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Hepatoprotective Effect of Ethanolic Extract of Oyster Mushroom (*Pleurotus ostreatus*) on acutely Administered Lead Poison in Albino Rats

Zitte, Leelee Famii and Tochi Ben

Department of Animal and Environmental Biology, Faculty of Science, University of Port Harcourt, Nigeria.
Corresponding Author Email: Leelee.zitte@uniport.edu.ng, lefzy@yahoo.com, 08037971401, 08079756415.

ABSTRACT

The hepatoprotective effect of ethanolic extract of Oyster mushroom (*Pleurotus ostreatus*) on acutely administered lead poison in albino rats was studied by assaying the concentration of liver markers enzymes: Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphate (ALP) and Total Bilirubin (TBL), Total Protein (TP), Albumin (ALB). A total of twenty-four albino rats were distributed into six groups of four animals each. Group 1 (control), group 2 (20mg/kg lead alone), group 3 (40mg/kg lead alone), group 4 (250mg/kg mushroom extract alone), group 5 (250mg/kg mushroom and 20mg/kg lead), and group 6 (250mg/kg mushroom and 40mg/kg lead). These animal groups were administered their respective treatments for a period of twenty-one days. Thereafter, various tests were carried out on the liver enzymes. The groups administered with lead (low and high dose) showed significant increase in levels of AST, ALT, ALP & TBL but reduced levels of ALB and TP respectively (59.00±1.63, 23.00±1.47, 61.25±2.49, 16.25±0.62, 31.75±1.18, 46.75±0.85, p <0.05), (63.75±0.48, 29.75±0.85, 72.25±1.03, 18.00±0.41, 27.00±0.71, 36.00±1.47, p<0.05) compared to the control with mean values of 37.75±1.88, 15.50±0.64, 46.50±1.55, 11.75±0.48, 39.00±0.71, 71.50±1.32. On the other hand, groups 4, 5 and 6 treated with oyster mushroom extract showed a hepatoprotective effect by maintaining the level of ALT (14.25±1.11, 16.00±0.91, 20.25±0.48, p<0.05), AST (24.25±1.49, 27.25±1.18, 31.25±1.65, p<0.05), ALP (34.25±1.11, 37.50±2.72, 39.50±0.87, p<0.05), TP (78.25±1.37, 72.25±0.47, 74.25±2.56, p<0.05), ALB (38.75±0.48, 40.00±0.41, 43.25±0.63, P<0.05) and TBL (11.00±0.41, 11.75±0.48, 13.25±0.48 p<0.05), around the normal level. Histological examination of the liver tissues showed that mushroom extract was hepatoprotective against the lead induced alterations, notably inflammation, necrosis and vacuolation. This result reveals that aqueous extract from *Pleurotus ostreatus* has significant hepatoprotection against lead induced hepatocellular injury.

Keywords: Lead, *Pleurotus ostreatus,* Hepatoprotection, Hepatotoxicity, AST, ALT, ALP, TP, ALB, TBL

INTRODUCTION

Lead poisoning is a serious and sometimes fatal condition that occurs when lead builds up in the body [1,2,3]. A toxic dose of lead poisoning may result in serious health problems which include; seizures, coma, malignancy, cell death, periportal inflammation, enlargement of the liver cells into hyperplasia (initiating the formation of tumors on the liver) and hepatocyte damage [4,5,6]. Lead is bio-accumulated and is most concentrated in the bone but has more toxic effects in the liver and kidney, and children are at a higher risk of lead poisoning [7,8,9]. The outbreak of lead poisoning was also discovered in 2010 at Zamfara State, Nigeria, which killed at least 400 inhabitants that were mainly children [10,11]. Lead is a common environmental pollutant, and its widespread use has resulted in extensive environmental contamination and adverse health effects, affecting almost every organ and system in the human body [12,13,14]. Lead

contaminates the environment through releases from mining industries and factories that use or produce lead, lead alloys, lead compounds, lead-acid batteries, lead wires, pipes and metal recycling [15,16,17]. Lead is readily absorbed by the body via primary routes of entry, inhalation and ingestion [18,19,20]. Some lead compounds are colorful and are widely used in paints [21] and children are at a greater risk as they're more likely to put objects in their mouth [22]. In adults, exposure at work is a common cause of lead poisoning, with certain occupations at particular risk [23].

