EFFECT OF SCENT LEAF (Ocimum gratissimum) AQUEOUS EXTRACT ON NEUROMUSCULAR FUNCTIONS OF ALBINO RATS (*Rattus norvegicus*)

ABSTRACT

Ocimum gratissimum (scent leaf) has been used for the treatment of various disease situations from ancient time because of its anti-convulsion, anti-diabetic anti septic, antibacterial, anticancer, antioxidant properties. in the present study, the effects of *Ocimum gratissimum* extract preparation was evaluated on the neuromuscular system of the albino Wister rat (*Rattus norvegicus)*. twenty five young animals of 40-90g were used for the experiment. They were divided into five groups of five animals per group. The different groups (groups 2, 3, 4, and 5) were administered with 100mg/kg, 200mg/kg, 400mg/kg, and 800mg/kg daily, respectively while group 1 was used as the control. The neuromuscular activities were assessed and studied by using scientific assays like the Handgrip test, inverted screen test, swimming test, and the beam walking for checking non-declarative memory function. From the result obtained , One way analysis of variance (ANOVA) was used to compare the mean values among the different groups. Differences were considered significant whenever the p-value was $P \leq 0.05$. From the different results evaluated it was observed that difference between the control and the treatment groups were not significant . This result reveals that the administration of *Ocimum gratissimum* does not have significant effect on the cognitive and neuromuscular functions of the rat which by extension may also be applicable to human.

Key words: *Ocimum gratissimum*, neuromuscular , cognitive , handgrip scent leaf.

INTRODUCTION

Ocimum gratissimum also known as African basil , clove basil or wild basil. it is commonly called scent leaf due to it pungent aroma (Kabir et all (2005). It has been used to spice African delicacies for centuries. in addition to its aromatic nature, researches have been conducted on some of its therapeutic properties especially in this era of researches on herbal therapy which are serving as replacement for synthetic drugs. Over the years, studies have shown that most of the synthetic drugs produced and approved for use on animals and humans are potential poison (Achinewu *et al*., 1995). For example, some over the counter anesthetic and analgesic drugs have been noticed to have active ingredients, called muscle relaxant; which have adverse and far reaching consequences on the skeletal muscle function and hence decreases muscle tone. Though it is used to alleviate symptoms such as muscle spasms, pain and hyperreflexia (Matasyoh et al 2007).

Scent leaf (*Ocimum gratissimum*) among other herbal therapeutic products , is seen to have promising nontoxic property even at a very high dose hence it serves as a good replacement for these synthetic products (Ijeh et al., 2004).

Studies have shown that scent leaf possess diverse therapeutic effects such as anti-microbial anti-bacterial, anti-oxidant, anti-viral, anti-plasmodial and anti-fungal (Ijeh et al 2005 Ubulom et al 2011). It has been seen to have anti-tumor, anti-diabetic properties (Aguiyi et al 2000)

amongst others (Edeoga and Friata, 2001). Scent leaf is a potential drug for so many health challenges, hence, its wide uses in many traditional herbal mixtures and remedies. Characterization of its ethanolic extracts revealed the presence of non-cyclic sesquitespenes and phenols (Matasyoh et al 2007).

Due to its potent therapeutic properties, its leaves are greatly used as "Muscle Relaxant". in this capacity it serves in two major therapeutic functions , viz; neuromuscular blockers and spasmolytic agents. While the neuromuscular blockers and spasmolytics are often grouped together as muscle relaxants; the term is commonly used to refer to spasmolytics only (Chen *et al.*, 1995, Madeira et al 2002, Chummy, 2006). This work is geared toward the investigation and further reduction in the dependency on risky and harmful synthetic drugs , which could cause serious neurological effect such as muscle cramps, weakness, poor coordination etc which could further be life threatening if taken over a long period of time (Carter et al 2001).

Though herbal medicine tends to look primitive and unscientific when compared to synthetic drugs nervertheless, herbal medicine is still the main stay of about 75-80% of the world population, mainly in the developing countries for primary health care (Kamboj, 2000). The aim of this research therefore is to determine and evaluate the therapeutic effects of *Ocimum gratissimum* on the neuromuscular function of Albino rats (*Rattus norvegicus*) using Handgrip test for neuromuscular function, Beam walking assay for non-declarative memory function, and Forced swimming test for depressive tendency.

