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Ameliorative Effects of *Ganoderma lucidum* on Pb-induced Toxicity in Wistar Rats

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ABSTRACT

Background and Purpose: *Ganoderma lucidum* is a medicinal mushroom that is used to promote health and longevity. This study was designed to evaluate the ameliorative effect of aqueous extract of *G. lucidum* (GL) on Lead (Pb)- induced hepatotoxicity in Wistar rats.
Methods: Thirty-five (35) Wistar rats were randomly allotted into seven groups (n=5). Group 1 served as control and was given distilled water *ad libitum*, group 2 was given (100 mg/kg) of Pb, group 3 was given Pb (100 mg/kg) plus GL (50 mg/kg), group 4 was given Pb (100 mg/kg) plus GL (100 mg/kg), group 5 was given Pb (100 mg/kg) plus GL (200 mg/kg), group 6 was given Pb (100 mg/kg) plus 50 mg/kg of 2,3-dimecarptosuccinic acid (DMSA), group 7 was given Pb (100 mg/kg) plus vitamin C (100 mg/kg). Treatment was daily by the oral route and for 28 days. Analyses of liver function enzymes, serum lipid profile, total protein and albumin were carried out.
Results: GL significantly reduced the level of ALT, AST, and ALP (P<0.001, P<0.001, P<0.05, respectively). Total cholesterol (TC), triglycerides (TG), and low-density lipoproteins (LDL) levels were significantly reduced (P<0.05, P<0.001, P<0.001, respectively), but high-density lipoprotein (HDL), albumin and total protein levels were significantly (P<0.001) increased. DMSA and Vitamin C significantly (P<0.001) reduced levels of TG, LDL, and the liver enzymes but significantly (P< 0.05) increased total protein but had no effect on albumin.
Conclusion: The aqueous extract of *G. lucidum* may ameliorate Pb poisoning by reducing ALT, ALP, AST, LDL, TC, TG and improving the levels of albumin, total protein, and HDL.

INTRODUCTION

Lead (Pb) toxicity has been known to humanity since ancient times **(**Sainath *et al,* 2011; Liu *et al.,* 2018**).** Lead is a ubiquitous, environmental and industrial pollutant that has been detected in every facet of the environmental and biological systems. It occurs in nature as oxides or salts (Ahmed, *et al,* 2012). It is a component of water pipes, paints, ceramic glasses, metal utensils and fixtures. It is also in cosmetic products like creams, powders, lipsticks and hair colorants (Wang, *et al* 2009; Imosemi *et al.,* 2020). Agricultural soil contamination may be responsible for the presence of Pb in some fungi and herbal medicines.

The detrimental effects of Pb on physiological, biochemical and behavioral dysfunctions on humans are alarming (Stewart *et al.,* 2006; Asiwe *et al.,* 2022). Lead toxicity occurs because of its high affinity for sulfhydryl groups which accounts for the involvement of multiple enzyme systems. Many of the toxic effects of Pb result from its inhibition of cellular function that require calcium since it binds to calcium-activated proteins with much higher affinity than calcium (Basha and Reddy, 2010). Once Pb enters the body systems, especially bone, it is very difficult to eliminate. Chelating agents which are used for decreasing concentration of Pb in blood are not without secondary toxic effects (Makrowitz *et al.,* 2000; Flora *et al* 2012). For example, the use of 2,3-dimecarptosuccinic acid (DMSA) as a Pb chelator has resulted in adverse effects such as rash, pruritus and mucocutaneous reactions (Ekpo and Johnson, 2021). Therefore, the search for safer antidotes continues. Previously, we reported that the concurrent administration of the aqueous extract of *G. lucidum* attenuated Pb-induced toxicity in rats (Sobowale *et al*., 2019). This medicinal mushroom with a glossy exterior and woody texture is used to promote health and longevity (Gonul *et al,* 2015). The extract reduces cholesterol level and eases allergy-related inflammation of the airways (Ajith *et al*., 2007; Cor *et al.,* 2018). It is rich in antioxidants which protect cellular components from oxidative damage, decrease the risk of mutations and protect immune cells (Collins 2005; Benzie *et al.,* 2009; Kirar *et al*., 2017). Although the antioxidant properties of *G. lucidum* have been elucidated (Wu *et al*, 2015; Sobowale *et al.,* 2019), there is little information on the ameliorative effects of low doses of the aqueous extract on Pb-induced liver toxicity. Therefore, this study evaluated the ameliorative effects of aqueous extract of *Ganoderma lucidum* on Pb-induced liver toxicity in Wistar rats.

MATERIALS AND METHODS

Drugs and Chemicals

Chemicals and reagents were of analytical grade and were obtained from internationally known suppliers such as Sigma-Aldrich (USA) and BDH (UK). Only freshly prepared solutions and reagents were used. Pb acetate was obtained from Nigerian German Chemicals PLC (Nigeria). 2, 3-dimercarptosuccinic acid (succimer) was manufactured by Sigma-Aldrich (USA). Vitamin C was obtained from the Pharmacy Department of the University of Benin Teaching Hospital, Benin City, Nigeria.