Chelation is a method used to treat metal poisoning. Chelating agents are not without side effects and can also remove beneficial metals from the body. Moreover, these chelating agents in turn are potentially toxic [14] and often fail to remove lead burden from body tissues, according to research report on chelators and lead toxicity management. In order to address these problems, natural therapies to promote chelation, detoxification and protection are gaining popularity because of minimal or no side effects [16]. Medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world due to their potent pharmacological activities, low toxicity and economic viability [19]. Thus, there has been an increased interest in the therapeutic potential of plant products and medicinal plants for having beneficial role in reducing lead poisoning in cells, tissues, and organs. Mushrooms are macrofungi that have distinctive basidiomata or ascomata large enough to be seen with the naked eye and to be picked with unaided hand. They are poor in calories, rich in proteins, fibers, carbohydrates and important vitamins and minerals [12]. They are considered as functional foods or nutraceuticals [14].

Liver disease is a serious problem worldwide and several studies have demonstrated the protective effects of herbs against experimentally induced liver injury. Additionally, a number of herbs such as the edible mushroom revealed promising properties which includes antioxidant, anti-cancer, anti-tumor and anti-inflammatory actions [15,17,18]. Therefore, this research is aimed at investigating the hepatoprotective effect of mushroom extract against lead induced hepatotoxicity in albino rats.

Liver damage is a widespread disease, which in most cases involves oxidative stress and it's characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. Recent research on functional foods such as nutraceuticals showed that many natural agents exert protective and therapeutic effects on the liver and some of the other herbal and nutritional supplements also have characteristics that make them beneficial to the liver [16]. According to the recent version of the United States Food and Drug Administration (FDA) guideline for industry drug induced liver injury, it is recommended to use a combination of four tests as Drug induced liver injury (DILI) biomarkers [5]. The diagnostic biomarkers for liver functions/damage (hepatotoxicity) are Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphate (ALP) and Total Bilirubin (TBL). These biomarkers are widely adopted by clinicians as recommended in the FDA guideline. Relative effects of graded levels of *Pleurotus ostreatus* concentrations on the neuromuscular activities of albino rats was studies and it was observed that at 250mg/kg , *Pleurotus ostreatus* exacted the most potent neuromuscular effect [19] This was further studied on the hepatoprotection against lead induced hepatotoxicity at different levels of *Pleurotus ostreatus*. This was also observed to have a greater hepatoprotection at the concentration of 250mg/Kg [20].

MATERIALS AND METHODS

This research work was carried out in Animal and Environmental Biology, Animal House in Choba Campus, University of Port Harcourt, Choba, Rivers State, Nigeria. Fresh *P. ostreatus* (Oyster Mushroom) was purchased from the mushroom farm in the University of Science and Technology, Nkpolu-Oroworukwo, Port Harcourt, Rivers

State, Nigeria. Twenty grammes (20g) of water soluble lead nitrate ($Pb(NO_3)_2$) was purchased from Geochem store in Choba, Rivers State, Nigeria. Twenty-four (24) albino rats of both sexes weighing 60-100g were obtained from the Department of Animal and Environmental Biology. The animals underwent acclimatization for a period of 7 days prior to the experiment. During the preparation of ethanolic extract of *P. ostreatus,* 870ml of ethanol was poured onto 300g of powdered *P. ostreatus* for maceration for 72 hours in a macerating jar, and filtered through the Whatman filter paper. The process of maceration and filtration was repeated to have a better recovery. The filtrate was placed in a rotary evaporator to separate the mushroom from the ethanol. The mushroom was placed in a water bath for further drying. Distilled water was then used to dilute *Pleurotus ostreatus* extract to produce 250mg/kg concentration.

