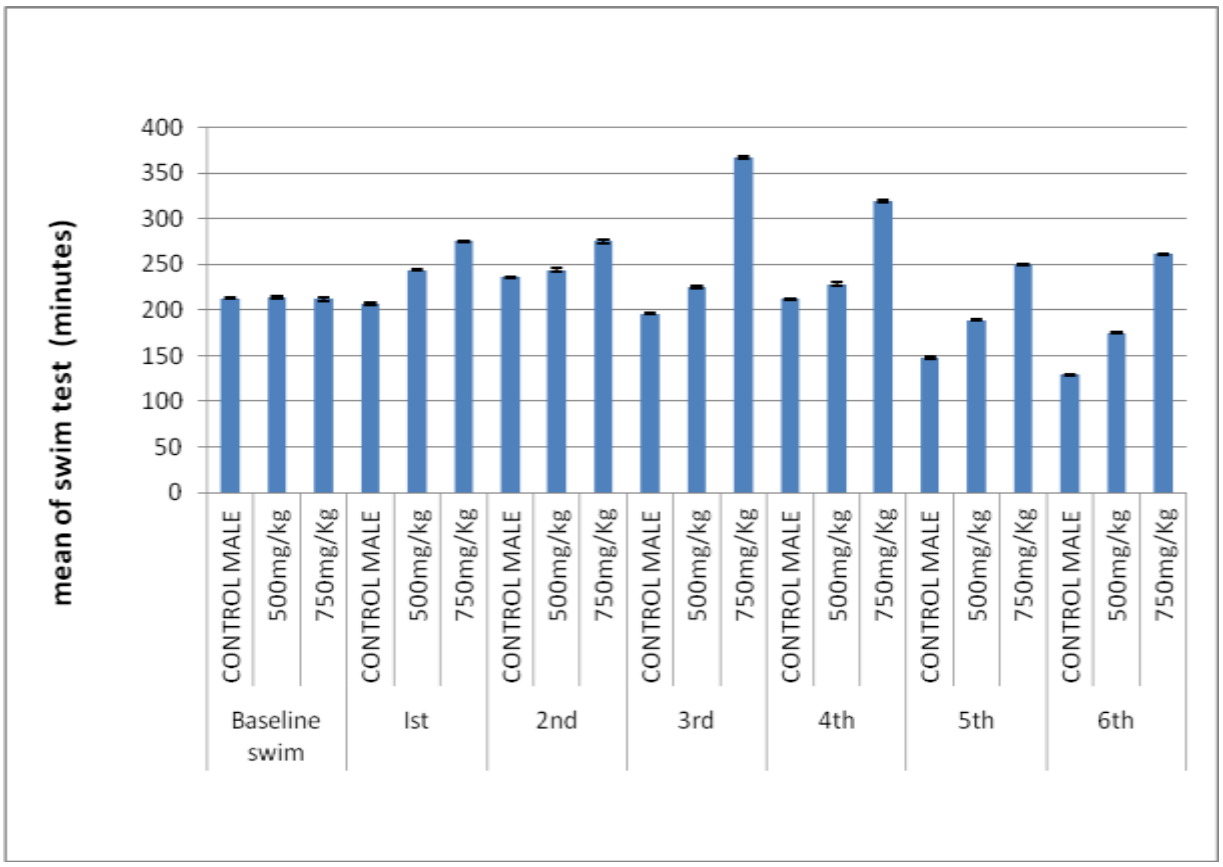

Figure 4a: Swim endurance time in male Wistar rats after treatment with *T. conophorum* aqueous nut extract
*The standard deviation was very less in all treatment groups because the values obtained for it were not significant enough to be easily seen on the bar chart.

In female rats, there was a remarkable ($p<0.05$) rise in swimming time, first noticed at the 2nd swim when matched with the untreated group of animals and those that took 500 mg/kg body weight of *T. conophorum* aqueous nut extract. A meaningful ($p<0.05$) elevation in swimming time was also noticed at the 3rd, 4th, 5th and 6th swim when measured with the untreated group and the group that got the 500 mg/kg dosage of the extricate. During the 4th, 5th and 6th swim, a glaring ($p<0.05$) difference was observed between groups that took 500 mg/kg 750 mg/kg doses of the extricate (Figure 4b).

