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Pleurotus tuber Regium: Ethanolic Extract Improves Spatial Learning and Memory in Rats but Alters Cerebral Cytology

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ABSTRACT

This work was carried out to determine possible toxic effects of *Pleurotus tuber regium* (an edible fungus) on the histology of the cerebral cortex (prefrontal cortex), spatial learning and memory in rats after its consumption. Twenty one adult Wistar rats weighing 150-200g were used. The animals were randomly divided into three groups: control, low and high test groups. The control group received distilled water while the test groups received 25mg/kg and 50mg/kg of the ethanolic extracts of *Pleurotus tuber regium* as 0.5 ml and 1ml for the low and high doses, respectively. Administration was done orally with the aid of orogastric tube for 4 weeks. The tests for visuo spatial learning and memory were carried out concurrently with the administration. The animals were sacrificed 24 hours after the last administration after being anaesthesized with diethyl ether. The brains were preserved in bufferred formaldehyde, and were processed and stained with haematoxylin and eosin method. Results showed dose dependent atrophy in the cells of the cerebrum. The swim latency and quadrant duration were significantly higher ($p < 0.05$) in the 50 mg/kg group (high dose group) implying that the extract had a dose dependent enhancing effect on learning and memory. In conclusion, the extract of *Pleurotus tuber regium* improved learning and memory in rats but presented untoward effect at a higher dose.

Keywords: *Pleurotus tuber regium, Ethanolic extract, Cerebral cytology, Neurobehaviour, Wistar rats*

INTRODUCTION

Mushroom resources are important in medicine, industry and agriculture (Olufokunbi and Chiejina 2013). It comprises a vast and yet largely untapped sources of powerful new pharmaceutical products as it is rich in bioactive compounds that help in the modulation of metabolic process which results in the promotion of better health (Opige et al. 2006; Okhuoya et al. 2013). The use of mushrooms in traditional ancient therapies dates back at least to the Neolithic age. For millennia, mushrooms have been valued as medicinal provisions for humans (Wasser 2014). *Pleurotus tuber* Regium, the king tuber mushroom, is an edible gilled fungus native to the tropics, including Africa, Asia and Australia. It has a history of economic importance in Africa as food and as a medicinal substance (Isikhuemhen and Le Bauer 2004). It is available as a white to brownish solid mass buried under the ground, with a dark brown on the outside and white on the inside (Jonathan et al. 2008). It is called Katala in Hausa, Umoho in Igede, Erousu in Igbo, Kukpatu in Yakurr

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and Isuo in Efik language.

Pleurotus tuber Regium is a saprotroph found on dead wood. As the fungus consumes the wood, it produces a sclerotium, or storage tuber, either within the decaying wood or in the underlying soil. It can also be produced from artificial substrates (Apertorgbor et al. 2013; Olufokunbi and Chejina 2013). Both the sclerotium and the mushroom are edible. The sclerotium, which is hard is peeled and grounded for use in preparation of soup. It is rich in protein, carbohydrate, ash, crude fibre and potential source of nutraceuticals (Ikewuchi and Ikewuchi 2008). Several workers have reported the antibiotic potentials of many macro fungi (Olawuyi et al. 2010). Some people use it as food while others use it as medicine (Aina et al. 2012; Jonathan et al. 2012). *Pleurotus* species belongs to phylum *Basidiomycota* and produce oyster shaped mushrooms (basidiocarps). *Pleurotus* species grow on various types of ligonocellulosic uncomposted agro-waste and produce mushroom rich in high value proteins, vitamins and minerals (Apertorgbor et al. 2013). Mushroom contains very low amount of carbohydrates, sugars and a very little amount of cholesterol (Barros et al. 2008; Oranusi et al. 2014). It is used as a partial replacement for melon seed in traditional preparations of sauces and soups (Nwokolo 1987). The sclerotium is inexpensive and used in vegetable soup and in the preparation of 'melon cake', a highly prized item considered a delicacy and presented at traditional marriage ceremonies in the south eastern part of Nigeria, particularly among the Annang tribe of Akwa Ibom State (Oral communication).

Phytochemistry revealed saponins, alkaloids, flavonoids, phytates, tannins and oligosaccharides. The ether extract showed crude protein, fibre, calcium, magnesium, potassium, sodium, copper, zinc, iron, manganese and phosphorus (Ijeh et al. 2009).