Experimental Design

Lead was diluted with water to produce concentrations of 20mg/kg (low dose) and 40mg/kg (high dose). A total of 24 rats weighing 60-100g were weighed and grouped into six (6) groups of four rats each. Group 1 served as the control and was given normal feed. Group 2 were administered 20mg/kg/day of lead (low dose). Group 3 were administered 40mg/kg/day of lead (high dose). Group 4 were administered mushroom extract alone (250mg/kg/day). Group 5 were administered mushroom extract (250mg/kg/day) and lead (20mg/kg/day). Group 6 were administered mushroom extract (250mg/kg/day) and lead (40mg/kg/day). This treatment was administered for a total of twenty-one (21) days, after which, various tests were carried out. At the end of the experiment, the animals were fasted for 24hrs, weighed and then blood samples were collected from the retro-orbital venous plexus under light ether anesthesia. After blood collection, all animals were sacrificed by cervical dislocation, the liver was extracted, rinsed in isotonic sterile saline, blotted and preserved in 10% neutral formalin solution at 4ºC for histological examination. The liver marker enzymes; Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphate (ALP) and Total Bilirubin (TBL), Total Protein (TP), Albumin (ALB), underwent different procedures for test results. Histological study was carried out on the liver for 5-7 days. Statistical analysis was carried out using the ANOVA. The results were reported as mean ± SEM and considered significantly different at $P \leq 0.05$.

RESULTS

The serum levels of AST revealed that group 1 to 6 had mean values of 37.75 ± 1.88, 59.00 ± 1.63, 63.75 ± 0.48, 24.25 ± 1.49, 27.25 ±1.18, and 31.25±1.65. Group 3 (lead alone at high dose) had the highest serum level while Group 4 (mushroom extract alone) had the lowest serum level for Aspartate Transaminase (AST). The mean values for ALT levels from Group 1 through 6 were 15.50 ± 0.64, 23.00 ± 1.47, 29.75±0.85, 14.25 ± 1.11, 16.00 ± 0.91 and 20.25 ± 0.48. The result showed that Group 3 had the highest mean serum level while Group 4 had the least mean value for Alanine Transaminase (ALT). The result for ALP levels across the groups from 1 to 6 were 46.50±1.55, 61.25±2.49, 72.25±1.03, 34.25±1.11, 37.50±2.72 and 39.50±0.87. From the result, group 3 had the highest mean value and group 4 had the least mean value for Alkaline Phosphate (ALP). The mean values for serum levels of total bilirubin (TBL) were 11.75 ± 0.48, 16.25 ± 0.62, 18.00 ± 0.41, 11.00 ± 0.41, 11.75 ± 0.48 and 13.25 ± 0.48 across the groups (from 1 to 6) respectively. From the result, TBL had the highest mean value recorded for group 3 and the lowest was recorded for group 4. The serum levels for Total protein (TP) for all groups (1-6) had mean values of 46.75 ± 0.85, 61.25 ± 2.49, 36.00 ±1.47, 78.25 ± 1.37, 72.25 ± 0.47 and 74.25 ± 2.56. Group 4 had the highest mean value for TP while group 3 had the least mean value for TP. The mean values for serum levels of albumin (ALB) across the respective groups (1 to 6) are 39.00 ± 0.71, 31.75±1.18, 27.00 ± 0.71, 38.75 ± 0.48,

40.00 ± 0.41 and 43.25 ± 0.63. The overall result for albumin showed that group 6 had the highest mean value while group 3 had the least mean value.

