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Effects of Hydro-Methanolic Extract of Oyster Mushroom (*Pleurotus Ostreatus*) on Muscle Strength and Coordination of Albino Rats (*Rattus Norvegicus*) using Inverted Screen Test.

Zitte[1] Leelee Famii, Konya[1] Roseline Sornayee and Olorunfemi[2] Oluwadare Joyce
[1]Department of Animal and Environmental Biology, Faculty of Science University of Port Harcourt, Nigeria.
[2]Department of Human Physiology, Faculty of Basic Medical Science, College of Health Science, University of Port Harcourt, Nigeria.

ABSTRACT

Edible mushroom (*Pleurotus ostreatus*) is known over the years for its nutritional properties and has been used in different food recipes. However, little is known of its cognitive and neuromuscular properties. To ascertain if in addition to its nutritional properties, it may have any neuromuscular effects on albino rats using its hydro-methanolic extract, fifty young rats were grouped into five groups (labeled groups 1-5) of ten animals per group. Groups 2, 3, 4, and 5 were treated with daily doses of 50 mg/kg, 250 mg/kg, 500 mg/kg and 1000 mg/kg respectively for one month while group 1 was used as the control, before they were subjected to inverted screen test. The test was repeated after another one month of continuous treatment to have two test periods namely- test one and test two. The result indicated that high doses and long duration of administration had significant effects on neuromuscular activities of albino rat. It was also found that midway into the dose concentrations, it had the highest neuromuscular responses in both test one and test two. In test one, group 1 had a mean holding time of 43.35 seconds while groups 2, 3, 4 and 5 had mean holding time of 87.79, 85.88, 43.17 and 27.22 seconds, respectively. In test two, groups 1, 2, 3, 4, and 5 had mean holding time of 35.18, 98.60, 81.93, 4030, and 23.64 seconds, respectively. This indicated that groups 2 and 3 had the strongest neuromuscular activities than all the groups being significantly different from the control and group 4, while group 5 was significantly lower than the control. It is therefore concluded that *Pleurotus ostreatus* extract had its highest neuromuscular effects between doses of 50mg/kg and 250mg/kg and its highest dose of 1000 mg/kg had a reversed effects on the performance.

Keywords: medicinal, edible, mushroom, cognitive.

INTRODUCTION

The inverted screen test is a neuromuscular test used to monitor sub-acute neuro-function on muscle coordination. It can be used to study neuro-muscular defects on both young and old animals. It tests the latency of the test animals to fall off the inverted screen under which they held unto with their four limbs. It tests how long it takes the animals to be exhausted and loose grip of the inverted screen [1].

The ability of rats to hold onto the inverted screen can also be used to study effects of drugs on neuromuscular functions[2]. Different drugs have different effects on the ability of the Central Nervous System (CNS) to influence muscular ability. Some are anxiogenic (pro-anxiety) while others are anxiolytic (anti-anxiety). These are expressed in several neuromuscular effects on the animals [3]

This test, among others, is used to determine the degree of anxiety and depression in animal. According to [4], anxiety is hardly precise, consisting of an intuitive combination of unpleasant feelings and sensations and more or less objective observations of disturbed breathing, increased heart activity, vasomotor changes and musculo-skeletal disturbances. Its influence on musculo-skeletal disturbance can be studied using the inverted screen test. The test is based

on the latency of the test animal to fall off inverted screen plain due to muscle weakness and exhaustion.

This test was used to determine the effects of hydro-methanolic extract of *Pleurotus ostreatus* on neuromuscular function and muscle strength. According to [5]"for phenotypic assessment of *mdx* mice, the hanging wire test is performed in order to demonstrate a motor neuromuscular impairment and motor coordination in a new strain, or as an *in vivo* preclinical tool, using models of neuromuscular disorders (e.g. *mdx* mouse).The test was also used in pharmacological studies, for evaluating the neuromuscular tone [6].

Muscle tone is the normal state of muscle tension. Abnormal state of muscle tone may result into hypotonia (less than normal) flaccidity (absent) or hypertonia (more than normal) which is also referred to as rigidity spasticity or tetany[7].

Muscle tone can be used to provide vital information about the condition of the central nervous system (CNS) as it relates to neuromuscular function. Hyperactive reflexes (hypertonia) may suggest disorder of the upper motor neurons (UMN) in the CNS, while *Hyporeflexia* or hypotonia suggests the presence of Lower Motor Neuron (LMN) disorder in the peripheral nervous system (PNS).