The polysaccharide of *Pleurotus tuber regium* attenuated hyperglycemia and oxidative stress in experimental diabetic rats (Huang et al. 2012). The anti-diabetic, anti-hyperlipidemic and antioxidant properties of *Pleurotus tuber regium* were examined in experimental diabetic rats. The polysaccharides inhibited the lipid peroxidation index (malondialdehyde) and restored superoxide dismutase and glutathione peroxidase activities in the liver of diabetic rats (Korivi et al. 2012). It exhibited antitumour activity against human tumour cell lines (Zhang et al. 2001). The polysaccharide extract also enhanced the immune system of mice by activating macrophages and related enzymes and inducing the release of cytokines (Wu et al. 2014). Dietary incorporation of *Pleurotus tuber regium* on body organs resulted in a non-significant effect on total triacylglycerol concentration and relative weight of vital organs (Ijeh et al. 2009). The hepatoprotective activity of *Pleurotus tuber regium* is reported in literature (Sukumar et al. 2015). Mushroom species including *Pleurotus* is described as a rich source of food nutrients with antitumour and anti-inflammatory properties (Chatterjee and Patel 2016).

Herbal preparation containing *Pleurotus tuber regium* is used against headache, stomach ailments, cold and fever, as well as asthma and high blood pressure (Oyetayo 2011). Herbal remedies are complex mixtures in which the active components may not be known or may be a small percentage of the total components. Some are believed to achieve their beneficial effects through the combined actions of several components. Little is known about the chronic toxicities that might be associated with their prolonged use. Moreover, there have been few research work on possible toxicity that might be associated with high doses or chronic consumption of *Pleurotus tuber regium* as a herbal remedy. Hence this study is worthwhile.

The aim of this study was to determine the effect of the ethanolic extract of *Pleurotus tuber regium* on the morphology and histology of the cerebral cortex of adult Wistar rats and neurobehavioural actions particularly, spatial learning and memory.

MATERIALS AND METHODS

Preparation of Extract

The sclerotium of *Pleurotus tuber* Regium was bought from Akpan Essiek market in Ukanafun local government area of Akwa Ibom State. It was identified by a taxonomist, Mr. Effa, Effa A. in the Department of Botany, University of Calabar. The sclerotium was grounded to powder (450 g), soaked for 72 hours and extracted using 60% ethanol to obtain a yield of 70 g. The filtrate was concentrated by evaporation at 40ºC in a water bath. The yield was a solid mass which was the extract used for this study.

Animals

Fifteen adult male and female Wistar rats were randomly divided into control, low dose (25 mg/kg) and high dose (50 mg/kg) groups. They were kept in the animal house of the Department of Anatomy, University of Calabar, for two weeks to acclimatize. The rats were fed daily with growers marsh produced by Vital Feeds Limited, Calabar. This work was approved by the Faculty of Basic Medical Sciences Ethical Committee on the Care and Use of Laboratory Animals in University of Calabar.

Extract Administration and Brain Removal

Control group received distilled water orally while the test groups received 25 mg/kg and 50 mg/kg of *Pleurotus tuber regium* extracts rats orally through orogastric tubes for twenty eight (28) days. Animal handling was in line with the guidelines given by the

Institutional Committee on Animal Care and Use and the National Academy of Sciences guide for handling Laboratory Animals. The animals were subjected to test for visuospatial learning and memory using the Morris water maze method. The experimental animals were anaesthetized with diethyl ether, perfused with normal saline and 10% buffered formaldehyde. The brains were dissected out and preserved in the same fixative. The frontal lobe was sectioned and processed for histological staining with haematoxylin and eosin stains.

Statistical Analysis

Data obtained from this study were analyzed using analysis of variance and a post hoc test, least square difference (LSD) was done using the SPSS package. Results were represented in bar charts, line graph and histogram

RESULTS

Histological Observations

Histologically the following results were obtained: The section of the cerebral cortex of the control group showed normal cerebral histology which comprised molecular layer (1), external granular layer (2), external pyramidal layer (3), inner granular layer (4), inner pyramidal layer (5) and polymorphic layer (6) designated as layers 1 to 6. The layers are not so well delineated physically but the cells distinguished them. The neuronal cells (NCB) were prominent with triangular to round shaped deeply stained nuclei and a rim of eosinophilic cytoplasm. The cells within the granular cells layers were deeply packed (Figures 1 and 2). For the 25 mg/kg of *Pleurotus tuber regium*, sections showed distinct cerebral cortex with intact layers. The neuronal cell bodies were prominent with well outlined cytoplasm and nuclei. There was hypertrophy of neuronal cell bodies observed (Figures 1B and 2B). For the 50 mg/kg of *Pleurotus tuber regium* ethanolic extract group. Sections showed abnormal histological features of the cerebrum. There was atrophy of cell bodies and mild vacuolations of cells observed in sections (Figures 1C and 2C).