The Histopathological analysis carried out showed that group 1(control) had structural integrity without necrosis, inflammation, or fibrosis. Group 2 (lead alone at low dose) showed inflammation, fatty change, and area of necrotic tissues. Group 3 (lead alone at high dose) showed high inflammation and slightly distorted liver architecture. Group 4 (mushroom alone) showed structural integrity without necrosis and inflammation. Group 5(mushroom and lead at low dose) showed alteration of portal tract while Group 6 (mushroom and lead at high dose) showed fatty change, vacuolar change and minimal inflammation.

Table 1: The mean response of Liver Marker Enzymes

LIVER MARKERS ENZYMES	AST	ALT	ALP	TP	ALB	TB
GROUPS						
Control	37.75±1.88	15.50±0.64	46.50±1.55	71.50±1.32	39.00±0.71	11.75±0.48
Group 2	59.00±1.63	23.00±1.47	61.25±2.49	46.75±0.85	31.75±1.18	16.25±0.62
Group 3	63.75±0.48	29.75±0.85	72.25±1.03	36.00±1.47	27.00±0.71	18.00±0.41
Group 4	24.25±1.49	14.25±1.11	34.25±1.11	78.25±1.37	38.75±0.48	11.00±0.41
Group 5	27.25±1.18	16.00±0.91	37.50±2.72	72.25±0.47	40.00±0.41	11.75±0.48
Group 6	31.25±1.65	20.25±0.48	39.50±0.87	74.25±2.56	43.25±0.63	13.25±0.48

Values are presented in mean ± S.E.M (n= 4), p <0.05 "*means values are statistically significant compared" to the control group.