Research has shown that *O. gratissimum* contain muscle relaxants, which acts on the neuromuscular system of the body. The neuromuscular system of an animal is very vital since all the activities of the muscle (response to stimuli) is under the controlled of the nervous system. It therefore deserves proper and careful treatment so as to sustain the vital organs of the body. Though, most muscle relaxants can have serious effect on the muscle tone of an animal, therefore, it is necessary to control the dose or stop the administration of such drugs, if found to have side effects (Maity et al., 2000.

Orafidiya et al., (2004) carried out studies on the acute and sub-chronic toxicity of the essential oil of *O. gratissimum*.and observed that *O gratissimum oil* is capable of evoking an inflammatory response on persistent administration.

There have been lots of research works carried out on the herb, *O. gratissimum*. Although, theeffect of *O. gratissimum* extract on the neuromuscular system of wistar albino rat is rare, however,, some few works related to this study have been documented. In a study carried out by a virologist, it was reported that the aqueous extract of *O. gratissimum* has the potential to inhibit HIV-1 strain $HTLVIII_B$ Cytopathicity (Farooque, 2001). In 2006, a research was conducted on the safety and hypoglycemic properties of aqueous leaf extract of *O. gratissimum* in Streptozotocin- induced diabetic rat for 28 days. The result showed a statistically significant reduction in plasma glucose level. The extract appears non-toxic as showed by normal serum levels of Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) and bilirubin. Also, in another study, *O. gratissimum* was administered orally on non-

diabetic rats for twenty-eight (28) days, but there was no significant variation in the plasma glucose concentration produced in the rat, compared with the control (Aguiyi et al 2000).

Cristiana *et al., (2009)* carried out the study to investigate whether seasonal variations in composition of essential oil of *O. gratissimum* are accompanied by changes in pharmacological properties; using experimental procedures to investigate the central nervous system activity. The essential oils obtained in each season were capable of increasing the barbiturate-induced sleeping duration. The greatest effect was obtained with the preparation from autumn, and the least effect was observed with that from winter, which was not active in the lesser dose administered.

MATERIALS AND METHODS

Experimental Materials

This research work was carried out in Animal and Environmental Biology museum, behind the general laboratory, University of Port Harcourt, Choba, Port Harcourt. Rivers State, Nigeria. Fresh leaves of *O. gratissimum* (scent leaf) were purchased from slaughter market, Trans-Amadi, Port Harcourt. The leaves were authenticated as *O. gratissimum* by Late Prof. Ndukwu, of the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Choba.

Twenty-five (25) wistar albino rats weighing between 40-90g were purchased from the animal house of Animal and Environmental Biology in Choba Campus, University of Port Harcourt. During the preparation of the aqueous extract of *O. gratissimum*, the leaves were rinsed with water to remove dirt and allowed to air-dry under shade at room temperature (28±2ºC) and was then ground to powder using a hand miller.

Two kilograms (2kg) of the powdered *O. gratissimum* was then taken to the pharmacology laboratory in the Faculty of Pharmaceutical Sciences, Department of Pharmacognosy, University of Port Harcourt, for the extraction of the aqueous extract. The crude aqueous extracts thus obtained was stored in sterilized amber colored bottles and maintained at 4ºC in a refrigerator.

Experimental Design

A total of 25 rats were weighed and divided into five (5) different groups of five rats per cage and fed with the grower's mash and water for the period of one week to acclimatize. The rats were re-weighed at the end of one week of acclimatization and their weights were used to quantitatively dilute and share the leaf extract (drug) into different doses of 50mg/kg, 100mg/kg, 200m/kg and 400mg/kg for groups 2,3,4and 5 respectively ; While group 1 served as the Control and was daily given 2ml of distilled water as placebo , group 2-5 were administered half of the daily doses of the drugs morning and another half in the evening At the end of one month of treatment, the neuromuscular behaviors of the rats were tested using different scientific experimental assays such as the Hand grip test, Beam walking, Inverted screen test, Force swimming test and swimming test.