During the UMN lesions, the damaged motor neurons lacks the functional ability to regulate descending pathways hence giving rise to disordered spinal reflexes, increased excitability of muscle spindles, and decreased synaptic inhibition (hypertonia). These are because motor neurons that influence the muscle fibres are directly activated through basal ganglia, cerebellum, thalamus and the primary motor cortex. Hence, for any given muscular activity, it requires the cooperation amongst many muscles, some acting as primary movers or agonists others as antagonists, fixators or synergists. These relationships are integrated in the spinal cord or the brain stem. An arrangement known as reciprocal innervation [8].Mal-functional or drug intoxicated UMN will therefore adversely affect LMN and invariably influence muscle contraction, muscle tone and tension.

Muscarinic acetylcholine effects in the central nervous system are complex. According to associated conditions, the stimulation of the post-synaptic muscarinic receptors elicits a depolarization or anhyperpolarization. In animals, their stimulation induces facilitation of training but also hypothermia, tremors and seizures.

MATERIALS AND METHODS

Inverted screen test was, basically, to evaluate the effects of oyster mushroom (*P.ostreatus)* hydro-methanolic extract on neuromuscular function of the albino rats. It measures the effect of the extract on muscle strength, demonstrating the animals' performance as they were placed with their four limbs under an inverted screen.

During hydro-methanolic extract preparation, dry fruiting body of oyster mushroom (*Pleurotus ostreatus)* was pulverized and macerated in 80% hydro-methanol for 72 hours, and then filtered through a whatman filter paper (No. 1). The process of maceration and filtration was repeated to achieve an effective extraction. The filtrate was then concentrated with rotary evaporator. Thereafter a little sample of it was measured and evaporated to dryness in an oven at 105 ^{0}C. The extract's concentration was then determined by ratio method.

A total of 50 rats between 70g and 100g were bought from the Animal House, Faculty of Pharmaceutical Sciences University of Port Harcourt, and taken to the Animal House, Faculty of Basic Medical Science for the study. They were weighed and grouped into five groups of ten rats per group. Thereafter they were acclimatized for two weeks before treatments. After the two weeks, the treatment commenced with administration of 50mg/kg/day, 250mg/kg/day, 500mg/kg/day and 1000mg/kg/day of the hydro-methanolic extract of *P. ostreatus* to groups two, three, four and five. Respectively while group one was given one milliliter of normal saline daily (to serve as the control). The treatment was given daily for thirty days before the neuromuscular test was carried out. The test was in two stages separated by a

period of one month (test one and test two). However, the treatment continued every day throughout the duration of the experiment.

During this test, a wire gauze was set at 35cm over a soft landing pad. Animals were weighed and made to hold under the wire gauze with their four limbs and the time taken by the animals to hold the wire gauze before falling was recorded. The test was repeated five times per animal. After one month, the animals were weighed and subjected to the test again. The fifty animals were tested on the inverted screen test for ten sessions (five sessions per test period).

RESULTS

The result showed that the *Pleurotus ostreatus* hydro-methanolic extract has a significant effect on the four limbs grip as they were tested. Groups two (50mg/kg), three (250mg/kg) and four (500mg/kg) performed comparatively better than the control by having increased neuromuscular activities demonstrated through the increased muscle tone and vigor. In test one, most of the treatment groups were significantly different from the control. In session one, groups two (50mg/kg) and three (250mg/kg) were significantly ($P<0.05$) higher than the control while group four (500mg/kg) and five (1000mg/kg) were significantly ($P<0.05$) lower than the control. In session two, groups two (50mg/kg), three (250mg/kg) and four (500mg/kg) were significantly ($P<0.05$) higher than the control while group five (1000mg/kg) was significantly ($P<0.05$) lower (Table1). From the result, it was found that groups two and three were significantly ($P<0.05$) different from the other groups and the control while control was not significantly ($P>0.05$) different from groups four (500mg/kg) and five (1000mg/kg) on the average of the first test.

During the second test, it was found that groups one, four (500mg/kg) and five (1000mg/kg) were not significantly ($P>0.05$) different but they were significantly ($P<0.05$) different from groups two (50mg/kg) and three (250 mg/kg) (Table 1).

Results of group performances on inverted screen test at different group levels were evaluated. In group one (control), test one session one was seen as the session with the longest time with 59.92±87.94s, while session five had the shortest time with 30.50±33.82s. On the second test, the first session was also seen with the highest demonstration of muscle strength expressed with the longest time of 49.89±69.28s, while session two with 28.38±21.14s was seen to be the shortest.

