

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

ABSTRACT

Ocimum gratissimum (O.G.) (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 O.G 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 in handgrip test, groups 2, 3 and 5 with hanging time of 11.7 ± 1.18 , 13.55 ± 1.37 and 11.5 ± 0.52 respectively were significantly different ≤ 0.05 from the control with 6.6 ± 1.13 second hanging time. Beam walking had a dose dependent slowness in walking across the beam among the treated groups as compared with the control. Inverted screen test has group 3 with the highest performance time of 20.55 ± 8.42 which was significantly higher than the control with 8.4 ± 1.98 seconds. This result reveals that the administration of O.G. have significant effect on the cognitive, coordination and neuromuscular functions of the albino rats. It also shows a window of effectiveness of the extract on neuromuscular activities.

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

INTRODUCTION

Ocimum gratissimum (O.G.) also known as African basil, clove basil or wild basil. It is commonly called scent leaf due to its pungent aroma (Kabir et al (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 properties 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).

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 nevertheless, 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 O.G. 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.G. 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).

There have been lots of research works carried out on the herb, O.G.. Although, the effect of O.G.extract on the neuromuscular system of wistar albino rat is rare, however,,

MATERIALS AND METHODS

Experimental Materials

This research work was carried out in Animal and Environmental Biology laboratory, University of Port Harcourt, Choba, Port Harcourt. Rivers State, Nigeria. Fresh leaves of O.G. (scent leaf) were purchased from the market in Trans-Amadi, Port Harcourt. The leaves were

authenticated as O.G. 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 aqueous extract preparation, the leaves were rinsed, air dried and pulverised. One kilograms (1kg) of the pulverized O.G. was macerated for 24 hours and filtered with Whatman filter paper (N0:1). Water was evaporated from the filtrate using rotary evaporator and water bath. The aqueous extract thus obtained was stored in sterilized amber colored bottles and maintained at 4°C 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 into different doses of 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg for groups 2,3,4 and 5 respectively; While group 1 served as the Control. Group 2-5 were administered half of the daily doses of the extract morning and another half in the evening. At the end of one month of treatment, 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.G. 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.G. 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.G. 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. Thereafter, 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 O.G. has been investigated on neuromuscular system of Albino Wistar rat, after been subjected to various scientific and investigative assays; each parameter is represented on the different graphs below.

Handgrip test:The rats in groups 3 and (200 mg/kg/day) exhibited high muscle strength by having a hang time of 13.55 ± 1.37 seconds compared to the control (Group one) with 6.6 ± 1.13 seconds . This can be attributed to the effect of the drugs on the muscle tone of the rat. Also in Group 2 (100mg/kg/day) 11.7 ± 1.18 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 (fig.1)

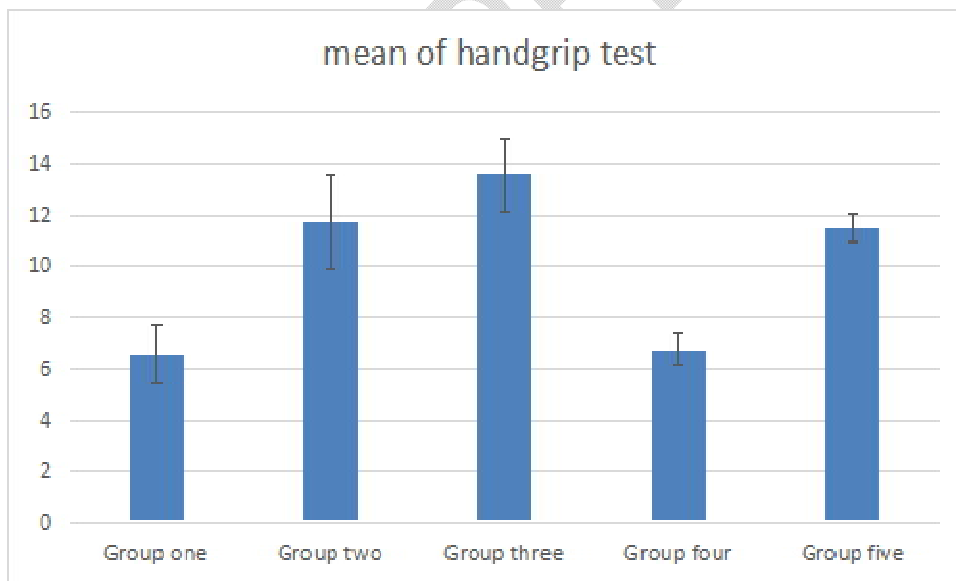


Fig. 1: Effect of aqueous extract of O.G. on Handgrip Test of Albino rats

Beam walking: Comparing the result gotten from Group one (control) with other results, it shows a trend, the control has the fastest time of 10.35 ± 2.63 seconds while the treatment groups have a dose dependent slowness from treatment group 2 to group 5 (Fig 2) the longer time taken to cross the beam the slower the animals and the less coordinated is the animal to walk on the beam.

