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Antitoxic Effects Of Ethanoic Extract Of Oyster Mushroom (*Pleurotus Ostreatus)* Against Neurotoxic Mercury Chloride In Albino Rats (Rattusnorvegicus) On A Navigational Maze Test

Zitte, Leelee Famii and Bernard, Seleke-Ere Favour

Department of Animal and Environmental Biology, University of Port Harcourt Nigeria.

Email: Leelee.zitte@uniport.edu.ng, lefzy@yahoo.com

ABSTRACT

The antineurotoxic effects of oyster mushroom (*Pleurotus ostreatus)* against neurotoxic mercury chloride was studied using the behavior of rats (*Rattusnorvegicus*) on a navigational maze test. Twenty-four young rats were grouped into six groups of four animals per group. Group one was used as the control while groups two, three, four, five and six were treated with mushroom extract and mercury chloride at concentrations of 250mg/kg, 50µg/kg mercury and 250mg/kg mushroom, 100µg/kg mercury and 250mg/kg mushroom, 50µg/kg mercury, and 100µg/kg respectively. The animals were treated for one month then subjected to navigational maze test. The result indicated that groups one, two, three , four, five and six had mean navigational times of 172.38±83.54, 77.31±73.62, 118.55±82.13, 182.7±64.04, 242.84±99.14, 281.75±41.34 respectively. The result shows a trend of mushroom extract effects on all the treatment groups having expressed the most remarkable effect on navigational competence in group 2 compared to group one the other two sets of groups: group 3 versus group 5 and group 4 versus group 6 also show another trend of mushroom competence against mercury- induced neurotoxicity

It is therefore necessary to affirm that mushroom extract has a neutralizing effect on mercury-induced neurotoxicity.

Keywords: navigational test, mercury toxicity, mushroom, aqueous extract, neurotoxicity.

INTRODUCTION

Over the years, mercury in different forms have been found to have several toxic effects on animals, ranging from immune disorders, sensory defects, different types of neurological dysfunctions, including motor and behavioral deficits like what is commonly found in austism spectrum disorders (ASDs). The effects are also observed in a neuroanatomical test affecting the neurotransmitters and other biochemical processes. It has also been implicated in antioxidant concentrations in cells which further leads to membrane integrity loss and cellularnecrosis [1].

Mercury is one of the heavy metals it has been studied severally and it has a well- documented report of its toxicity profile. It has been implicated for inducing public health disasters in Minamata Bay Japan [2] and in Iraq [3], [4], [5]. Mercury exist in several forms: firstly, inorganic mercury such as metallic mercury and mercury vapor (Hg^0), mercurous (Hg^+) or mercuric (Hg^{++}) salts. Secondly organic mercury in which mercury is bound to carbon containing organic compound like the methyl , ethyl, phenyl groups etc) it has been noticed that in all these forms, mercury can express some degree of toxicity even at a low dose [6]. It has been reported that there are some degrees of interconversion in-vivo between the various forms of mercury. Inhaled elemental mercury vapor, for example, is easily absorbed through

mucus membranes and the lung and rapidly oxidized to other forms (but not so quickly as to prevent considerable deposition of elemental mercury in the brain. Methyl mercury is easily absorbed through the gut and deposited in many tissues, but does not cross the blood-brain barrier as efficiently as elemental mercury; however, on entering the brain it is progressively demethylated to elemental mercury [7]. On entry to the bloodstream, MeHg adheres to sulfhydryl groups, particularly to those in cysteine, and it is deposited throughout the body [8].

Human sources of mercury toxicity include contamination from eating of contaminated fishes. Atmospheric elemental mercury settling in water, where it is converted by microorganisms into organic (methyl or ethyl) mercury, which is ingested by smaller creatures and further gets bio accumulated in the food chain. At the top of the food chain are fishes like tuna, swordfish or sharks. These are finally eaten by human. Other sources of mercury include outgassing of mercury from dental amalgam, or occupational exposure to mercury [9] [10] coal burning, and mining of mercury and gold [9]. Acute exposures have been observed to have a latency period of one or more weeks; once acquired, toxic doses are cleared slowly, if at all [11]. Massive prenatal poisoning may induce a form of cerebral palsy [12]. Lesser prenatal doses have been associated with neurodevelopmental delays and cognitive deficits [13], [14], [15]