Handgrip test: This was carried out to test the effects of *O. gratissimum* on the muscle strength of the fore-limb of the rat; it measures the hang time with respect to body weight. In this test, a string was taut to two wooden poles and the rats were made to Hang using its forearms and suspended in the air. A stopwatch was started simultaneously at the start of the hanging and the time taken for it to fall off was recorded. The test was conducted for five consecutive times for each treatment.

Beam walking: This assay was carried out to assess the effects of *O. gratissimum* on the motor balance and coordination in rats. In this test, the animals were kept on a horizontal beam to see his ability to walk across an elevated narrow beam to a safe platform. The time taken and the number of claw-slip that occur during the process was noted. The experiment was repeated for five consecutive times for each treatment.

Inverted screen test**:** This assay, also known as "four limb hang test", was carried out to test the effects of *O. gratissimum* on the muscle strength (muscle tone) of the animals and also, to test the models of neuromuscular disorders. This test was done by taking note of the body weight of the animal in respect to hang time and routinely recorded for each session. This correlation provided important information on the efficiency of the treatment in increasing body weight and improved ability to produce sustained tension in limb flexors.

Force swimming test**:** This test was carried out to show the distinct patterns of active behaviors as a result of the treatment. A behavior sampling technique was developed to score the active behaviors; climbing, swimming, diving trying to find a safe and escape route. In this test, the rats were dropped in a bowl of water containing a raised platform and the time taken to locate the safe platform was noticed. This was done to train the animals to locate the safe platform. There-after, the water made opaque by adding a handful of powder milk, the safe platform was submerged. The animal were tried again to see how they can locate the safe platform.this was carried out five time per animal.

Swimming test**:** This assay was carried out to test the brain coordination and muscle tension of the animals from the treatment. This test is similar to the force swimming test but considers longer period of time. In this test, the rats were submerged into bowl of water fill with 15cm height of water and was left there to observe the limb movement, swimming ability, mobility and immobility in respect to the time taken.

All results were collated and data analysis was performed using SPSS version 16.0. Data were recorded as mean±SEM. The mean values obtained from the different groups were compared using ANOVA and differences were considered significant at $P \leq 0.05$.

RESULTS

The effect of *Ocimum gratissimum* has been investigated on neuromuscular system of Albino Wistar rat, after been subjected to various scientific investigative assays; each parameter is represented on the different graphs below.

Handgrip test:The rats in groups 2 and (100 mg/kg/day) exhibited high muscle strength by having a hang time of 17.0 ± 7.77 seconds compared to the control (Group one) with 9.8 ± 2.87 seconds (Table 1 and Fig.1). This can be attributed to the effect of the drugs on the muscle tone of the rat. Also in Group 3 (200mg/kg/day) 15.6 ± 7.51 and 15.6 ± 9.88 seconds, when compared to Group one (control) had relatively high muscle strength. This could be as the result of the concentration of the doses of the drugs (table 1 and fig.1)

Table 1; Effect of Ocimum gratissimum on The Neuromuscular Function of Rats Using Handgrip Test

Treatments	Trial one (sec)	Trial two (sec)	Trial three (sec)	Trial four (sec)	Trial five (sec)
Group one	4.0 ± 0.44	5.0 ± 1.30	6.6 ± 2.40	5 ± 1.51	9.8 ± 2.87
Group two	10.4 ± 4.14	9.8 ± 2.31	17.0 ± 7.77	11.0 ± 3.36	9.0 ± 2.44
Group three	11.6 ± 4.04	13.2 ± 5.64	9.8 ± 3.67	15.6 ± 7.51	15.6 ± 9.88
Group four	8.6 ± 0.92	8.4 ± 3.48	7.0 ± 2.09	6.0 ± 1.64	5.6 ± 0.87
Group five	7.4 ± 2.11	10 ± 1.34	12.8 ± 2.63	12.4 ± 3.00	11.6 ± 2.22

Values are represented in mean ± SEM (n=5), p 0.05; * means values are statistically significant compared with the control group