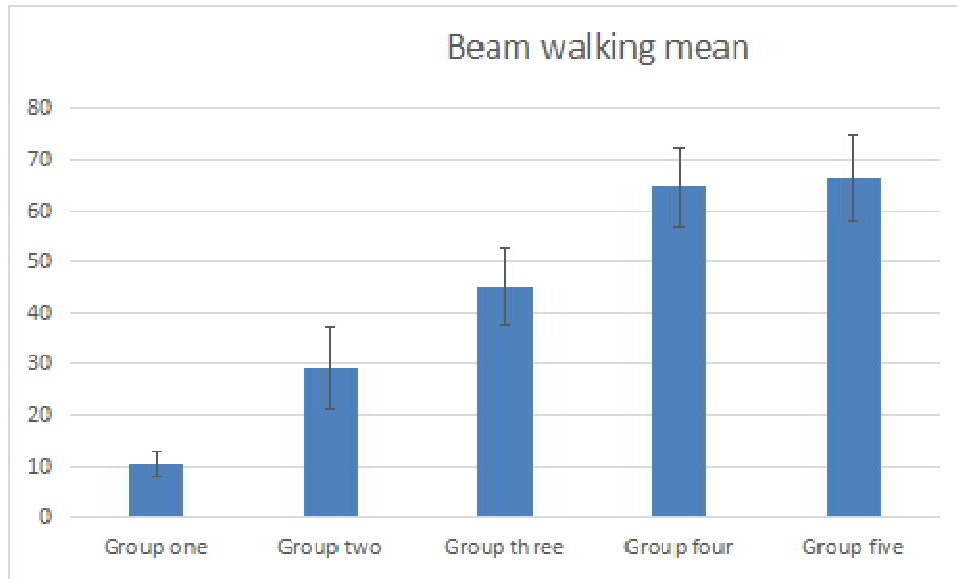


Fig. 2: Effect of aqueous extract of O.G. on Beam walking Test of Albino rats

Inverted screen test: When comparing the result gotten from Group one (control) with other results it shows that group 3 had the highest muscle strength holding unto the inverted screen with a holding time of 20.55 ± 8.42 seconds hanging on the screen. Group 5 (800mg/mg/day) was further seen as the group with the second highest holding time with 12.05 ± 3.46 seconds, while the control had a spent time of 8.4 ± 1.98 holding unto the inverted screen (Fig.3).

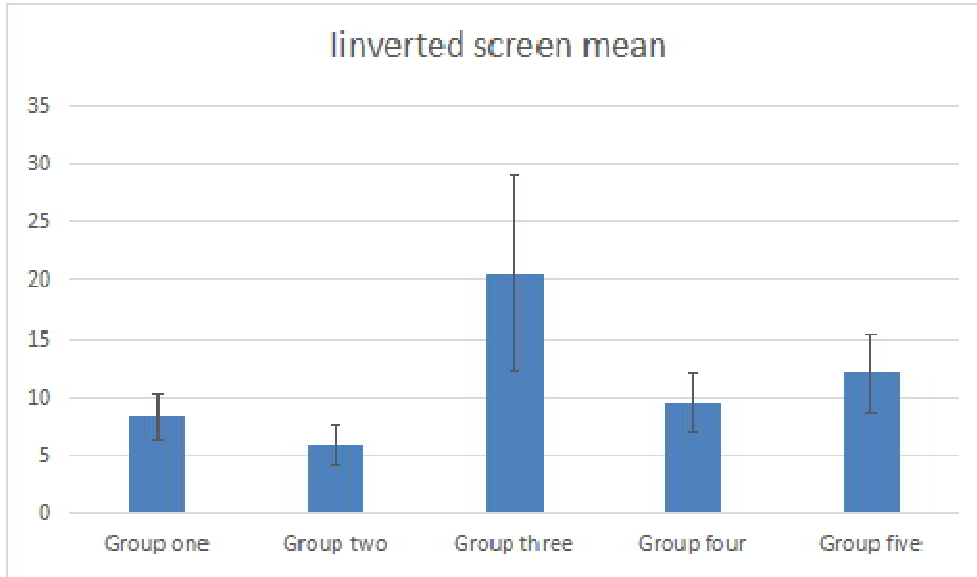


Fig. 3: Effect of aqueous extract of O.G. Inverted screen Test of Albino rats

Force swimming test: The rat in group 3 (400mg/kg/day) had the longest swimming time of 99.65 ± 9.09 seconds, group 4 with the shortest time of 36.35 ± 7.29 , compared to the control a swimming time of 72.6 ± 23.27 . The responses were not in an observable dose dependent fashion and the results were not statistically significant across the groups (Fig. 4).