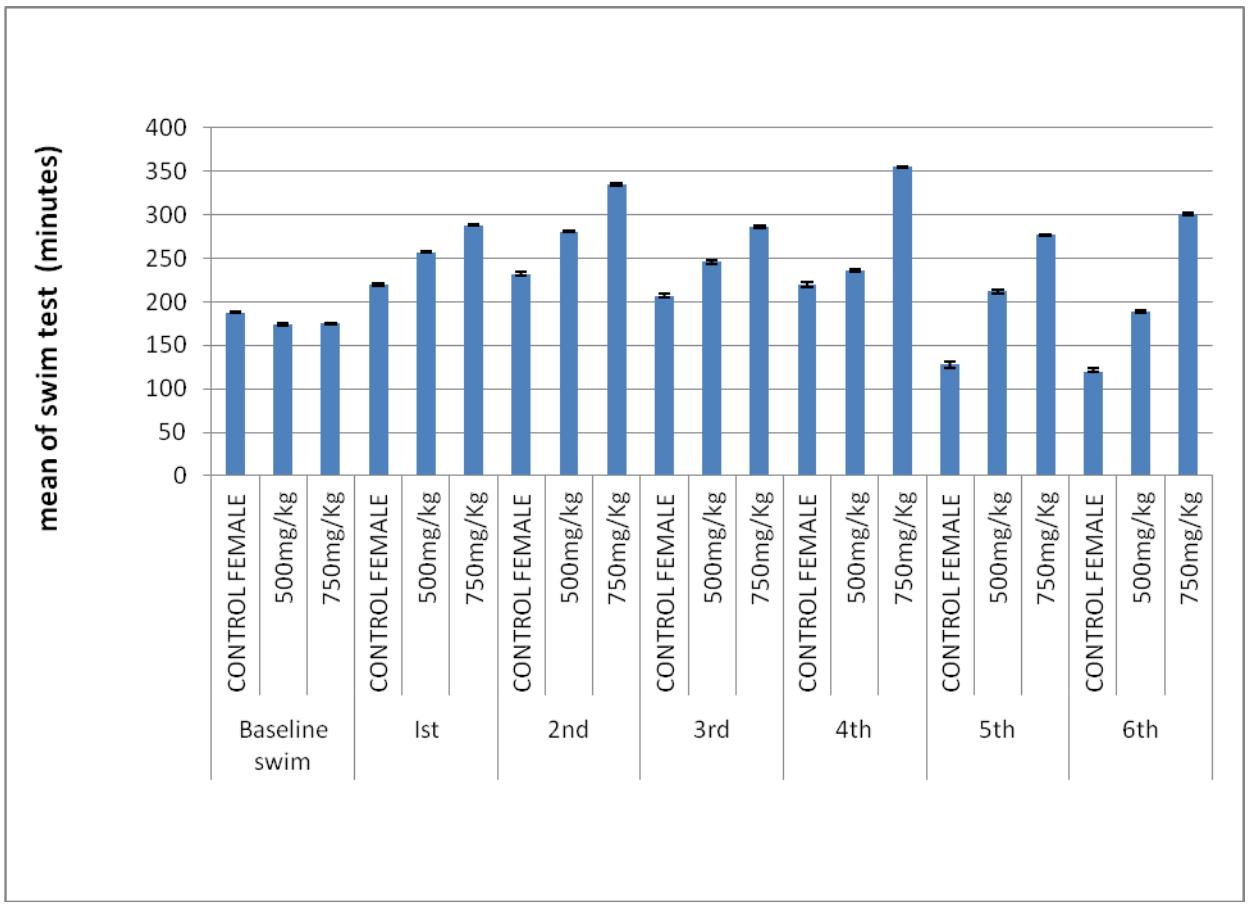

Figure 4b: Swim endurance time in female albino rats following treatment with *T. conophorum* aqueous nut extract

*The standard deviation was very less in all test categories because the figures obtained for it were not significant enough to be easily seen on the bar chart.