Result in test one indicating a gradual reduction in the time of the inverted screen test (fig 1). In test two, session one was the longest, while session two was the shortest. This suggested that in the second test, the animals lost much strength after the first session hence the time of the second session was drastically reduced.

Group two (50mg/kg) was seen to have a relatively longer time in the test. In test one, session one with 121.65±185.50s was seen to hold on to the inverted screen with the longest time in group two test one, while session four with 51.97±124.20s used the shortest. In test two, session one with 138.85±185.50s was the longest time while session three with 67.56±58.92 was the shortest. This indicated that the second test was relatively higher than the first, which means that at the second test, the animals had much muscle strength to hold on to the inverted screen than it was obtained in the first test (Fig 2).

Result of group three (250mg/kg) indicated that in test one, there was a gradual decline in the expression of the muscle strength with session one having the highest muscle strength expression (145.30±113.84s) and the fifth session having the least value (51.58±32.98s). In the second test it was found that the first session with 162.05±113.84s was the longest while 37.06±18.28s of the fourth session was the least performance (Fig.3).

Group four (500mg/kg) results indicated that the extract at 500mg/kg had an effect on the performance of the animals in group four. Group four, test one had the longest time in session two with 54.61±37.16s while the shortest time was found in session five with 28.30±18.67s. In test two, the longest time was session one with 46.52±55.82s, and the shortest time was session five with 31.26±18.67s (fig.4)

Group five (1000mg/kg) had the lowest set of values. This showed that the extract had a negative effects at high dose on the

muscle strength, having made group five (1000mg/kg) with the highest dose to have the shortest time. In test one the longest time was in session one with 29.79±17.11s, while the shortest time was in session two with 23.70±21.01s. However, in test two the longest time was in session one with 29.01±15.69s and the shortest time was in session five with 14.67±5.49s (fig.5).
Results of the different groups expressed in sessions showed the relative performances of all the groups according to their sessions. Group two (50mg/kg) and group three (250mg/kg) had longer time in all the sessions while group five (1000mg/kg) was found to have the shortest time consistently (fig.6).

Performances of the different groups expressed in tests one and two were also evaluated. It was observed that groups two (50mg/kg) and three (250mg/kg) held onto the inverted screen rail more than groups one (Control), four (500mg/kg) and five (1000mg/kg). This showed that between the doses of 50mg/kg and 250mg/kg, the extract had the greatest effects. This could be said to be the window of effectiveness (Fig 7)
Percentage performance expressed the percentage differences between session one and session five of the different tests in the groups. This analysis was used to study if there was improvement in the subsequent session over the previous session. In test one, group three (250mg/kg) had the greatest degree of change with -60.9 % while group five (1000mg/kg) had the least degree of change with -6.57. During the test two, it was found that group three (250mg/kg) also had the greatest change with -77.1% while group four (500mg/kg) had the least change with -32.8 % (change can be negative change or positive chance, the farther away from, zero the greater is the change in this test). A negative graph indicated that the animals had relatively lower responses along the five sessions of a test period. It did not show which of the animals held onto the hand grip test for the longest duration (Fig 8). The negative change expressed in the test showed a natural trend of weaker muscle as the test progressed.
A study of the effect of the extract on the relative performances of the different groups compared to the control was carried out. The result showed that, generally, test two held on to the inverted screen for a longer time than test one. Specifically it was found that group two (50mg/kg) test two had the most positive percentage performance with 180.25% followed by group three (250mg/kg) test two with 132.87% while group five (1000mg/kg) had the least percentage performance with 32.82%. During test one study, it was also observed that group two(50mg/kg) had the highest performance 102.5%, closely followed by group three (250mg/kg) with 98.07 % while group five(1000mg/kg) still had the least performance with -37.21% (fig. 9).

Table 1: Effect of hydro-methanolic extract of *Pleurotus ostreatus* extract on inverted screen test at two different test period (sessions in seconds)