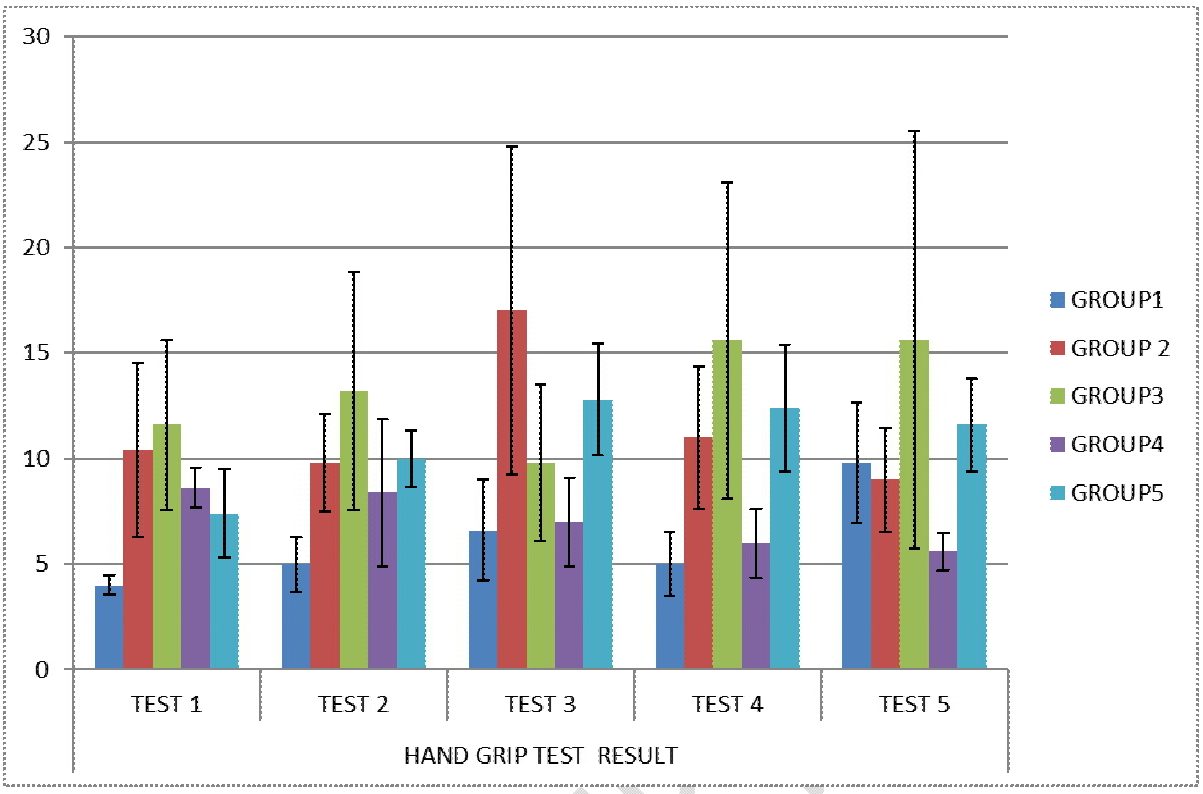

Fig. 1: Effect of aqueous extract of Ocimum gratissimum on Handgrip Test of Albino rats

Beam walking: Comparing the result gotten from Group one (control) with other results, it shows a trend, the control has 19.2 ± 22.07 and 90.2 ± 12.53 seconds as its lowest and highest time taken respectively to cross the beam successfully whereas Group 4 (400mg/kg/day) showed 5.4 ± 1.12 and 17.8 ± 13.05 seconds as lowest and highest time taken respectively, to cross the beam successfully to the other end, with this it be thought that, the drugs is psychedelic. However, this thought is countered by the result of Group 5 (800mg/kg/day) which showed 27.4 ± 20.28 and 51.4 ± 42.52 seconds as lowest and highest time taken respectively (table 2 and fig.2)

Table 2, Effect of Ocimum gratissimum on The Neuromuscular Function of Rats Using Beam Walking Test

treatment	Trial one (sec)	Trial two (sec)	Trial three (sec)	Trial four (sec)	Trial five (sec)
Group one	90.2 ±12.53	55.4 ±13.42	91.2 ±22.07	57.6 ±17.50	60.6 ±24.52
Group two	39.8 ±17.51	45.6 ±21.33	17.8 ± 4.85	13.0 ± 2.58	39.6 ±17.93
Group three	70.2 ±22.07	59.8 ±20.97	61.4 ±16.19	87.0 ±19.80	50.2 ±22.06
Group four	6.4 ± 1.16	5.4 ± 1.12	8.6 ± 3.10	9.6 ± 2.69	17.8 ±13.05
Group five	51.4 ±42.52	27.4 ±20.28	65.0 ±53.26	44.2 ±45.18	44.0 ±37.17

