

Effects of Methanolic Extract of Bitter leaf (*Vernonia amygdalina*) on the Neuromuscular functions of Albino rats. (*Rattus norvegicus*)

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ABSTRACT

This research was conducted to determine the effects of methanolic extract of bitter on the neuromuscular system of albino rats. Twenty-five (25) albino rats were grouped into five groups (A, B, C, D and E) of five (5) rats per group. Group A served as control while groups B, C, D and E were treated with different concentrations of methanolic extract of bitter leaf (100mg/kg/day, 200mg/kg/day, 400mg/kg/day and 800mg/kg/day) at two different times (morning and evening with half the daily dose per administration) respectively for a period of one-month. Thereafter the rats were subjected to five different tests: handgrip test, beam walking test, inverted screen maze test, swimming test and force swimming test. Statistical Analysis showed that the effects of methanolic extract of bitter leaf, exhibited significant ($p < 0.05$) effect on the nerves of the muscle tone when compared with the control. In hand grip test, group C, showed mean values which were significantly ($p < 0.05$) higher than the control. In beam walking test the treatment groups (B, C, D, and E) were slower than the control in a dose dependent manner. The treatment groups were not significantly different from the control though there observable trends expressing treatment-induced effects. Only group C was seen to be significantly different ($p < 0.05$) in swimming test. In forced swimming test, groups B, C, D were dose dependently smarter than the control to locate the safe platform faster. However, group E was not so influenced. The result expressly showed that *Vernonia amygdalina* has significant effect on neuromuscular, memory and other cognitive functions and in proper dosage is applied it can enhance effective neuronal functions.

Keywords: *Vernonia amygdalina*, neuromuscular effects, *Rattus norvegicus*

INTRODUCTION

Vernonia amygdalina (bitter leaf), is a member of the Asteraceae family (Compositae) [1]. In Sudan, *V. amygdalina* is locally called gharibe. The leaves are green with a characteristic odour and bitter taste [2], and has been domesticated in some parts of West Africa, e.g. Nigeria, where it is locally known as bitter leaf [3] and [4]. Wide array of phytochemicals has been shown to be present in *V. amygdalina*. The presence of oxalates, phytates and tannins have been reported [5]; [6]; [7], flavonoids [8]; [5], as well as anthraquinones, saponins,

polyphenols, and alkaloids [9],[10] [11]. In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredients are extracted, while in traditional medicine, a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food [12]; [13][14]. Several herbal preparations from different parts of plants (leaves, roots, barks and twigs) have become popular for the treatment of a variety of diseases such as arthritis, epilepsy, paralysis, muscle weakness, Parkinson disease, hypertension, alzheimer

disease, schizophrenia etc. [15]; [16]. One of such plants suspected to possess medicinal value is bitter leaf (*V. amygdalina*). It is a small tree of about 1 - 3 m tall, occurs as an herb or shrub in Madagascar and Asia [17]. Bitter leaf is a common plant in the tropical African region, it is domesticated in different parts of Nigeria and it is used in preparing different delicacies with different tribes and languages having names for it. The Edo people call it "Oriwo", Hausa "Chuserdek", Ogoni "Elobiri", Yoruba "Ewuro", and the Ibos "Olugbo" [18].

Studies on the nutritional composition of the bitter leaf (*V. amygdalina*) are numerous [19]. *V. amygdalina* has been found to be rich in minerals, especially phosphorus, calcium, potassium, magnesium, zinc, iron and some vitamins like vitamin A, C and E. Scientific and pharmacological studies have revealed the antihyperglycemic action of the roots [19]. In folkloric medicine, it was used to cure a lot of diseases including eczema, measles and anaemia [20]; [21]; [22]. *V. amygdalina* is one among many plants that have multiple medicinal values in different parts of Africa. Scientific and pharmacologic studies have revealed antihyperglycemic action of the roots [19] and leaves [23]; [24] and hypoglycemic effect of the leaves of this plant. Extracts from the leaves have been reported to possess hypolipidemic property [25] and to protect the kidneys and the liver of alloxan-diabetic rats against complications. *V. amygdalina* extract have been previously reported to have prebiotic properties [26]; [27]; [28]. Studied by [29] and [30] revealed that *Vernonia amygdalina* possessed some properties, with anti-oxidant, anti-inflammatory, anti-biotic, anti-septic, anti-allergic and analgesic effects. Maintaining good levels of brain and muscle function across the lifespan is crucial to achieving a good quality of life. There is substantial evidence showing an association and a role