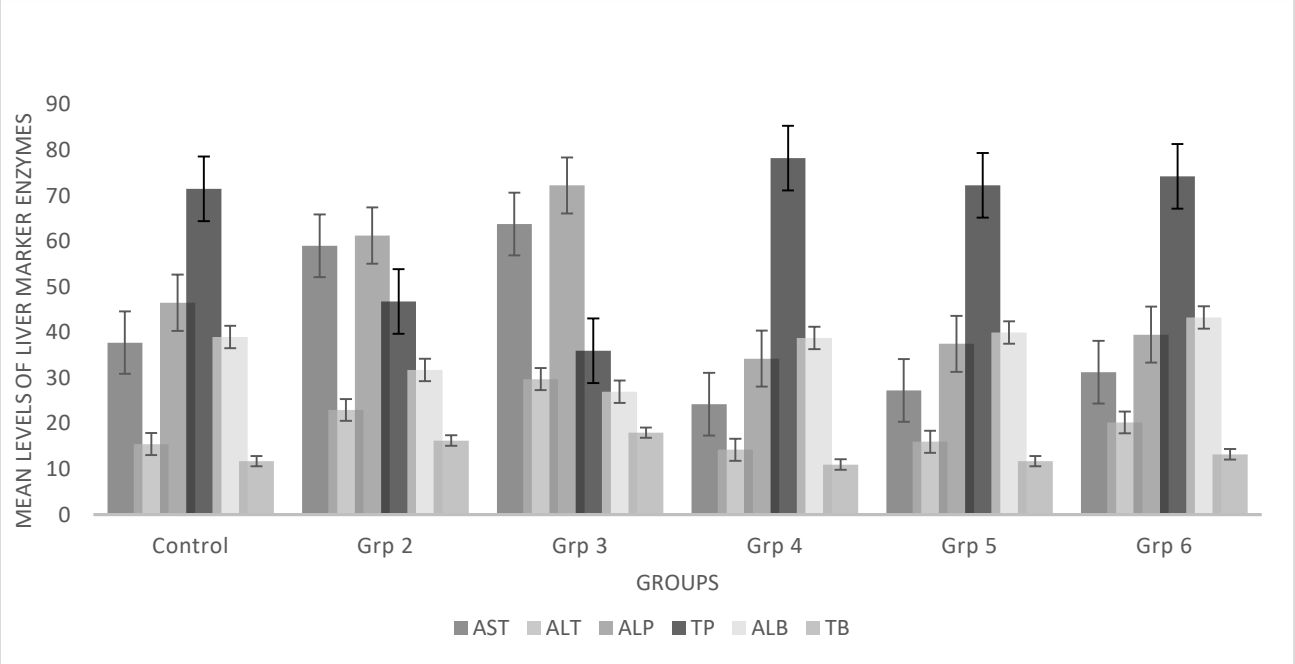

Figure 1: Mean levels of Liver Marker Enzymes

DISCUSSION

Lead poisoning has been recognized as a major public health issue, particularly in developing countries with children mostly susceptible to it as they're most likely to put objects in their mouth [3]. The experimental intoxication induced by lead is widely used for modelling liver injury in rats because of the ability of lead to cause severe cell impairment. *P. ostreatus* (oyster mushroom) on the other hand have been reported to possess potent antioxidant, immunomodulatory and anticancer properties [7,9]. Research has also shown it to have potential hepatoprotective effects [11].

From the results shown, the administration of lead alone (20mg/kg and 40mg/kg) in group 2 and 3 increased serum levels of acute liver damage indicators (AST, ALT, ALP, TBL) and decreased protein content indicators (TP, ALB), which was supported by histological examination of the liver section, revealing distorted architecture of hepatocytes and major generalized vacuolations. However, pre-treatment with ethanolic extract obtained from the oyster mushroom was shown to provide protection against hepatic injury caused by lead as seen in groups 5 and 6 which was also supported by histological examination, showing liver with structural integrity and minimal inflammation. The data obtained revealed that the concentration of these parameters were statistically significant ($P<0.05$) in the different groups pre-treated with mushroom extract. This was result was also supported by the findings of [4].

In the different groups, it could be seen that the liver marker enzymes, total protein and albumin decreased significantly in group 2 and 3, which could be concluded as the inability of the liver to synthesize protein due to liver damage. While group 4, 5, and 6 pre-treated with oyster mushroom extract showed elevations in the total protein and albumin level. Histopathological studies also confirmed the protective effect of *P. ostreatus* extract against lead induced liver damage. The histological appearance in group 4 (mushroom alone) and group 5 (mushroom and lead with low dose) appeared normal with minimal inflammation seen only in group 6 (mushroom and lead with high dose). The other groups (2 & 3) induced with lead alone showed areas of necrosis, fatty changes and scattered lymphocytes, which was reported by [4,7] that lead overdose causes ultrastructural changes in the liver.

CONCLUSION

It can be concluded that ethanolic extract of oyster mushroom is hepatoprotective against acutely administered lead toxin in albino rats. The serum levels showed a decrease in acute liver damage indicators and the histological studies also showed significant alleviation in the alteration caused by lead intoxication, which proves that oyster mushroom has hepatoprotective effect.

REFERENCES

1. FDA (2009) FDA guidance for industry Drug-induced liver injury Premarketing clinical evaluation.Gilman,
2. Henretig FM. (2006). Lead in Toxicologic Emergencies. (8th ed.). New York: Mc Graw-Hill, 1308-1324.
3. Janmeda, P., Sharma, V., Singh, L., Paliwal, R., Sharma, S., & Yadav, S. (2011). Chemo-preventive Effect of hydro-ethanoic extract of *Euphobia nerifolia)* leaves against DENA-Induced hepatic and renal carcinogenesis in mice. Asian Pacific. Journal. *Cancer Prevention* ., 12, 667-683.
4. Jones, S., & Jarnadhanan, K.K. (2000). Antioxidant and antitumor activity of *Ganoderma lucidum* (Cart. Fr.) P.Karst.-Reishi (Aphyllophoro mycetidae) from South India. *International journal of Medicinal Mushroom*, 2, 195-200.
5. Kues, U., and Liu, Y. (2000). Fruiting body production in basidiomycete. *Applied Microbiology*
6. Lozano, M., Darrigo, M., Guillamon, E., Villares, A. (2012). Anti-inflammatory activity of

methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. *Food Chem.*, 130, 350-355.