Neurobehavioral Observations

Swim latency: There was a significant difference between the values obtained for control (44 seconds) and the 50 mg/kgw group (26 seconds) but not in the 25 mg/kgw group (30 seconds), both in the acquisition training and reversal training. This implies that

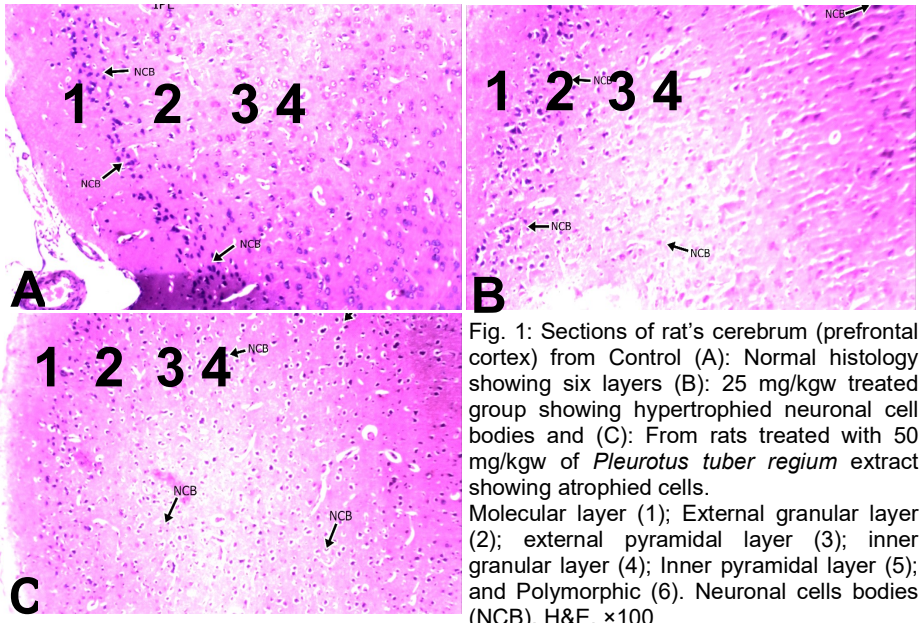

Fig. 1: Sections of rat's cerebrum (prefrontal cortex) from Control (A): Normal histology showing six layers (B): 25 mg/kgw treated group showing hypertrophied neuronal cell bodies and (C): From rats treated with 50 mg/kgw of *Pleurotus tuber regium* extract showing atrophied cells.
Molecular layer (1); External granular layer (2); external pyramidal layer (3); inner granular layer (4); Inner pyramidal layer (5); and Polymorphic (6). Neuronal cells bodies (NCB). H&E, ×100