played by the brain between cognition and muscle function. A greater understanding of this role will help to improve current knowledge of the mechanisms linking brain and muscle function over the life of an individual [31].

It is instructional to briefly outline the traditional views on the mechanisms of contraction and relaxation of muscles. The contraction in the stretch position, although not moving the muscle's origin or insertion, creates a small degree of muscle fibre shortening while elongating the musculotendinous junction [32]; [33]. This is considered to reduce the tension in the muscle spindle and stretch the Golgi tendon organ (GTO) at the musculotendinous junction. The combination of the muscle spindle suppression and the inhibitory effects of the GTO result in inhibition potentiating, it also results in determining the available length and the degree of muscle stiffness. This has been indicated by the findings of [34]. According to them, the changes in muscle stiffness (muscle displacement in response to a brief torque) follow a passive stretch and follow an isometric contraction. They found that the stretch reduced the muscle stiffness and the contraction increased the stiffness. Importantly, these changes were accompanied by changes in electromyographic pattern and recorded responses from individual muscle spindles, indicating a neurological alteration rather than simply an alteration in the viscoelastic property of the intramuscular connective tissue. When compared with the results from Hutton and [35], [34] offer substantial evidence in support of the concept that the active components are critical in determining the available muscle length and the degree of muscle stiffness in awake, neurologically normal, human subjects. Bitter leaf extract is known to calm the nerves, strengthens the muscles, cleanses the system, eradicates joint pains associated with

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rheumatism, stroke and arthritis and help maintain healthy and strong bones. Therefore, this research is of great interest to the whole world and particularly to the benefits of the people having some of these

diseases. This research is aimed at studying the effects of *Vernonia amygdalina* (bitter leaf) methanolic extract on neuromuscular functions in albino rats.

MATERIALS AND METHODS

This research was carried out in the laboratory at the Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt Campus. Twenty-five wistar albino rats of both sexes weighing between 60-90g were bought from the Department of Animal and Environmental Biology, animal house at Choba Campus, University of Port Harcourt for the study. The animals were kept in well aerated laboratory cages at the Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt Campus. They were acclimatized for a period of one week before the commencement of the experiment.

Fresh *V. amygdalina* leaves were harvested from a farm in Gwara Community, Khana Local Government Area, Rivers State, Nigeria. The required quantity of the plant was carefully collected from the farm and the plant was botanically identified and confirmed by Mr. Suleiman Yusuf, a Botanist and Lecturer at the Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Science, University of Port Harcourt. The plant was dried to a constant weight, ground to powder and extracted with 75% alcohol (methanol). A total of twenty-five Wistar albino rats were randomly divided into five different groups (A, B, C, D & E) of five rats each. Each group was given different concentrations of bitter leaf extract. Group A served as the control. Groups B-E were administered 100mg/kg/day, 200mg/kg/day, 400mg/kg/day and 800mg/kg/day of bitter leaf extract at two different doses (morning and evening)

respectively for one month. Thereafter, the rats were subjected to different neuromuscular tests: hand grip, beam walking, inverted screen test, swimming and force swimming test.

Hand grip test: (Also called Weights Test). The rats were to hang on a string with their forelimb and the time taken by the animal to hang there was recorded.

Beam walking test: The beam-walking (BW) test was used to evaluate motor coordination, integration, balance performance and motor skills. The animal were made to walk on a horizontal pole of about 90 cm long and of a predetermined diameter the time taken to walk across was recorded.