There have been several heavy metal chelating agent which are primarily used for the removal of heavy metal from the body. Some of the most commonly used are 2,3-Dimercapto-1-propanesulfonic acid (DMPS) and Dimercaptosuccinic acid (DMSA), also called succimer [16]. Heavy metal toxicities are also corrected using some plant extracts in recent years. Mushroom are widely known for its anti nociceptic, [17] ,antiinflamatory [18] hypoglycemic, hepatoprotective, effects [19] neuroprotective effects of berberine against Mercury-induced neurotoxicity [2]. The chelating effects of the mushroom -Pleurotus tuberregium extract against arsenic and chromium toxicity in albino wistar rats has also been reported [7]

MATERIALS AND METHOD

This study was conducted at the Animal house of Department of Animal and Environmental Biology Faculty science, University of Port Harcourt, Port Harcourt, Nigeria. Twenty-four albino rats weighing between 50 and 100g were used for the study. They were bought from the Animal House, of Faculty of Basic Medical Science University of Port Harcourt.

The oyster mushroom (*Pleurotus ostreatus*) was purchased from a commercial farm and it was air dried at room temperature. It was further pulverized then macerated in 70% for 72 hours it was thereafter filtered through the number 1 whatman filter paper. The process of macerationand filtration was repeated to have a greater extract recovery. Liquid recovered was then concentrated in a rotary evaporator thereafter it was further dried in a water bath. The dry extract was diluted with distilled water to the required concentration.

Mercury chloride was purchased from Goechem Choba, and diluted based on the percentage mercury content to a mercury concentration of 50µg/kg and 100µg/kg, having weighed the animals to determine their actual weights.

Experimental design

The twenty four rats were divided into 6 groups, of four rats each. Group 1 was given normal feed (control).Group 2 was given mushroom alone (250mg/kg). Group 3 was given mercury (50µg/kg) and mushroom (250mg/kg). Group 4 was given mercury (100µg/kg), and mushroom (250mg/kg).Group 5 was given mercury alone (50µg/kg). Group 6 was given mercury alone (100µg/kg). Procedures used in previous studies [19]. Cognitive behavior tests (memory test with navigational maze) were then carried out after 30 days of treatment.

Navigational maze test: This test was about the cognitive awareness and memory. It was used to test the rats in order to know how the mushroom extract controls neurotoxic effects of

mercury in rats. Navigational maze was constructed to have an entrance and an exit. The maze had a zigzag pathway to get to the exit. The animals were placed at the entrance and the time taken to get to the exit was recorded. The test was repeated five times to know the cognitive awareness of the animals and how much they could easily trace the route to the exit. Animals which could not trace the exit in five minutes (300 s) were removed and 300s recorded as their time. The result was analyzed for cognitive function using their ability to trace the exit point quickly as a sign of enhanced cognitive function.

RESULTS

Navigational maze test: In this test, the faster the animals went round the puzzle and found its exit the more enhanced memory the animal was considered. Group 1 (control) had 197.99 ± 66.00s and 157.75± 82.12s as its highest and lowest time respectively (with a mean response time of 172.38±83.54) to navigate its way out of the maze. Group 2 (mushroom alone 250mg/kg) had 139.87 ± 20.75 s and 12. 06 ±4.49 s as its highest and lowest time spent to navigate its way out of the puzzle (with a mean response time of 77.31±73.62).Group 3 (mercury 50 µg/kg and mushroom 250mg/kg) had highest and lowest values as 167.43 .47±65.85s and 54.38± 13.71s (with a mean response time of 118.55±82.13) Group 4 (mercury 100 µg/Kg and mushroom 250mg/Kg) had 300.00 ±0 and 96.06 ± 34.80 seconds as its highest and lowest time respectively (with a mean response time of 182.7±64.04) group 5 (mercury alone 50 µg/kg) had it highest and lowest time to be 300.0±0.00 and 162.5± 79.38s (with a mean response time of 242.84±99.14). Group 6 (mercury alone 100 µg/kg) had its highest and lowest time as 300.00 ± 0.00 and 258.98± 41.02 (with a mean response time of 281.75±41.34) (Fig 1) the result indicated that while mercury toxicity was noticed in groups five and six as causing weakness with poor response times the two groups even used up the allocated 300seconds for the experiment without success, as some trials indicates that mercury toxicity is a serious neurological problem. Be that as it may, mushroom extract as an antidote of this mercury was also demonstrated. This was seen when the two groups (groups three and four) at which mushroom was used as an antidote, they had a relatively faster rate compared to their corresponding groups without mushroom (groups five and six).To further buttress the efficacy of mushroom extract as an enhancer of neurological performance , group two, of which mushroom was used alone, was seen to have the fastest response with a mean response time of 77.31±73.62 seconds even faster than the control with a mean response time of 172.38±83.54 seconds.