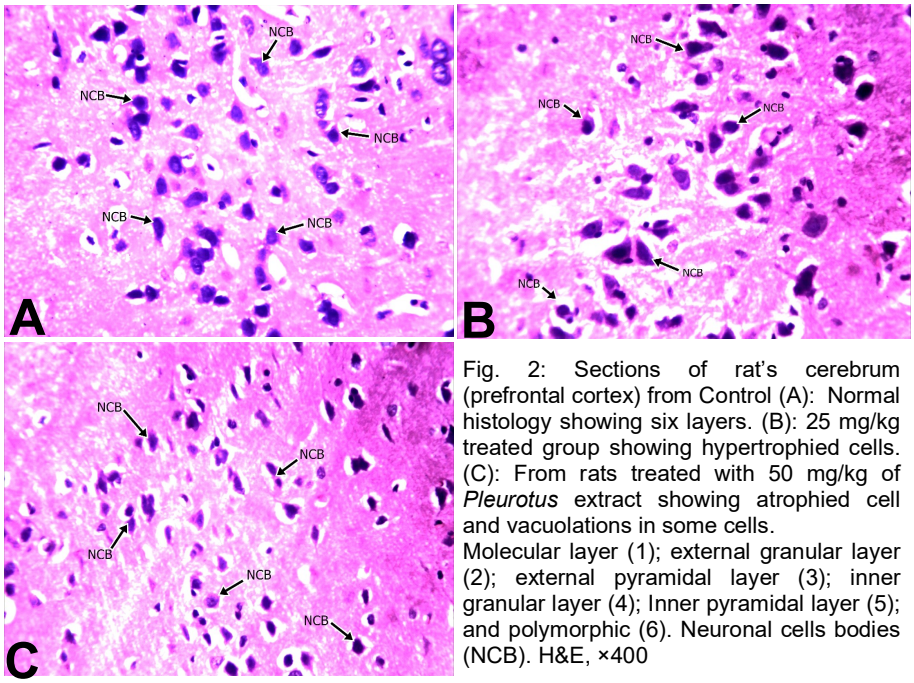

Fig. 2: Sections of rat's cerebrum (prefrontal cortex) from Control (A): Normal histology showing six layers. (B): 25 mg/kg treated group showing hypertrophied cells. (C): From rats treated with 50 mg/kg of *Pleurotus* extract showing atrophied cell and vacuolations in some cells.
Molecular layer (1); external granular layer (2); external pyramidal layer (3); inner granular layer (4); Inner pyramidal layer (5); and polymorphic (6). Neuronal cells bodies (NCB). H&E, ×400

animals in the test group that received high dose of the extract had improved memory and learning activities when compared with animals in the control group (Figures 3 and 4).

The quadrant duration between the tests and control rats is shown in Figure 5. Animals in the Group that received the extract at the dose of 25 mg/kg weight of rat spent more significant time ($p<0.05$) (28 seconds) in the North-East quadrant than the control (13 seconds) and the animals that received extract 50 mg/kg weight of rats (16 seconds). However, in the South-West quadrant the quadrant duration of the 50mg/kg group (24 seconds) was significantly ($p <0.05$) higher compared to the control (15 seconds) and low dose group (16 seconds). In the North-West quadrant there was reduced quadrant duration in the low dose and control groups compared with high dose with values, 24, 10 and 11 seconds for control, low dose and high dose groups respectively. Animals in the group that were tested with the extract at a dose of 50 mg/kg weight (high dose) had increased learning and memory activities (Figure 5).

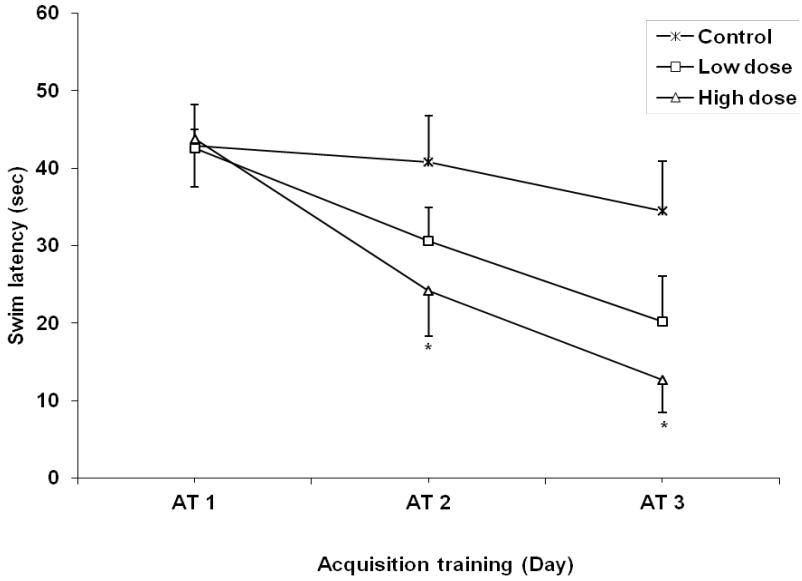

Fig. 3: Comparison of swim latency in the different experimental groups during acquisition training on days 1, 2 and 3 of Morris water maze test. Values are expressed as Mean ± SEM, n=5. * Significantly different from the control at $P < 0.05$