Inverted screen test: The inverted screen was a 43 cm² wire mesh consisting of area of 12 mm² woven with a 1 mm diameter wire. The wire mesh was placed flat over a plastic container of 50 cm high. The rats were made to hang under the wire mesh with their four limbs the time taken by rats to hang were recorded. The whole setups were placed over the cushion (rubber basket) which protected the fallen rat from injury. Five trials were performed per animal on each experiment,

Swimming test: Rats were forced to swim in the plastic basin (height: 60 cm, diameter: 40 cm) containing tap water, maintained at room temperature (28°C). Depth of the water in the plastic basin was 30 cm. Experiments were done between 10 am and 12 noon to minimize hypothermal shock. The rats were kept to find out how long they can swim before getting

tired and start to attempt to keep afloat.

Force swimming test: The forced swim test was a rodent behavioral test used for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that were aimed at rendering or preventing the expression of depression. Rats were placed in an inescapable transparent tank that was filled with water to its 2/3 capacity. A safe platform was placed at a corner of the tank and the animals were given a pretest trial to locate the safe platform. Thereafter, powder milk was mixed

with the water to make it opaque and water was filled to cover the safe platform. The animals were then tested to know if it could discover the position of the safe platform the time taken to locate the safe platform was recorded. Data collected were subjected to Analysis of variance (ANOVA) and differences among groups were considered significant at $p < 0.05$. Mean were carried out by one-way analysis of variance and Duncan's multiple test was used to determine the level of significant difference among the groups.

RESULTS

The evaluation of the effects of methanolic extract of bitter leaf on the neuromuscular system of albino rats indicated that different doses of the bitter leaf extract administered to different groups of rats create different effects of extracts on the animals according to their different groups. Result of the handgrip test, indicates that test 1 and 2, there was no significant differences ($p > 0.05$) between the five groups was so observed. However, Groups C, and E were significantly different ($p < 0.05$) from the control in tests 3, 4 and 5 while groups B and D were not significantly different ($p > 0.05$). (Table 1). The mean of the handgrip test indicate that group C had the greatest handgrip strength (Fig 1). Beam walking tests showed that groups B, C, D and E responses were slowed significantly ($p < 0.05$) in a dose dependent manner compared to the control in the respective test (table 2). This was also expressed in their Mean results (Fig 2). In inverted screen test group C, D and E were significantly

different ($p < 0.05$) from the control in tests 1-5. However group B was not significantly different when treatment compared with the control (Table 3). Mean group result indicates that groups C and E had the highest inverted screen strength compared to the control and groups B, and D (Fig3). In Swimming test, the treatment groups were not significantly different ($p > 0.05$) from the control except in test 1 where groups B, C and E were significantly different ($p < 0.05$) from the control (table 4). Relative values of the mean group results showed that there were no significant difference among the groups though the treatment group were slightly higher than control group (Fig 4). Forced swimming test result suggests that *V. amygdalina* could serve as an antidepressant due to the fact that alertness increases with increase of the treatment concentrations though not at significant difference except group D. (table 5), this was also demonstrated in the groups mean performances (Fig 5).

Table 1: Effects of hydromethanolic extract of bitter leaf at different concentration on the performance of rats on handgrip test.

Hand grip test							
GROUPS	DOSE	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	MEAN
CONTROL	0mg/kg/ day	10.67±1.74	11.33±1.57	8.67±1.02	6.33±1.35	8.33±1.30	11.35±1.74
GROUP B	100mg/kg/day	6.80±1.62	4.40±1.03	7.20±3.28*	6.60±2.98	8.00±2.88*	8.25±2.94
GROUP C	200mg/kg/day	6.40±1.29	7.60±2.34	25.80±13.92*	14.60±4.04*	23.60±10.57*	19.5±8.0
GROUP D	400mg/kg/day	6.60±2.40	5.60±1.75	8.60±1.69*	7.80±2.58	8.80±2.08	9.35±2.62
GROUP E	800mg/kg/day	7.20±1.49	7.20±1.24	13.40±2.50*	16.00±4.74*	13.20±3.25	14.25±3.3