	Dose (mg/Kg)	SESSION 1	SESSION 2	SESSION 3	SESSION 4	SESSION 5	% PERFORMANCE
control	1 ml normal	59.92±87.94	46.37±38.99	43.06±26.47	36.93±48.30	30.50±33.82	-49.1
GRP2	50	121.65±185.50*	74.08±76.913*	97.90±57.97*	51.97±124.20*	93.33±58.92*	-23.3
GRP3	250	145.30±113.84*	93.13±69.55*	86.46±32.34*	51.58±32.98*	52.88±18.28*	-60.9
GRP 4	500	46.77±55.82*	54.61±37.16*	45.32±34.23	40.80±38.66	28.30±18.67	-39.5
GRP 5	1000	29.79±17.11*	23.70±21.01*	29.05±9.18*	25.72±15.69*	27.83±5.49	-6.57
		TEST TWO					
	Dose(mg/kg)	SESSION 1	SESSION 2	SESSION 3	SESSION 4	SESSION 5	% PERFORMANCE
control	1ml normal saline	49.89±69.28	28.38±21.14	33.89±33.52	32.03±20.18	31.73±28.56	-36.40
GRP2	50	138.85±185.50*	95.78±76.913*	69.57±57.97*	121.26±124.20*	67.56±58.92*	-51.3
GRP3	250	162.05±113.84*	100.12±69.55*	62.46±32.34*	47.96±32.98	37.06±18.28	-77.1
GRP 4	500	46.52±55.82*	36.69±37.16*	45.11±34.23*	43.57±38.66	31.26±18.67	-32.8
GRP 5	1000	29.01±15.69	28.69±21.01*	17.278±9.18	27.26±17.11	14.67±5.49	-49.4

Values are presented in mean ± S.E.M (n= 10), p <0.05 " *means values are statistically significant compared" with the control group.

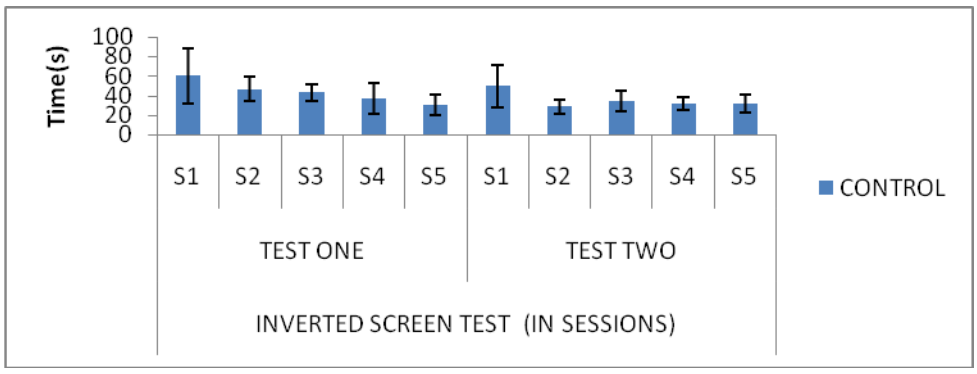

Fig 1: Inverted Screen Test Group One Results Indicating the Trend of the Different Sessions in the Test Groups.

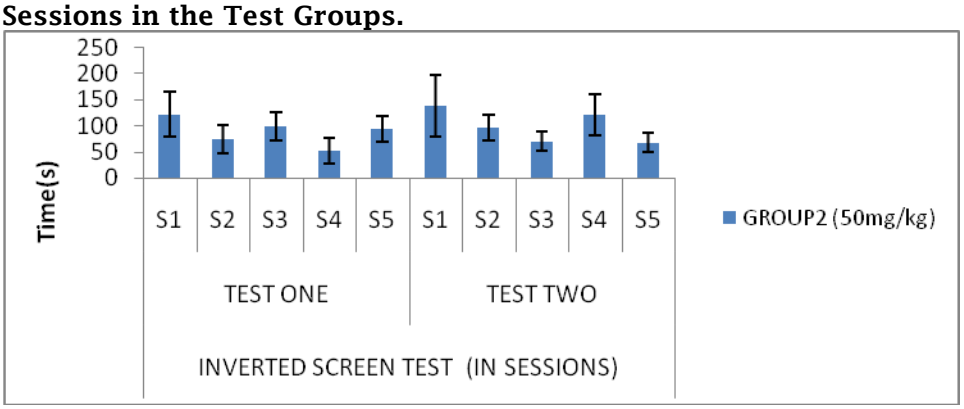

Fig 2: Effects of Hydro-Methanol Extract of *Pleurotus ostreatus* at 50mg/Kg on the Performance of *Rattus norvegicus* on Inverted Screen Test at Two Different Test Periods.