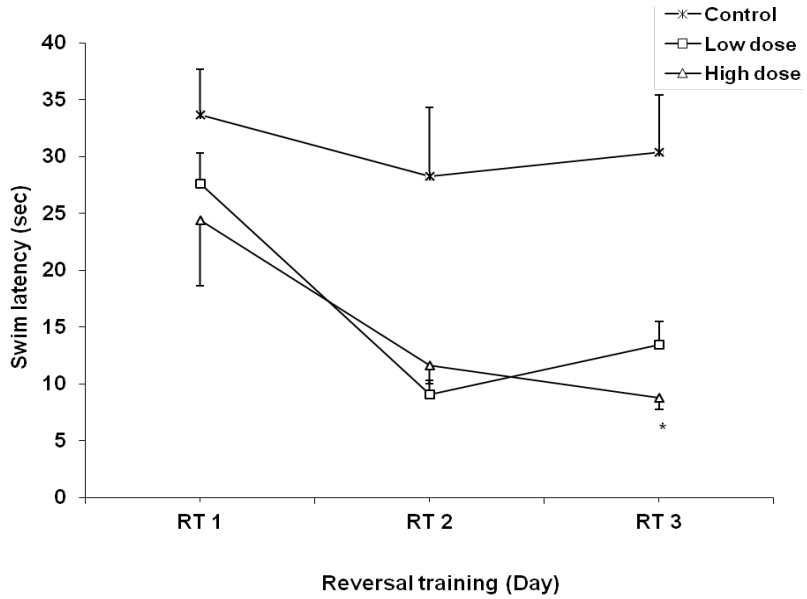

Fig. 4: Comparison of swim latency during reversal training on days 4, 5 and 6 of Morris water maze test in the different experimental groups. Values are expressed as Mean ± SEM, n=5. * Significantly different from the control at $P < 0.05$

DISCUSSION

Mushroom is one of the sources of food nutrients and pharmacologically useful substances (Oyetayo 2011). The cell wall of mushroom fruiting body contains some non digestible macromolecules that are a rich source of dietary fibre with biological functions that are beneficial to human health (Lei and Cheung 2014).

The results of this study revealed that *Pleurotus tuber regium* has an enhancing effect on memory and learning. This may be attributed to its constituents. Mushroom contains protein, fibre, vitamins, mineral elements (such as potassium phosphorus, calcium, magnesium, copper iron zinc, selenium) and low fat. It contains amino acids with glutamic acid and aspartic acids being the most abundant (Barros et al. 2008; Valverde et al. 2015). Amino acid plays an important role in protein synthesis and metabolism. Glutamic and aspartic acids are excitatory neurotransmitters in the central nervous system (CNS). Glutamic acid is important in sugar and fat metabolism and supplies glucose to the brain. It plays a role in thinking and memory and facilitates potassium transfer through blood brain barrier (Lohrey 2015). These constituents are proposed to be responsible for the observed improvement in the learning and memory potential in the test group of the experimental animals which received a higher dose of the extract, 50 mg/kg weight.

Research has shown that the plant kingdom harbours a great number of active ingredients which are valuable in the management of many diseases. Extracts

of mushrooms and the isolation of certain chemicals from them have been used independently or in combination with other chemical substances to produce antibiotics and drugs (Pöder 2005). The mushroom isolates; lentinan, polysaccharide krestin and polysaccharide peptide are licensed pharmaceuticals in certain countries (Pöder 2005). The mushroom isolates actually of great pharmacological and economic importance (Stadler and Hoffmeister 2015).

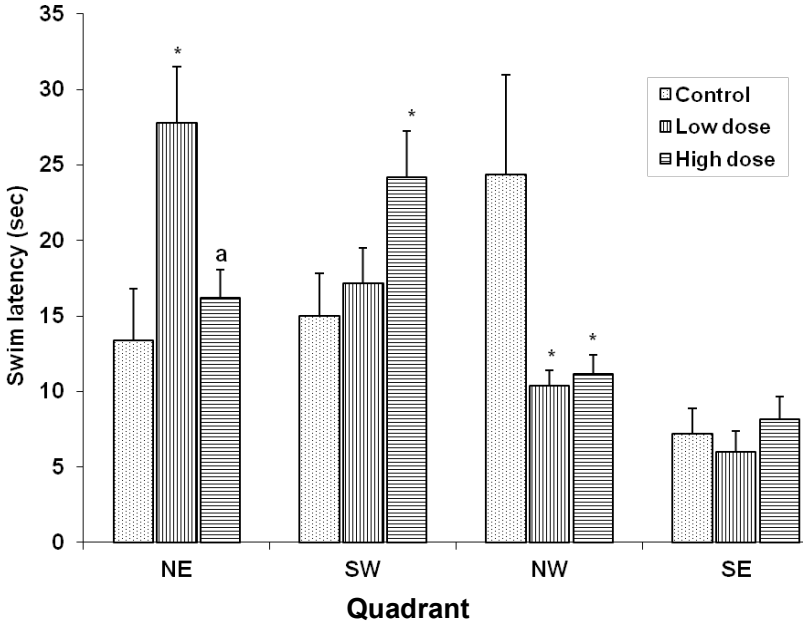

Fig. 5: Comparison of swim latency different groups during probe trial on day 7 of Morris water maze test. Values are expressed as Mean ± SEM, n=5. * Significantly different at P < 0.05; ** P < 0.01 compared with the control. a= p < 0.05 compared with the low dose.