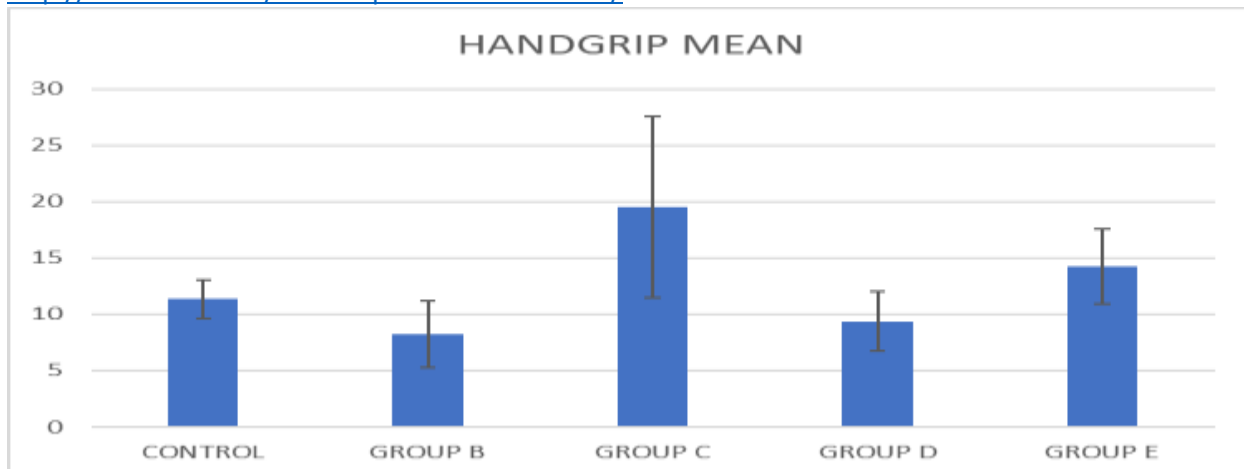


Fig 1: mean performances of the different groups on a hand grip test

Table 2: shown the effects of hydromethanolic extract of bitter leaf at different concentration on the beam walking test.

Beam walking test							
GROUPS	DOSE	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	MEAN
CONTROL	0mg/kg/day	6.40±1.91	5.20±1.59	5.00±1.26*	5.20±2.03	7.00±2.12	7.2±2.22
GROUP B	100mg/kg/day	11.00±3.73	22.60±7.84	18.20±7.99*	15.60±5.36	10.80±3.65	19.55±7.14
GROUP C	200mg/kg/day	3.00±22.65	26.20±20.51	26.80±20.88	22.60±14.69	17.40±13.19	24±22.98
GROUP D	400mg/kg/day	31.40±22.30	32.40±22.13	32.00±19.05*	31.40±22.36	29.00±18.29	39.05±26.03
GROUP E	800mg/kg/day	67.00±22.93	42.00±19.24	35.80±22.16	31.20±16.99	38.00±20.71	53.5±25.50

Values are Mean ± S.E.M, n=5, significance is set at p<0.05 mean values are statistically significant compared with the control group.