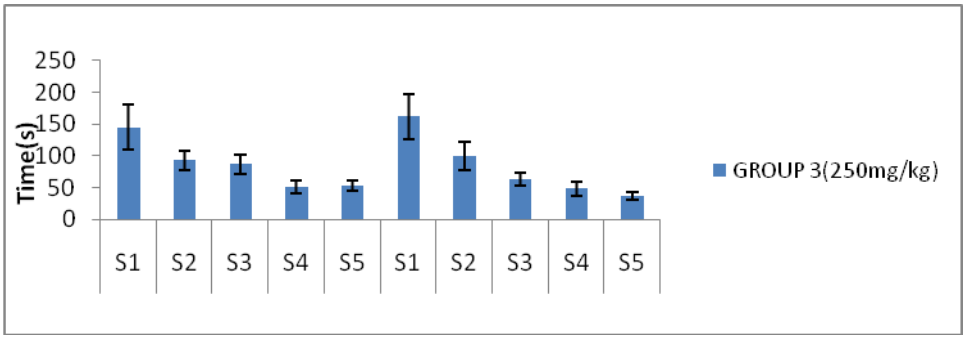

Fig. 3. Effects of Hydro-Methanolic Extract of *Pleurotus ostreatus* at 250mg/Kg/Day on The Performance of *Rattus norvegicus* at Two Different Inverted Screen Test Periods

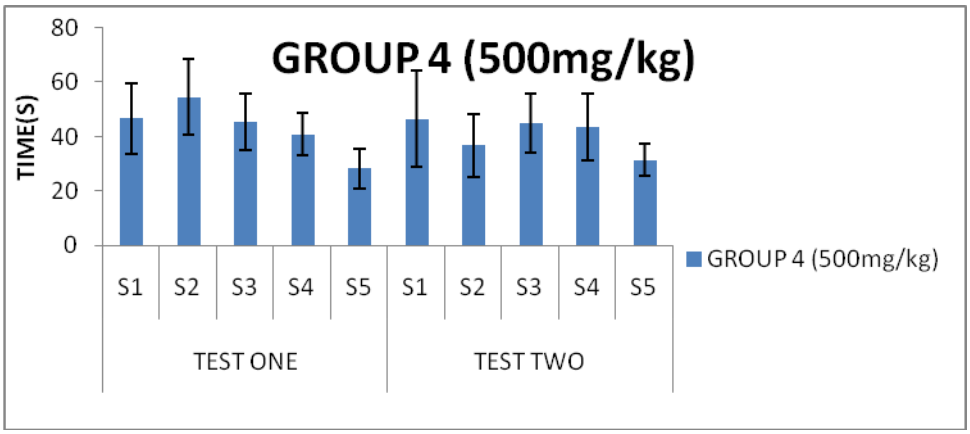

Fig 4: Effects of Hydro-Methanolic Extract of *Pleurotus ostreatus* at 500mg/Kg/Day on the Performance of *Rattus norvegicus* at Two Different Inverted Screen Test Periods

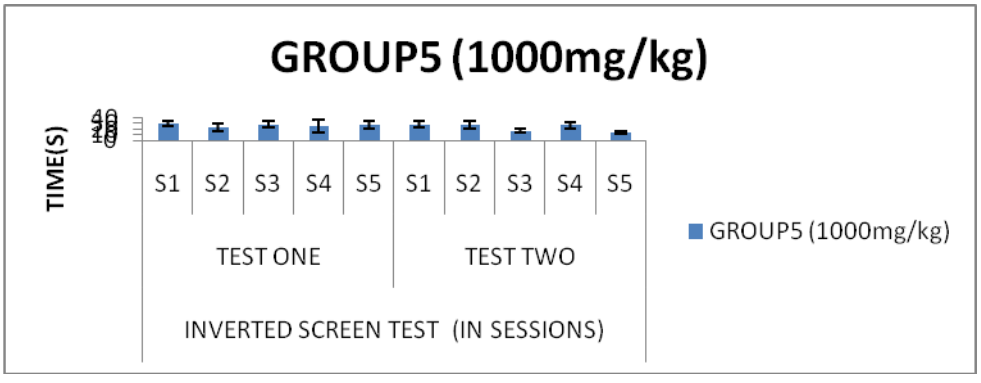

Fig. 5: Effects of Hydro-Methanolic Extract of *Pleurotus ostreatus* at 1000mg/kg/day on the Performance of *Rattus norvegicus* at two Different Inverted Screen Test Periods.