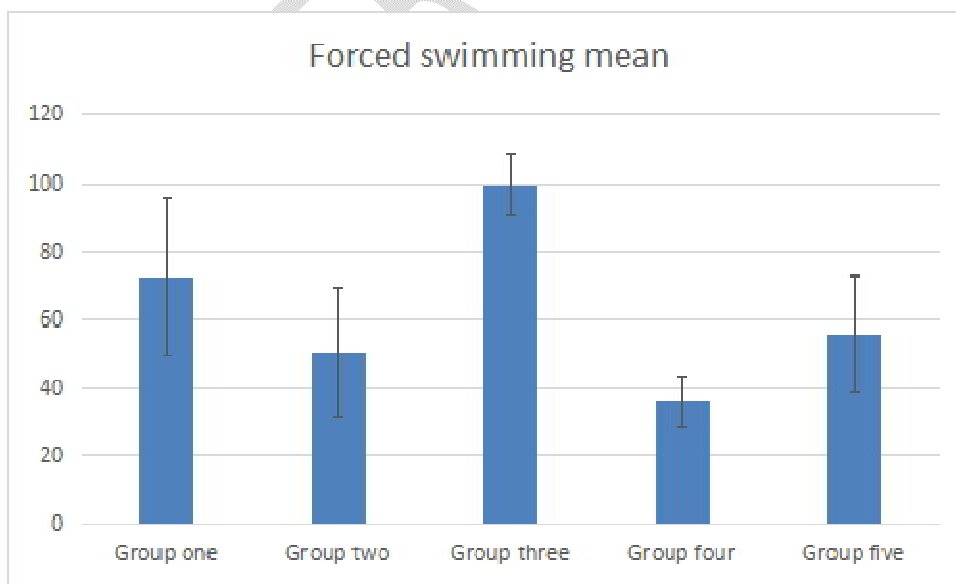


Fig. 4: Effect of aqueous extract of O.G. on Force swimming Test of Albino rats

Swimming test : In swimming test the Group 1 (control) had 46.2 ± 13.72 seconds compared to the Group 3 (200mg/kg/day) with 71.8 ± 18.70 seconds. Group 4 and 5 also had swimming times of 66.2 ± 19.92 and 65.15 ± 15.27 respectively. The differences in results were not statistically significant among the different groups. (Fig. 5).

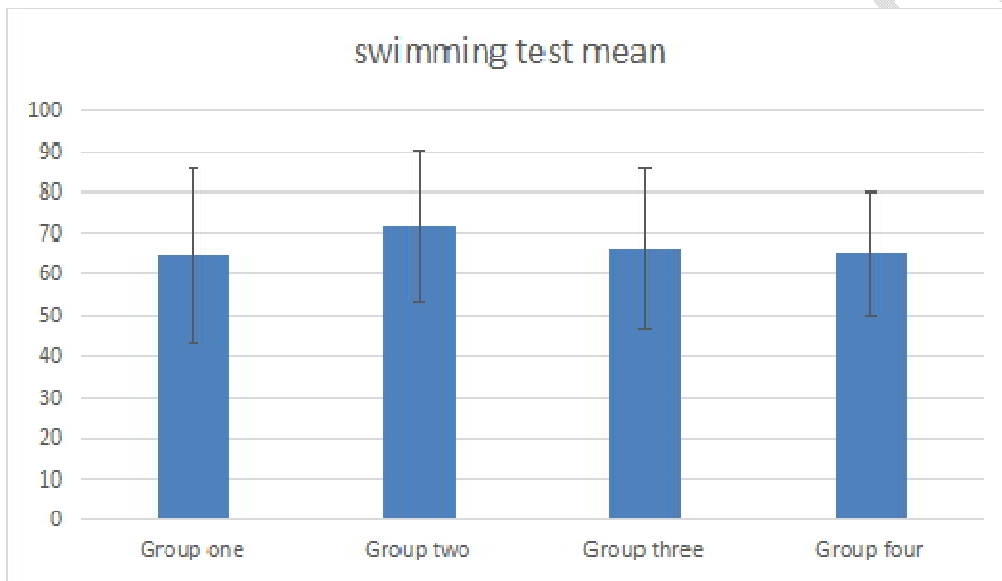


Fig. 5: Effect of aqueous extract of O.G. Swimming Test of Albino rats

Discussion

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.