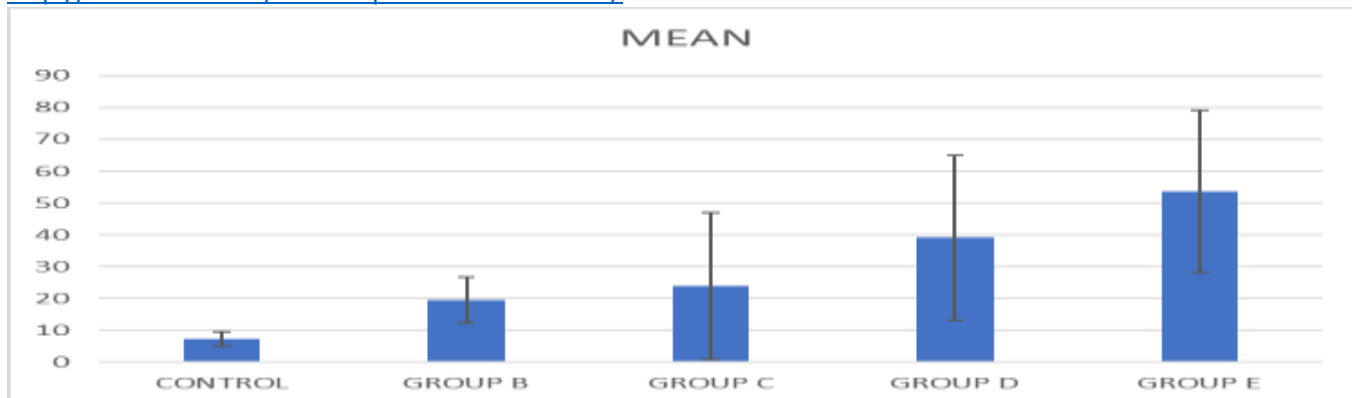


Fig 2: mean performances of the different groups on a beam walking test

Table 3: shown the effects of hydromethanolic extract of bitter leaf at different concentration on the inverted screen maze test.

Inverted screen maze test							
GROUPS	DOSE	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	MEAN
CONTROL	0mg/kg/ day	13.20±7.12*	9.80±2.75	10.20±3.76	9.00±3.29	13.80±6.64	10.7±8.39
GROUP B	100mg/kg/day	33.40±17.14*	12.80±4.58	12.80±4.42	14.00±7.86	12.00±5.29	12.9±9.82
GROUP C	200mg/kg/day	27.60±10.91	42.60±20.86	20.20±9.15	42.00±20.33	23.80±8.15	39.05±17.35
GROUP D	400mg/kg/day	16.00±7.12	26.80±8.12	11.80±1.56	21.20±11.34	18.00±4.43	23.45±10.64
GROUP E	800mg/kg/day	30.20±8.13	18.40±4.20	35.00±9.57	33.40±8.86	30.80±8.09	36.95±9.71

Values are Mean ±S.E.M, n=5, significance is set at $p < 0.05$ mean values are statistically significant compared with the control group.

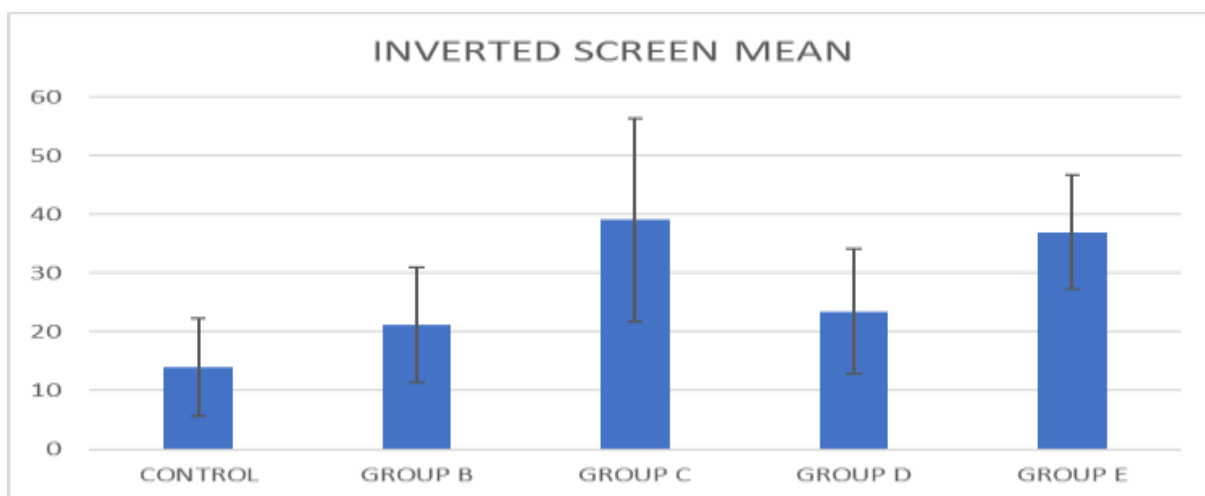


Fig 3: mean performances of the different groups on an inverted screen test

Table 4: shown the effects of hydromethanolic extract of better leaf of different concentration on the swimming test.