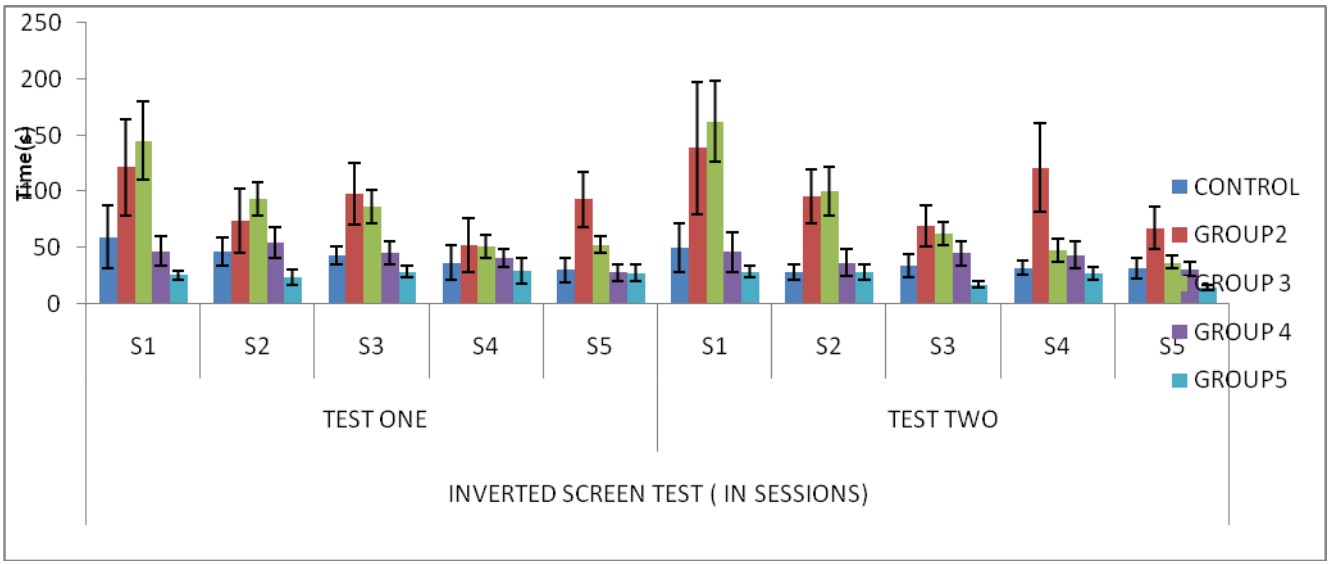

Fig. 6: Relative expression of the effects of hydro-methanolic extract of *Pleurotus ostreatus* on the performance of different experimental groups of *Rattus norvegicus* in an inverted screen test at two different test periods.