Values are represented in mean ± SEM (n=5), p 0.05; * means values are statistically significant compared with the control group

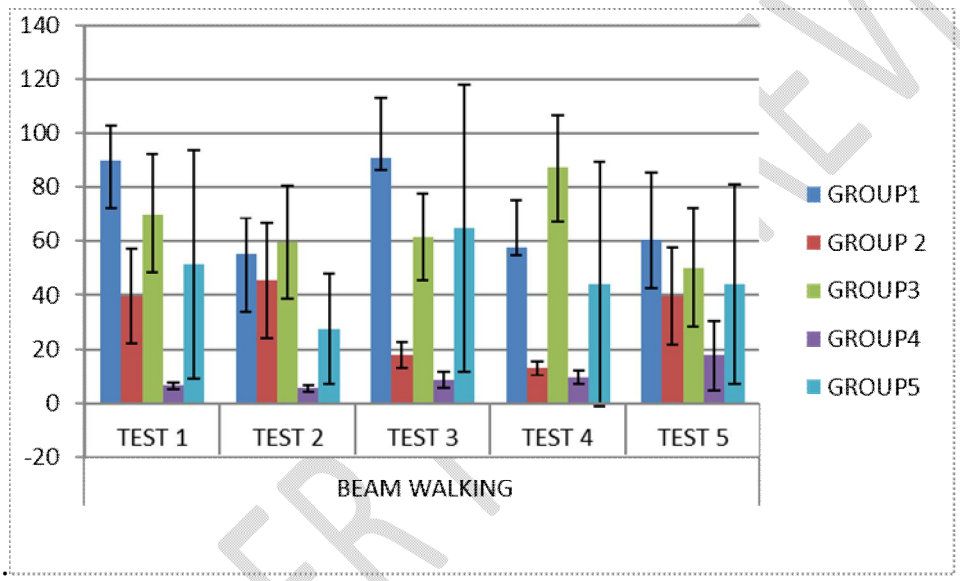

Fig. 2: Effect of aqueous extract of Ocimum gratissimum on Beam walking Test of Albino rats

Inverted screen test:When comparing the result gotten from Group one (control) with other results it shows 6.2 ± 0.86 and 10.4 ± 2.80 seconds as its lowest and highest time spent hanging on the screen , Group 4 (400mg/mg/day) showed 10.0 ± 2.60 and 33.2 ± 13.29 seconds lowest and highest time spent hanging on the screen respectively. It could have been right to say that the drugs had a significant effect on the muscle tone of the rat, but in Group 5 (800mg/kg/day) with

an even higher dose, had a staggering 8.4 ± 1.43 and 15.2 ± 6.55 seconds, which was at defiance to the group four result (Table 3 and Fig.3).

Table 3, Effect of *Ocimum gratissimum* on The Neuromuscular Function of Rats Using Inverted Screen Test

Treatment	Trial one (sec)	Trial two (sec)	Trial three (sec)	Trial four (sec)	Trial five (sec)
Group one	8.8 ± 1.52	10.4± 2.80	7.8 ± 1.71	9.2 ± 2.57	6.2 ± 0.86
Group two	7.4 ± 2.60	6.40± 0.74	8 .6 ± 1.80	7.4 ± 1.74	9.8 ± 2.63
Group three	10.0± 2.60	19.4± 9.88	13.0± 4.84	33.2±13.29	16.6 ± 5.67
Group four	17.0 ± 4.34	13.8 ± 3.76	9.6 ± 2.40	6.4 ± 2.01	8.4 ± 1.93
Group five	15.2 ± 6.55	8.4 ± 1.43	12.2 ± 4.12	14.2 ± 5.77	13.4 ± 2.52

Values are represented in mean ± SEM (n=5), p 0.05; * means values are statistically significant compared with the control group