Percentage enhancements of the mushroom extract were calculated in the two groups of 50 µg/Kg and100 µg/Kg mercury. When group three (50 µg/kg mercury and 250mg/kg mushroom) was compared with group five (50 µg/kg mercury alone) there was a 51.18% memory enhancement . it was also observed between groups four (100 µg/kg mercury and 250mg/kg mushroom) and group six (100 µg/kg mercury alone) that there was percentage memory enhancement of 35.15%. These expressed that the mushroom really shows a remarkable effect on the cognitive performance of rats in a navigational maze test.

In another comparative analysis, where every group was compared with the control, it was seen that groups 2,3,4,5, and 6 had results of 55.15%, 31.22%, -5.99%, -40.87% and -63.45% respectively. Group 2 with no mercury produced the highest percentage performance when compared with the control, while the percentage performances decreased according to the relative concentrations of mercury and mushrooms with group six with the highest concentration of mercury alone had the least performance of -63.45%. Table 1, fig 2

Table 1; Navigational maze test results indicating similarities and differences between groups

Treatment	Dose	T1 (s)	T2 (s)	T3 (s)	T4 (s)	T5 (s)	mean	% enhancement by mushrooms	% performance of the groups compared to the control
Group 1	Control	168.99 ±75.66	157.75 ±82.12	157.75 ±82.14	197.99±66.00	179.44±71.87	172.38 ±83.65		Reference value
Group 2	M.A 250mg/kg	139.87 ±20.75	112.5± 63.53	37.86± 17.71	12.06±4.49	84.25 ±72.07	77.31± 75.32		55.15%
Group 3	M.M 50µg/kg	54.38± 13.71	100±67.43	167.43 ±74.05	108.47±65.86	162.47±79.60	118.55 ±81.87	51.18 %	31.22%
Group 4	M.M 100µg/kg	131.56 ±31.56	300±0	272.25 ±27.75	96.06±34.80	113±63.766	182.7± 79.47	35.15%	-5.99%
Group 5	M.A 50µg/kg	266.48 ±33.52	162.5± 79.38	300±0	226.25±73.75	258.98±41.02	242.84 ±82.98		-40.87%
Group 6	M.A 100µg/kg	274.09 ±25.90	278.09 ±21.90	300±0	300±0	258.98±41.02	281.75 ±49.24		-63.45%

Values are presented in mean ± S.E.M (n= 4), means are statistically significant compared with the control group at $p \leq 0.05$

Key:

T1-T5 is number of trial

M.A 250mg/kg = mushroom alone

M.M= mercury + mushroom

M.A 50µg/kg and 100µg/kg = mercury alone 50µg/kg and 100µg/kg

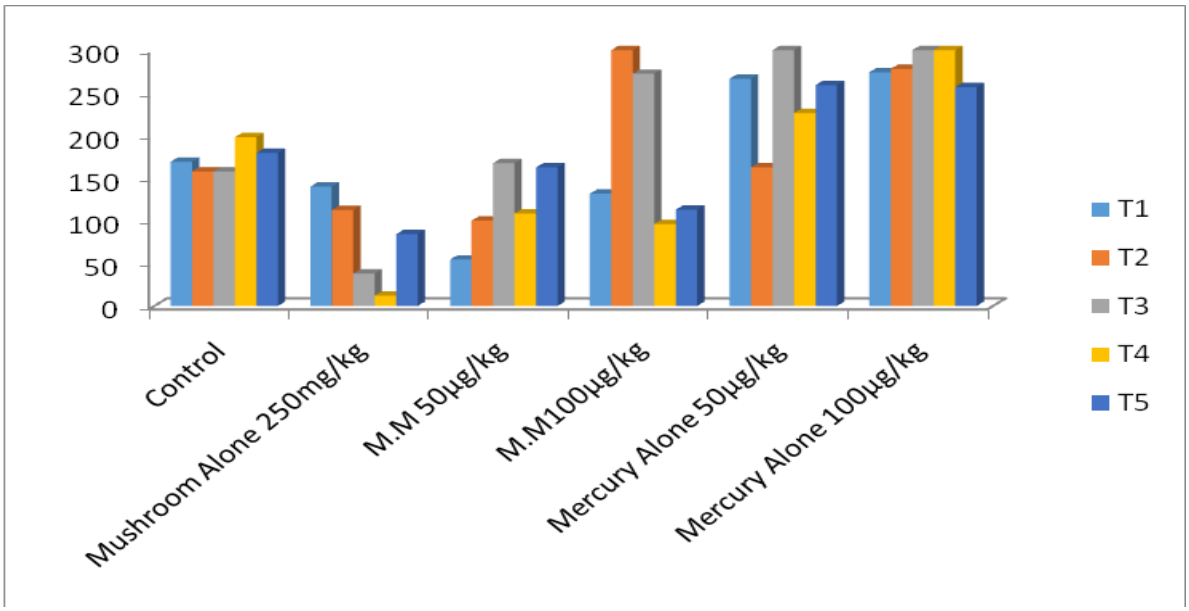

Fig 1; Effects of *P. ostreatus* extract on mercury induced neurotoxicity using navigational maze test.

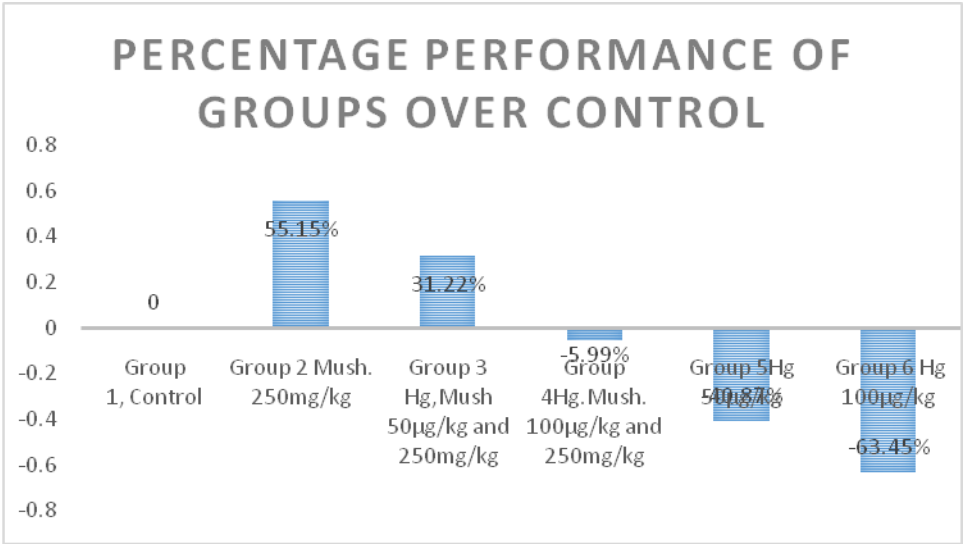

Fig 2: Percentage performance of the groups over the control

DISCUSSION

The result shows that *Pleurotus ostreatus* has a good therapeutic property against neurotoxicity of mercury, this was demonstrated in the navigational maze test conducted. From the result, it was seen that ethanolic extract of *Pleurotus ostreatus* increased the ability of the rats to navigate the puzzle, hence, getting to the exit point faster than any other group. This was observe in group two with the fastest record. It was also seen that group 6 with the highest concentration of mercury and without mushroom remedial effects has the slowest record thereby demonstrating low mental and cognitive function. This differential rates in the performance of the animals have a link to their mental ability and their sense of judgment. These are mostly influenced by environmental factors especially sense of reward, the hypothalamus is implicated as highly affected by neuronal toxicities, which

limit the sense of drive, pleasure, reinforcement and cognition of the internal homeostasis. This engenders floppy lifestyle and lack of enthusiasm, hence not being able to accomplish the task at the stipulated time. This result indicates that though mercury has a high recorded neurotoxic effects [17] ethanolic extract of *Pleurotus ostreatus* in seen to demonstrate anti-toxic effect on mercury induced neurotoxicity.

CONCLUSION

Pleurotus ostreatus extract has a potency as antitoxic agent against mercury toxicity hence serving as a good therapeutic agent in treating neurotoxic conditions related to heavy metals like mercury.it is also observed that it does not only protect the nervous system against the mercury toxicity but also enhances neuronal alertness by increasing the performances of the positive control.

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