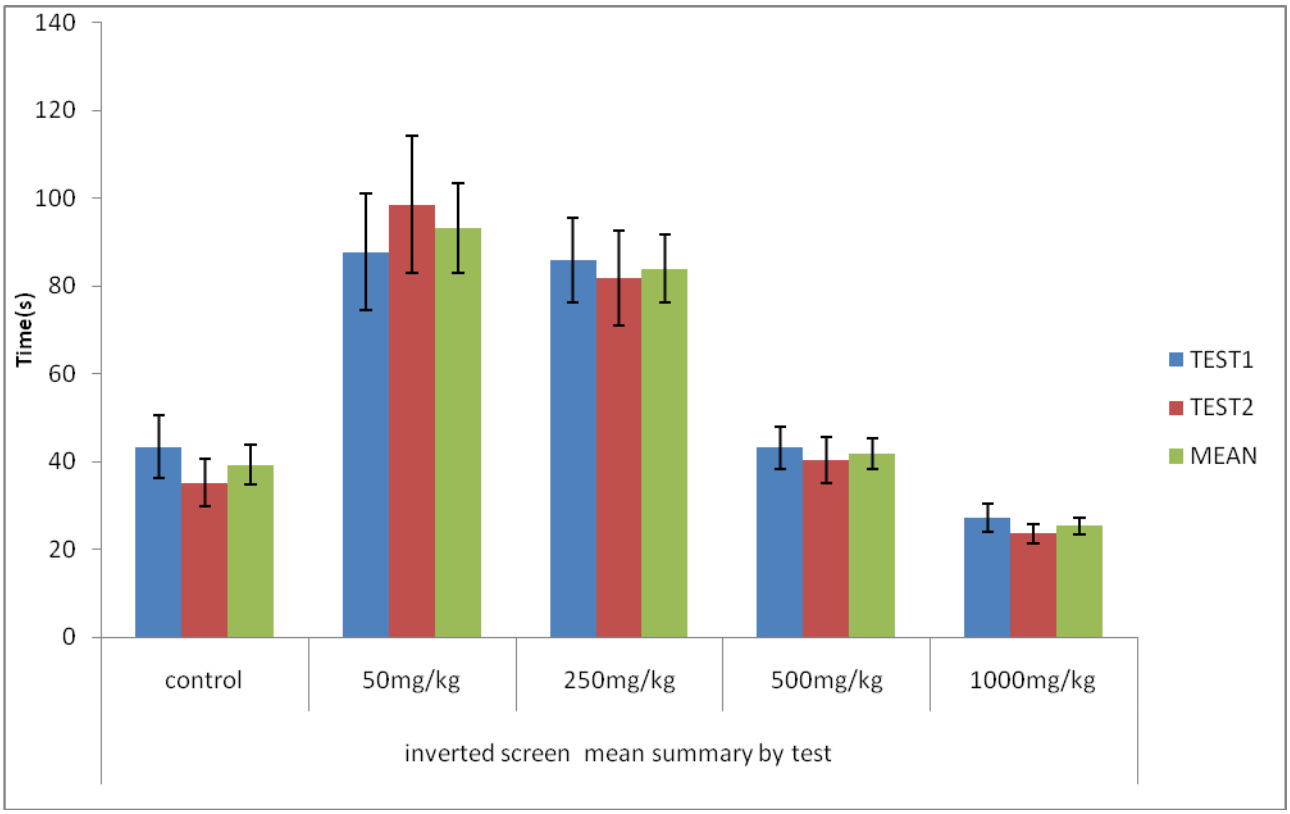

Fig. 7: Effects of *Pleurotus ostreatus* hydro-methanolic extracts on inverted screen test of different treatment groups expressed in means of test one and two

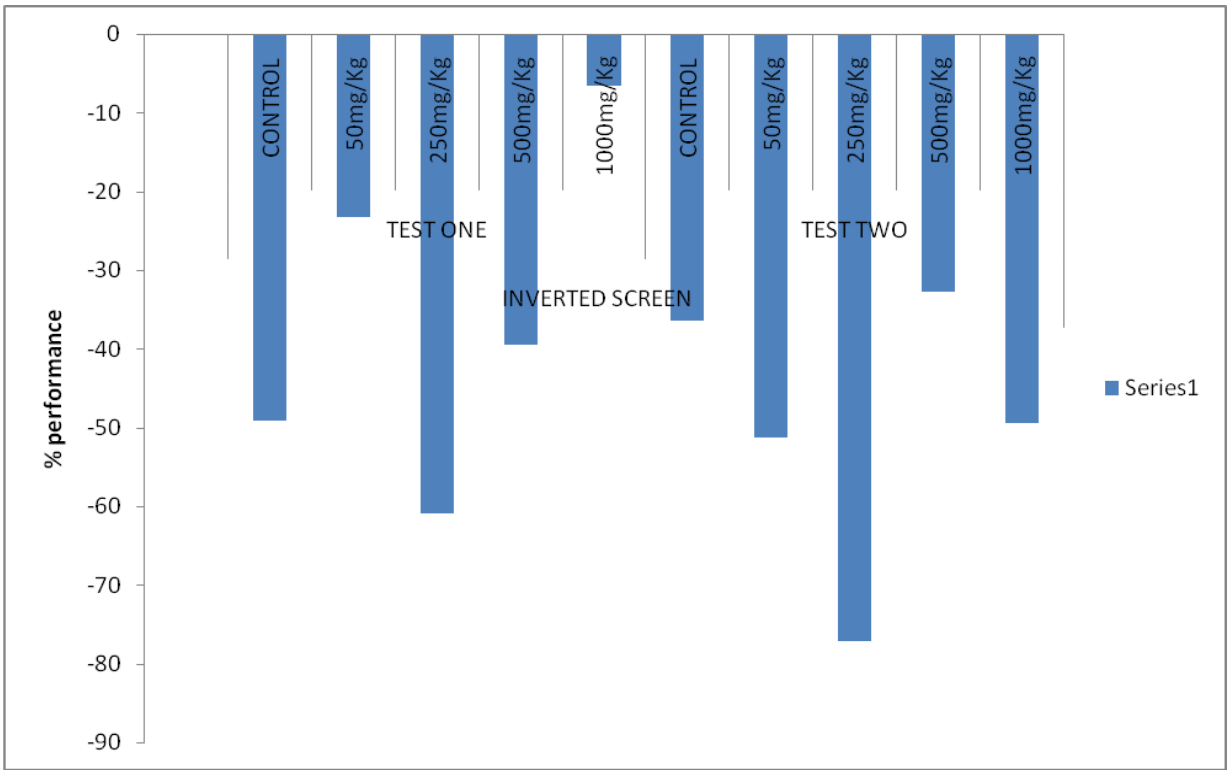

Fig.8: Percentage performance of the fifth sessions of inverted screen tests over the first sessions