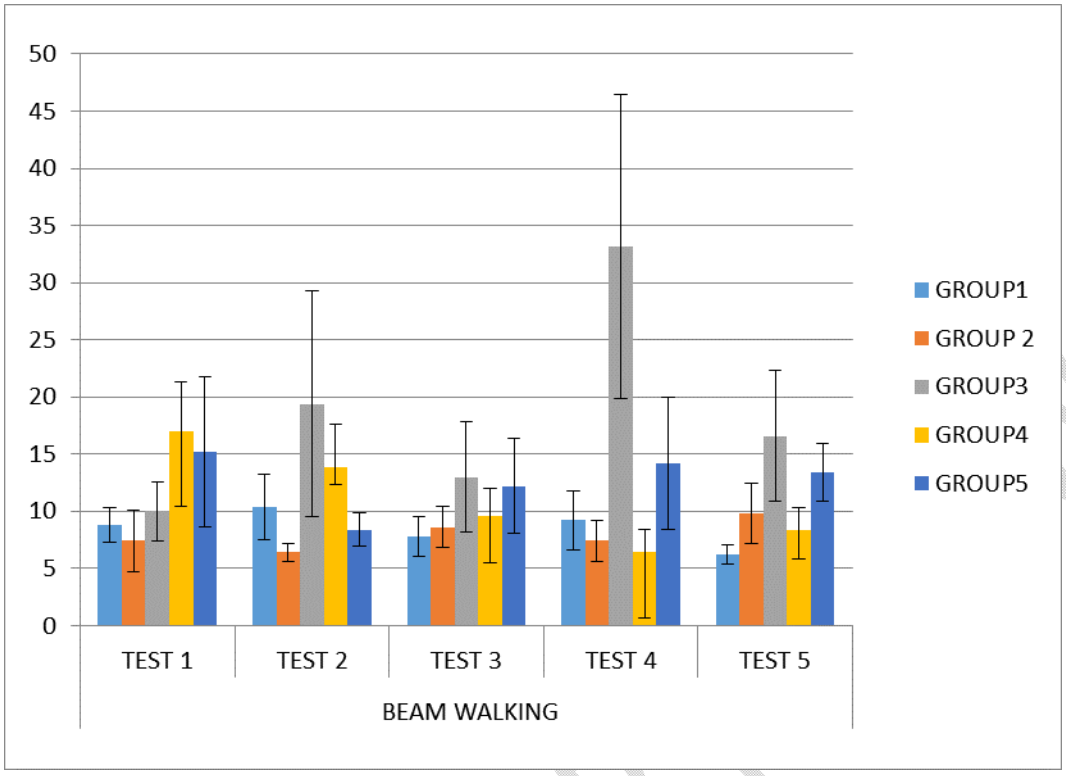

Fig. 3: Effect of aqueous extract of Ocimum gratissimum Inverted screen Test of Albino rats

Force swimming test:The rat administered with 400mg/kg/day dose of the drugs showed 84.0 ± 15.03 and 114.2 ± 3.95 seconds as its lowest and highest time taken to locate the safe platform, whereas Group one (control) had 15.0 ± 22.71 and 82.6 ± 16.62 seconds as its lowest and highest time respectively taken to locate the safe platform . It have been good to say that the drugs had a depressant effect, but what happen in group 5 (800mg/kg/day) with 51.4 ± 20.12 and 72.6 ± 9.73 seconds lowest and highest time spent at separate trials was different from the general rule (Table 4 and Fig. 4)

Table 4; Effect of *Ocimum gratissimum* on The Neuromuscular Function of Rats Using Force Swimming.

Treatment	Trial one (sec)	Trial two (sec)	Trial three (sec)	Trial four (sec)	Trial five (sec)
Group one	82.6 ± 16.62	51.0 ± 22.71	80.0 ± 23.61	81.4 ± 21.02	78.0 ± 25.76
Group two	70.2 ± 15.50	46.4 ± 19.55	52.6 ± 18.21	61.0 ± 18.48	41.8 ± 20.04
Group three	91.6 ± 15.97	90.2 ± 12.12	84.0 ± 15.03	114.2 ± 3.95	110.2 ± 5.29
Group four	25.6 ± 6.45	40.6 ± 8.84	40.2 ± 5.72	38.6 ± 11.18	26.0 ± 3.34
Group five	72.6 ± 9.73	59.4 ± 11.31	56.6 ± 18.27	51.4 ± 20.32	55.2 ± 18.58