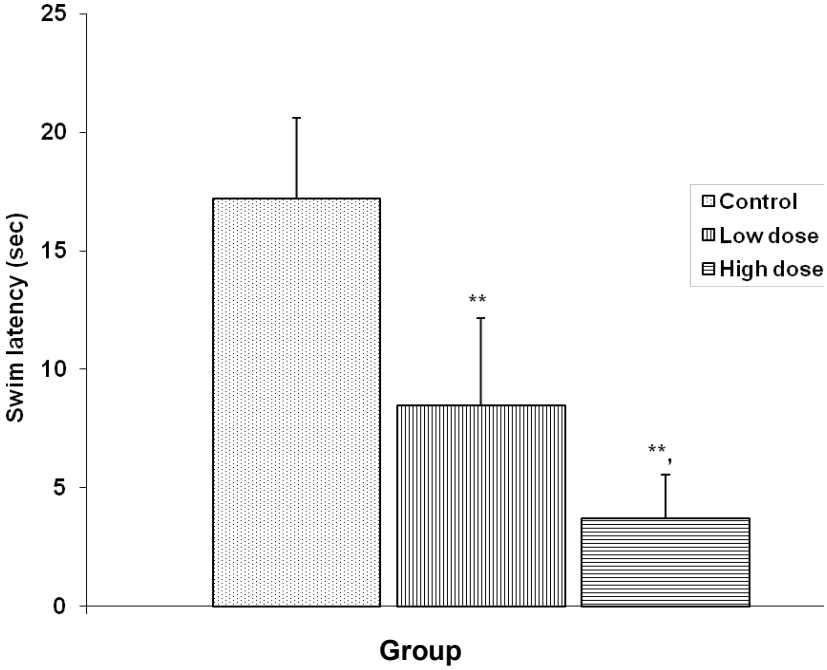

Fig. 6: Comparison of swim latency during visible platform on day 7 of Morris water maze test in the different experimental groups. Values are expressed as Mean ± SEM, n=5. * Significantly different; P < 0.05. ** P < 0.01; *** P < 0.001, compared with the control. a= p < 0.05 compared with the low dose

Herbal remedies of medicinal mushrooms have been used as food or in dietary supplements which have proved to be medicinally beneficial. In the United States for example, an extract from the mushroom *Coriolus versicolor* has been formulated into a product that is sold as a dietary supplement (Roupas et al. 2012). Nowadays, medicinal mushrooms are used as dietary food and dietary supplement products (the product is fast growing and has a value of more than 18 billion US dollars per year) (Wasser 2010; Chang and Wasser 2012). Awareness on the importance of several mushroom species namely, *Ganoderma lucidum* is increasing (Wasser 2014). There is also a long history of traditional use of mushrooms as curatives in Meso-America (especially for species of the genus *Psilocybe*), in Africa (Yoruba populations in Nigeria and Benin), Algeria, and Egypt (Chang and Wasser 2012). Mushroom polysaccharides prevented oncogenesis, showed direct antitumour activity against various synergetic tumours and prevented tumour metastasis (Wasser 2014). Their activity is especially beneficial when used in conjunction with chemotherapy. Mushroom polysaccharides were also able to induce gene expression of various immunomodulating cytokines and cytokine receptors (Lee and Kim 2014).

The observed dose dependent vacuolations in the neuronal cells may be regarded as an adverse effect of the mushroom when taken at high doses. This study may not have enough scientific explanations to this but literature has shown that *Pleurotus tuber regium* has mineral contents including lead and copper at about 0.2-0.8 mg/kg (Nnorom 2011). Lead is a known neurotoxic substance (Sander et al. 2009). The authors however, explained that these concentrations are within the normal tolerable weekly levels but we suggest that a higher concentration may be attained in the body from chronic consumption leading to toxicity from cumulative effect.

CONCLUSION

Ethanolic extract of *Pleurotus tuber regium* improved learning and memory in rats in a dose dependent manner but presented dose dependent side effects seen as atrophy and vacuolations of cells found in the layers of the cerebral cortex.

Conflict of Interest

None declared.

Acknowledgment

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