7. Manay, N., Cousillas, A.Z., Alvarez, C., & Heller, T. (2008). Lead contamination in Uruguay: the Teja. Reviews of Environmental contamination and toxicology, 195, 93-115.Moro, C., Palacios,
8. Nada, S.A., Omara, E.A., Abdel-Salam, O.M.E., & Zahran, H.G. (2010). Mushroom insoluble polysaccharides prevent carbon tetrachloride induced hepatotoxicity in rat. *Food Chem. Toxicol.*, 48, 3184-3188.
9. Needleman, H. (2004). "Lead poisoning". Annual Review of Medicine. 55: 209-212.
10. Padilha,M.M., Avila, A.A.L., Sousa, P.J.C., Cardoso, L.G.V., Perazzo, F.F., Carvalho, J.C.T. (2009). Anti-inflammatory activity of aqueous and alkaline extracts from mushrooms (*Agaricus blazei* Murril). *J. Med. Food*, 12, 359-364.
11. Preeti, A., Pushpa, S., Sakshi, S., Jyoti, A. (2012). Antioxidant mushrooms: A review. Int. Res. J. Pharm., 3, 65-70.
12. Rall A.G., T.W., & Nies, A.S. (2008). *Goodman and Gilman's. The Pharmacological basis of therapeutics*. New York: Pregamon.
13. Refaie, F.M., Emat, A.Y., Daba, A.V., Osman, W.M., & Taha, S.M. (2010). Hepatoprotective activity of polysaccharopeptides from *Pleurotus ostreatus* mycelium on thioacetamide-intoxicated mice. Micologia Aplicada International., 22(1), 1-13.
14. Sharma, S., Sharma, V., Pracheta, & Sharma, S.H. (2011). Therapeutic potential of hydromethanolic root of *Withania somnifera* on brain. J. medicine, 22, 417-433.*technology*, 54, 141-152.
15. Timbrell, J. (2008). Biochemical Mechanism of Toxicity: Specific examples. (4th ed.). Informal Health care.
16. United States Food and Drug Administration (2015). Elemental impurities guidance for industries.
17. Williams, A.L. & Hoofnagle, J.H. (2002). Ratio of serum aspartate to alanine amino-transferase in chronic hepatitis: relationship to cirrhosis. *Gastroenterology*, 95, 734-739.
18. World Health Organization (2000). "Lead". *Air Quality Guidelines for Europe*, 149-153.
19. 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.
20. Zitte L.F., Konya, R.S. and Olorunfemi O.J. (2019). Effect of hydro-methanolic extract of oyster Mushroom (*Pleurotus ostreatus*) on muscle strength and coordination of Albino Rats (Rattus norvegicus) using inverted screen test. IDOSR Journal of Biochemistry biotechnology and Allied field 4(1) 11-20.
21. Afiukwa CA, Oko AO, Afiukwa JN, Ugwu OPC, Ali FU and Ossai EC (2013). Proximate and Mineral Element Compositions of Five Edible Wild Grown Mushroom Species in Abakaliki, Southeast Nigeria. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*,4(2):1056-1064.
22. CA Afiukwa, Okechukwu PC Ugwu, LN Ebenyi, HA Oketa, JN Idenyi, EC Ossai (2013). Phytochemical analysis of two wild edible mushrooms, Auricularia polytricha and Pleurotus ostreatus, common in Ohaukwu area of Ebonyi State, Nigeria. *Res J Pharm Biol Chem Sci* 4(2): 1065-1070.
23. OU Orji, UA Ibiam, PM Aja, AJ Uraku, OR Inya-Agha, PC Ugwu Okechukwu (2015). Hepatoprotective activity of ethanol extract of Vernonia ambigua against carbon

tetrachloride induced hepatotoxicity in albino rats. *IOSR J. Dental Med* Sci,14:9-22.