Values are represented in mean ± SEM (n=5), p 0.05; * means values are statistically significant compared with the control group

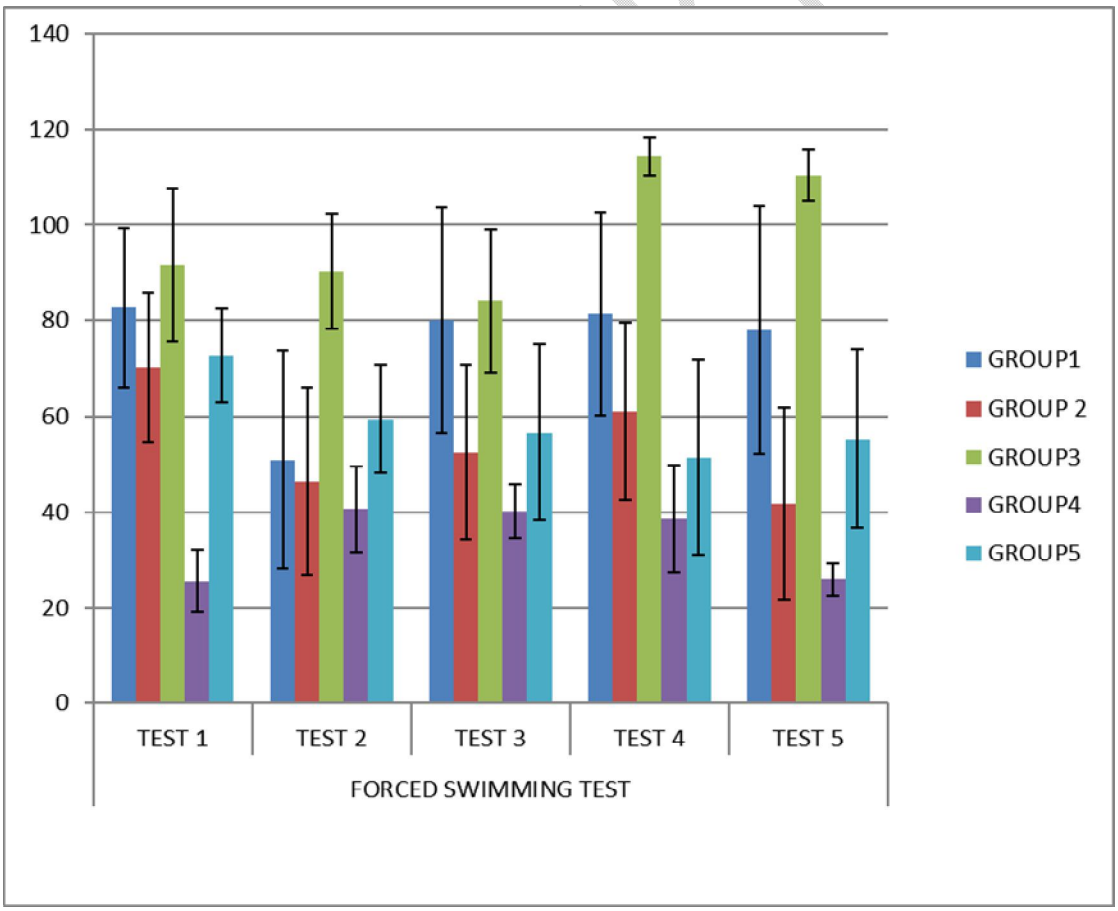

Fig. 4: Effect of aqueous extract of Ocimum gratissimum on Force swimming Test of Albino rats

Swimming test **:** In swimming test the Group 1 (control) had 43.2 ± 20.40 and 78.0 ± 20.59 seconds compared to the Group 4 (400mg/kg/day) with 59.2 ± 15.74 and 92.4 ± 20.92 seconds. It is seen that Group 4 trials spent more time in the bowl moving, swimming, and navigating, whereas Group 1, lacked that skill, it could have been right to suggest that the drugs had a significant effect on the motor and swimming skill of the rat, but Group2 (200mg/kg/day) countered such suggestion owing to its results that showed 35.0 ± 13.78 and 53.0 ± 10.90 seconds as its lowest and highest time spent during the swimming session. (Table5 and Fig. 5).