Swimming test							
GROUPS	DOSE	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	MEAN
CONTROL	0mg/kg/ day	14.00±2.07	3.80±0.80	2.60±0.40	3.60±1.12	5.00±1.84	7.25±1.55
GROUP B	100mg/kg/day	20.40±10.44	7.2±3.98	6.00±0.95	7.60±4.00	7.20±2.73	12.1±5.52
GROUP C	200mg/kg/day	31.20±3.76	5.00±0.84	4.40±0.98	5.40±2.14	5.40±1.72	12.85±2.36
GROUP D	400mg/kg/day	9.80±2.71	2.6±0.60	9.40±4.02	7.00±1.05	6.60±1.94	8.85±2.58
GROUP E	800mg/kg/day	20.60±6.69	4.00±1.30	4.80±1.39	6.00±1.45	8.60±3.60	11±3.60

Values are Mean ± S.E.M, n=5, Significance is set at p<0.05 mean values are statistically significant compared with the control group.

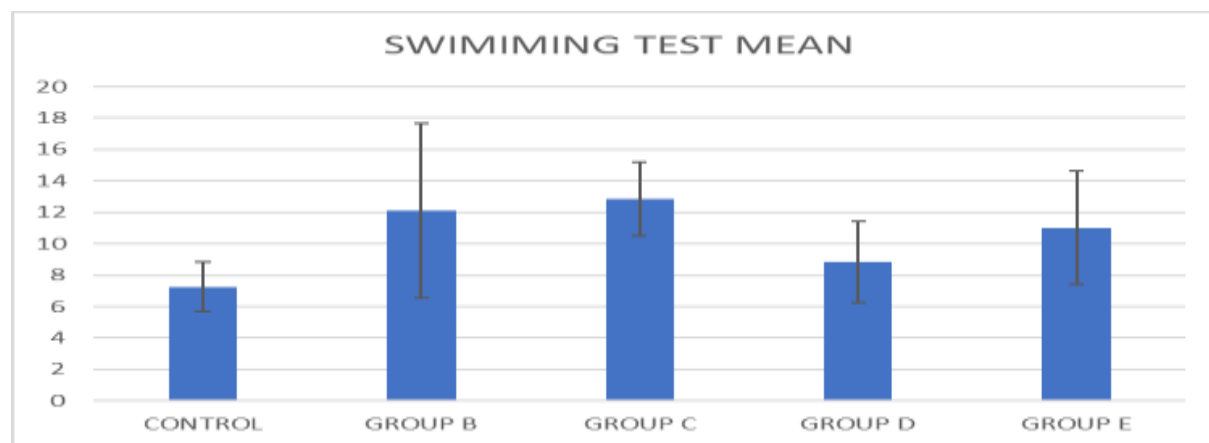


Fig 4: mean performances of the different groups in a swimming test

Table 5: shown the effects of hydromethanolic extract of bitter leaf of different concentration on the force swimming test.

Force swimming test							
GROUPS	DOSE	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5	MEAN
CONTROL	0mg/kg/ day	19.80±4.15	32.60±5.49	27.20±6.88	24.80±5.15	24.00±6.30	32.1±6.99
GROUP B	100mg/kg/day	21.60±5.82	23.00±5.74	20.40±3.53	18.00±6.79*	12.40±2.04	23.85±5.98
GROUP C	200mg/kg/day	33.20±7.44	19.40±6.21	13.20±4.04	11.40±3.76*	12.20±3.44	22.35±6.22
GROUP D	400mg/kg/day	9.80±1.99	19.00±1.52	8.40±2.40	15.60±2.32*	13.60±2.25	16.6±2.62
GROUP E	800mg/kg/day	16.80±2.62	24.20±5.59	26.00±5.84	20.60±6.27	19.80±3.53	26.85±5.96

Values are Mean ± S.E.M, n=5, Significance is set at p<0.05 mean values are statistically significant compared with the control group.