DISCUSSION

Studies have shown that glucose is easily absorbed by the body, thereby generating energy and eliminating accumulated fatigue-related metabolites (Wang *et al*., 2008). Fatigue, also known as tiredness/weakness, is an aggregate of functional and biological procedure. After a strenuous exercise, hepatic glycogen decomposes to maintain glucose levels in blood, and glycogen reserves decrease (Coyle *et al*., 1983). When the human body produce large amount of lactate, H^+ concentration rises while pH of muscular tissue drops, causing a series of physiological and biochemical reactions that promote fatigue. Exercise tolerance experiments (e.g., the animal swimming endurance test/exercise tolerance test) and biochemical indicator tests (e.g., hepatic glycogen, blood glucose, BUN, blood lactic acid and LDH activity) are key methods commonly used to measure the level of weakness (Saurez *et al*., 1993; Nozawa *et al*., 2009; Zhang *et al*., 2010). Therefore, the elevation in level of blood sugar, in male rats facilitated by *T. conophorum* aqueous nut extract at 750 mg/kg dose might indicate elevated hepatic glycogen catabolism. The increase could also mean that *T. conophorum* nut extract may have influenced glucose metabolism factors like; gluconeogenesis and enhanced glucagon activity.

The improved swim endurance capacity of male and female rats by the extract at all doses could be attributed to the presence of saponins, flavonoids and phenol which have antioxidant properties which could counter the effect of free radicals which is one important reason for physical fatigue (Jin and Yin, 2012; Ayodele, 2003). Alkaloids might be helpful in ameliorating oxidative issues caused by exhaustive physical exercise-induced fatigue (Feng *et al*., 2011). Flavonoids may prevent fatigue by facilitating anaerobic glycolytic rate of muscular lactate. Fatigue is caused by lipid peroxidation induced by free radical oxidative damage to the cell membrane. Saponins lower free radical induced peroxidative damage, and thereby protects the functioning capacity of the hepatic plasma layer. This facilitates the homeostasis of the hepatocyte, hastens locomotive action, and speed-up recuperative process after gymnastics in animal models (Cigremis *et al*., 2009; Yan and Wang, 2010; Kohler *et al*., 2014).

During anaerobic glycolytic activity, lactate is greatly stored in the body, resulting in both lowered muscular agility and physical activity-mediated tiredness. LDH acts as an enzyme

that participate in lactate clearance from the biological system (You *et al*., 2011). Therefore, the increase in LDH activity in male rats by the 500 mg/kg extract dose suggest elevated reversible potential of LDH in reconverting lactate to pyruvate, and back to glucose thereby managing exercise-induced fatigue (Robergs *et al*., 2004; Tan *et al*., 2012). LDH is a precise marker for muscle distortion and catalyzes the transformation of pyruvic acid and lactic acid (Brooks, 1986; Kim *et al*., 2003). The aqueous extract of *T. conophorum* nut may have also acted by inhibiting the activity of LDH thereby delaying the increase in lactate production. The non-significant effect at 750 mg/kg dosage of the extricate suggests that *T. conophorum* nut may not affect LDH activity.

Extracellular Urea Nitrogen (BUN) is a major biomarker to ascertain the enduring threshold of experimental models from physical activity owing to protein and amino acid degradation. Proteins and amino acids are a good alternative for energy generation when the body has used up glucose and lipid store. There is therefore a good relationship between *in vivo* urea nitrogen level and exercise endurance (Wei *et al*., 2010). The decrease in BUN in both groups at 750 mg/kg extract dosage suggests that *T. conophorum* nut extract could result in reduced catabolism of proteins/amino acids leading to a decreased production of urea by the urea cycle. The extract may therefore reduce catabolic decomposition of protein for energy by this mechanism.

The prolonged swimming time in female rats by 750 mg/kg dosage of the extricate as well as the increase in male rats at the 3rd 4th 5th and 6th swim by 500 and 750 mg/kg extract dosage indicate that *T. conophorum* nut extract could induce increase in swim tolerance ability and facilitate recuperation from tiredness (Matsumoto *et al*., 1996; Carvalho *et al*., 2005; Jiang *et al*., 2013; Yan *et al*., 2014). *T. conophorum* nut at 500 and 750 mg/kg body weight exhibited anti-fatigue activity in male and female rats by extended forced swimming time, decreased BUN, increased blood glucose contents LDH at 500 mg/kg dose in male animals.

CONCLUSION

The anti fatigue effect could result from single or synergistic action of phytocomponents which may have acted by delaying increase in lactate, enhancing antioxidant enzyme activity, increasing swim endurance time and reduction in catabolism of protein for energy as well as lipid peroxidation. *T. conophorum* nut should therefore be consumed not just as a snack but as a remedy for fatigue.

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STATEMENT OF CONCERNS

We declare that this investigation was conducted following active participation from all the authors with no issues of concern.

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