Table 5, Effect of Ocimum Gratissimum on The Neuromuscular Function of Rats Using Swimming Test

Treatment	Trial one (sec)	Trial two (sec)	Trial three (sec)	Trial four (sec)	Trial five (sec)
Group one	63.4 ±20.35	43.2 ±20.40	78.0 ±20.59	70.0 ±20.97	68.0 ±22.67
Group two	42.0 ±13.92	35.0 ±13.78	48.4 ±14.00	53.0 ±10.90	48.4 ±16.21
Group three	70.2 ±17.66	73.2 ±20.37	73.4 ±18.21	60.8 ±16.08	79.8 ±20.15
Group four	92.4 ±20.92	74.8 ±24.66	63.8 ±23.20	59.2 ±15.74	67.0 ±16.09
Group five	77.2 ±18.08	62.0 ± 8.00	68.6 ±15.58	60.0 ±19.87	70.0 ±17.66

Values are represented in mean ± SEM (n=5), p 0.05; * means values are statistically significant compared with the control group.

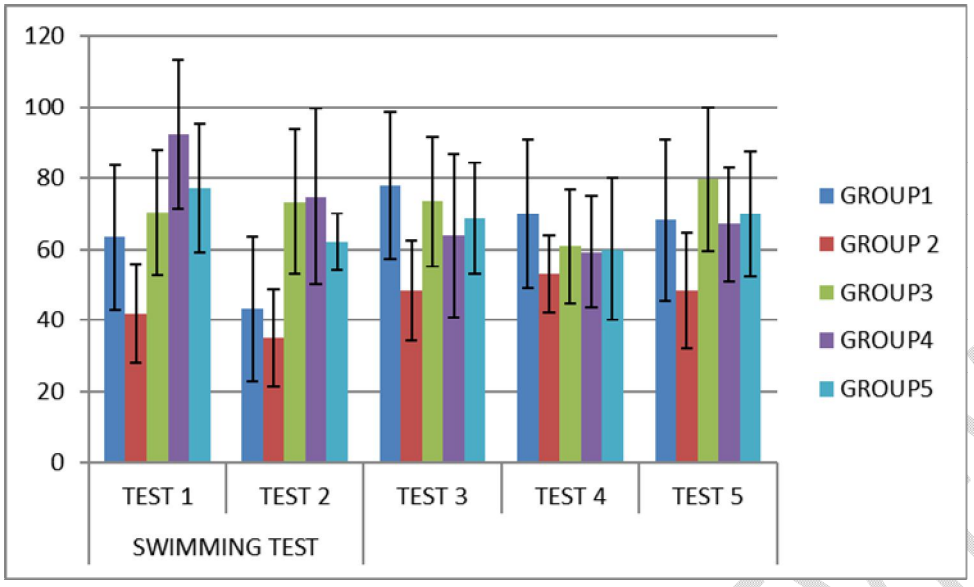

Fig. 5: Effect of aqueous extract of Ocimum gratissimum Swimming Test of Albino rats

The experimental models used to evaluate neuromuscular activity, such as; non-declarative memory and muscular flexor and coordination are assumed to identify the effects of the drugs against generalized tonic–clonic, partial seizures and generalized chronic seizures, respectively. These and many other cognitive skills can be detected using the assays such as beam walking for non- declarative memory, swimming test for depression while hand grip and inverted screen test

It is also observed that group five with the highest dose Ocimum gratissimum has a relatively less effect compared to the lower doses . this has been observed also in behavioral test by (Zitte et al 2019) where the highest dose was seen to be relatively less potent compared to the middle dose of mushroom extract on neuromuscular functions

Finally, it could be concluded that aqueous extracts of *Ocimum gratissum* has a duration and dose dependent effect on the neuromuscular functions of the wistar albino rat (*Rattus norvegicus*), after a month of constant administration. The result also confirm that herbal products have a window of effectiveness which are not always at the highest dose rather a particular dose according to the different extracts

From the result of this study, it is recommended that further studies be carried out to unravel the therapeutic effect (negative and positive) of this herbal product., to enhance and maintain its efficacious usage and it effectiveness on the body.

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