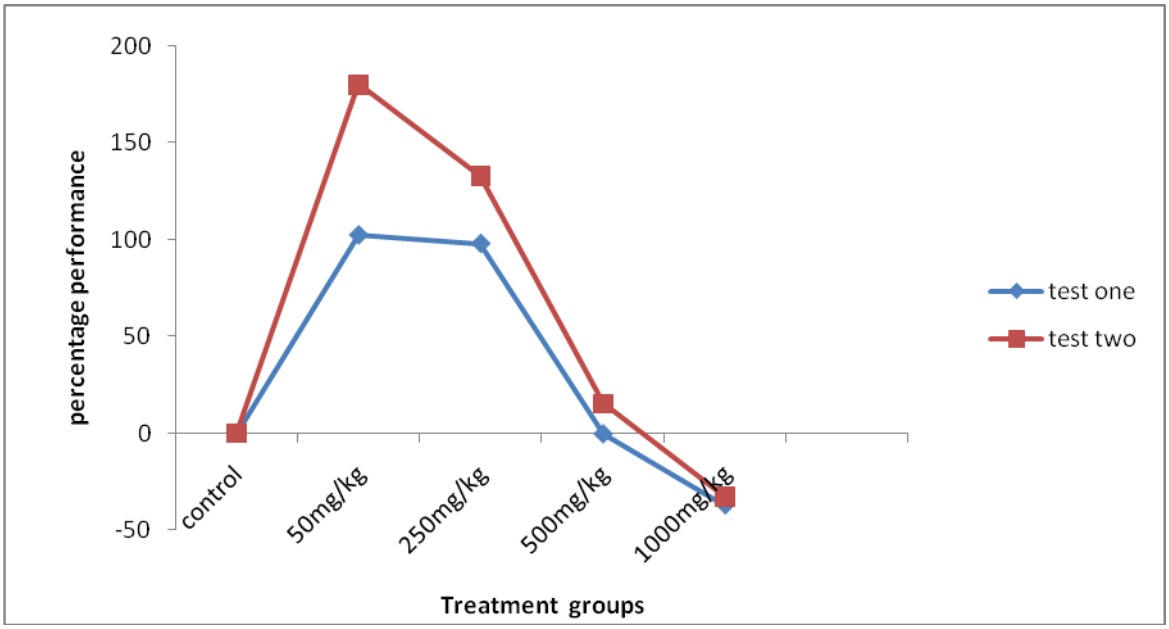

Fig. 9: Percentage performance of different treatment groups over the control group on inverted screen test at two different test periods.

DISCUSSION

From the result, it is seen that the extract exacts a strong positive effect on muscle strength of albino rats as demonstrated in the inverted screen test. This can be as a result of the presence of steroid in the extract [9]. It is observed from the study that the extract expressed concentration dependent influence on the neuromuscular

performance of the animal. This was found to be between the concentrations of 50mg/kg/day and 250mg/kg/day. It was also noticed that chronic administration of seemingly nontoxic food supplement like *Pleurotus ostreatus* can elicit neuromuscular responses with effects similar to toxic drugs. Even as [10] opined thatcontinued drug use causes cognitive deficits that aggravate the difficulty of establishing sustained abstinence, when he worked with addictive drugs. From the result, it is worthy of acknowledging that effects of continued drug administration could also be expressed in the administration of food supplement like *Pleurotus ostreatus*. The manifestation of this chronic effect and its associated indication of high response on a definite dose range which was noticed in the neuromuscular contraction as the animals held onto the inverted screen test, also strongly suggest that the extract has a significant effects on the neuronal substrate with influence on muscular activities. Due to the fact that dopaminergic neurons from the substantianigra pars compacta to the dorsal striatum, (the *nigrostriatal pathway*) was enhanced hence playing significant roles in the control of motor function[8]. This enable the animal to hold onto the inverted screen longer within the extract doses of 50mg/kg and 250mg/kg range [11].

CONCLUSION

From the result it is concluded that *Pleurotus ostreatus* hydro-methanolic extract exact much potent effects on neuromuscular function within the dose range of 50mg/kg and 250mg/kg. It is also found that high doses of the extract above this range elicit receptor insensitivity hence producing poorer responses.

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