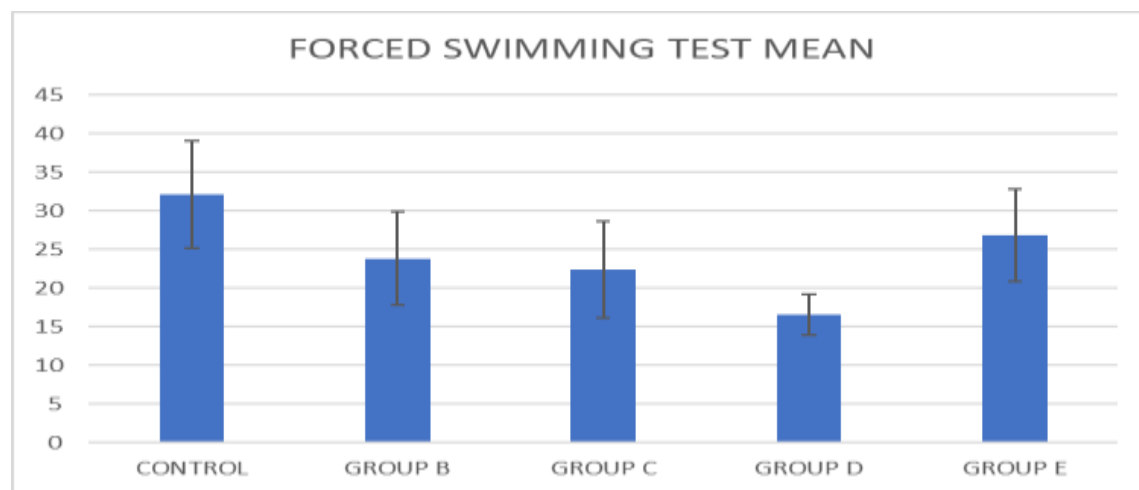


Fig 5: mean performances of the different groups in a forced swimming test

Results of handgrip, beam walking, inverted screen, swimming test and forced swimming test indicated that individual behavioral differences were so pronounced that the result has a very wide standard error of mean. This wide mean error suggests that group distinction was not observed across the treatment groups of the different experimental studies, in spite of these there were some observed groups performances among the different studies.

Handgrip test group C had the longest handgrip time which was observed in group C was not distinctive enough to cause significant difference between it and the other groups. Beam walking expresses slower result as the treatment dose increases. This could be attributed to the non psychedelic effects of the treatment hence the more the treatment doses the slower the performance rate.

Result of the mean value of Inverted screen test also follow similar trend, however, group C and E expressed higher strength to hold onto the inverted screen when compared to the other groups. This means that the effects of bitter leaf extract have high levels of functionalities on the neuromuscular system of the body. It also implies that the effects of bitter leaf extract have high levels of

functionalities on the neuromuscular system of the body. Studied by [29] and [30] revealed that *Vernonia amygdalina* possessed some properties, anti-oxidant, anti-inflammatory, anti-biotic, anti-septic, anti-allergic and analgesic effects. Which protect the brain cells and tissues from being damage, since that the brain is a complex organ the control the entire body parts. The brain comprised 100 billion neurons or nerve cells which linked in networks, thereby give rise to an amazing collections of cognitive functions. The inability to performed cognitive tasks for worked. The neural functions begin to reduce into a mixed of knots that interfered with neurotransmitters in the nerve cells. Although, problems with memory and cognition come in different forms and for many reasons from chronic or acute stress to brain trauma or aging, neurotransmitters do a wide range of things, and excess or deficiency of them plays a role in many different diseases. This study suggests that methanolic extract of bitter leaves have a potential of neuronal cured in the cerebral cortex and muscle nerves, thereby stimulate the uptake of neurotransmitters in the neuronal environment and sensory functions of the muscle tones. Based on this study, it is recommended that people should

develop the habit of eating bitter leaf because it has emmensed medicinal

values in the treatment of arthritis, stroke and rheumatism.

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