Result in handgrip test, inverted screen test and forced swimming test demonstrated that muscular flexor were influenced by the doses of the extract as it was demonstrated in the overwhelming performances of animals in group 3 at a significant differences when compared to the control.

It is also observed that group five with the highest dose O.G. 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.

conclusion

It could be concluded that aqueous extracts of O.G. 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

Recommendation

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.

References

- Achinewu S. C., Aniena M. I., (1995): Studies of spices of food value in the South Eastern states of Nigeria: Journal of African medicinal plants; vol. 18, Pg. 135- 139.
- Aguiyi , J. C. , Obi, C. I. , Gyang, S. S., Igweh,A. C. (2000). Hypoglycaemic activity of *Ocimum gratissimum* in rats. *Fitoterapia* .71(4): 444-6.
- Carter A.P, Clemons W.M (Jr), Brodersen D.E, Morgan Warren R.J, Hartsch T.(2001) Assessment of sensorimotor and cognitive deficits induced by a moderate traumatic injury in the right parietal cortex of the rat. *Neurobiol Dis* 2001.8(6): P.1082- 93.10
- Chen, M. D., Lin, P. Y.; Tsou, C. T.; Wang, J. J. and Lin, W. H. (1995). Selected Metal Status in Patients with non insulindependent diabetes Mellitus. *Biology of Trace Element Research*. 50(2): 119 – 124.
- Chummy S. S. (2006): Last's Anatomy, Regional and Applied, 11th Edition: Churchill Living stone, London. Pg. 225. Ford, R. U. (1999).

- Edeoga H. O., Eriata D.O. (2001): Alkaloids, Tannins and contents of some Nigerian medicinal plants: *Journal of medicinal aromatic plants science*; vol. 23, Pg. 21-25.
- Ijeh I. I, Njoku O. U., Ekenza E. C. (2004): Medicinal evaluation of *Xylopiya aethiopica* and *Ocimum gratissimum*: *Journal of medicinal aromatis science*; vol. 26 (1) Pg. 44-47.
- Ijeh I.I, Omodamiro OD, Nwanna IJ.(2005) Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimum gratissimum* and *Xylopiya aethiopica*. *Afr J Biotech* 2005; 4: 953-6.
- Kabir OA, Olukayode O, Chidi EO, Christopher CI, Kehinde AF.(2005) Screening of crude extracts of six medicinal plants used in South- West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complement Altern Med* ; 5: 1-7.
- Kamboj V. P.(2000,) Herbal medicine are the synthesis of therapeutic remedies. *Current Science Alert* 78 (1)p.35. 10,
- Madeira SVF, Matos FA, Leal-Cardoso JH, Criddle DN.(2002) Relaxant effects of the essential oil of *Ocimum gratissimum* on isolated ileum of the guinea pig. *J Ethnopharmacology* ; 81: 1-4.
- Maity A(1), Pore N, Lee J, Solomon D.O, Rourke D. M., 2000. Epidermal growth factor receptor transcriptionally up-regulates of cancer. *Cancer Res.* 2000 Oct 15:60 (20): 5879-86.
- Matasyoh, Lexa G., Matasyoh , Josphat C., Wachira Francis N., Kinyua, Miriam G., Thairu Muigai, Anne W., and Mukiama, Titus K. (2007) Chemical composition and antimicrobial activirtiy of the essential oil of *Ocimum gratissimum* L growing in Eastern Kenya. *African Journal of Biotechnology* 6(6)pp760-765.
- Ubulom P., Akpabio, E., Udobi, C.E. and Mbon, R. (2011). Antifungal Activity of aqueous and ethanolic extracts of *Picralima nitida* seeds on *Aspergillus flavus*, *Candida albicans* and *Microsporum canis*. *Research in Pharmaceutical Biotechnology*, 3(5): 57-60.]
- Zitte L.F. and Unegbu C.E. (2019) Hepatoprotective activity of aqueous extract of oyster mushroom (*Pleurotus ostreatus*) against lead -induced hepatotoxicity in albino wister rat (*Rattus norvegicus*). *World journal of advanced Reviewed and Reviews* 3(3) 33-37