Mushroom and Method of Extraction

Ganoderma lucidum (GL) was sourced from a forest in Iwaro Oka Akoko, Ondo State by a Botany technologist, Mr. Festus Ologunorisa of the Department of Plant Science and Biotechnology, Faculty of Life Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. The mushroom was identified by Prof. John Okhuoya, the Director of Mushroom Research Centre, University of Benin, Benin City, Edo State, Nigeria. Adulterants were carefully picked out and the mushroom was thoroughly rinsed in tap water, cut into smaller pieces and allowed to dry under shade for 15 days. Thereafter, the mushroom was grounded into powder and the aqueous extract was prepared by adding 500 g of the powder to 2000 mL of tap water for 30 min at 60^{o}C. The content was filtered with a plain white handkerchief and the filtrate was concentrated over a water bath at 55^{o}C to obtain the extract. The extract was stored in a refrigerator and reconstituted in distilled water before administration according to the experimental design.

Animals

Adult Wistar rats (186-254 g) of both sexes were obtained from the Animal Unit, Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria. They were housed in standard plastic cages (males separated from females) and allowed access to rat chow (Top Feeds and Flour Mills Limited, Nigeria) and tap water *ad libitum*. Animals were exposed to day/night light cycle and room temperature. The animals were handled according to standard protocols for the use of laboratory animals (National Institutes of Health USA; Public Health Service Policy on Human Care and Use of Laboratory Animals, 2002). The study was approved by the University Ethical Approval Committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria with reference number EC/FP018/24.

Treatment Protocol

The Wistar rats were randomly distributed among seven groups of 5 animals each. Group 1 was given distilled water *ad libitum*. group 2 were given Pb (100 mg/kg/day), groups 3, 4 and 5 were given Pb (100 mg/kg/day) and extract of *G. lucidum* at doses of 50, 100 and 200 mg/kg/day, respectively, group 6 rats were administered Pb (100 mg/kg/day) and 50 mg/kg/day of (DMSA) (El-Tantaway, 2015), group 7 rats were given Pb (100 mg/kg/day) and 100 mg/kg/day of Vitamin C (Flora *et al*., 2007; El-Tantaway,

2015) .Treatment was by the oral route and lasted for a period of 28 days.

Assessment of Body Weight Changes

The percentage change in the body weight of the treated animals was determined by using the formula:

$$\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Tissues and Blood Sample Collection

The rats were anesthetized and in their unconscious state, blood was obtained from the abdominal aorta using hypodermic needles attached to 5 mL syringes and distributed into plain bottles. The contents of the plain bottles were centrifuged at 3500 rpm for 10 min and the sera were decanted into new plain tubes. Kidney, liver, heart, lungs and spleen were excised, and the livers were homogenized in 5 mL of deionized water. The homogenates were centrifuged at 3500 rpm for 10 min. All the supernatants (sera and liver) were stored in a deep freezer.

Relative Organ Weight

The excised liver, heart, spleen, lungs and kidney were weighed on a digital weighing balance to determine the weight of each of the organs.

Assay of Pb Concentration in Tissue Homogenate

The concentration of Pb in each organ was estimated by modifying the method described by Szkoda and Zmudzki (2005). In brief, 10 mL each of 1 M nitric acid and 1 M perchloric acid in ratio 5:2 were introduced into a conical flask with 0.5 mL of the tissue homogenate. The mixture was heated until the volume of the sample with the mixed acids was reduced to half of the original with the flask becoming clear. The digested sample was filtered using Whatman filter paper (No. 1 size) into a 100 mL volumetric flask and was finally marked up with deionized water. The samples were analyzed at 283.3 nm using flame atomic absorption spectrometer (Buck Scientific 210 VGP, USA) and the data obtained were expressed as µg/g of the wet tissue.

Biochemical Assays

The biochemical assays were performed using reagent kits manufactured by Randox Laboratories Ltd (UK). The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in the sera of rats were assayed based on the method of Reitman and Frankel (1957) which measures spectrophotometrically at 546 nm, the quantity of pyruvate or oxaloacetate produced following the reaction of sample with 2,4-dinitrophenylhydrazine. Alkaline phosphatase (ALP) was assayed spectrophotometrically at 405 nm based on the formation of yellow-colored p-nitrophenol by the action of ALP on p-nitrophenyl phosphate (Bassey *et al* 1946; Wright *et al*., 1972

Serum levels of lipids including total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and low-density lipoproteins (LDL) were assayed using commercial kits (Randox Laboratories Ltd, UK) and following manufacturer's guideline (Trinder, 1969).

Total protein and albumin were assayed in liver homogenates using the method described by Misra and Fridovich (1972) which depends on the auto-oxidation of adrenaline (epinephrine) in aqueous solution to adrenochrome. The concentration of the adrenochrome was monitored spectrophotometrically at 420 nm.

Statistical Analysis and Data Presentation

Data are presented as mean ± SEM (standard error of mean) and "n" represents the number of rats per group. Comparisons were made between treated and control groups using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc*. All data were analysed using GraphPad Prism version 5 software (GraphPad Prism, UK). $P<0.05$ indicates statistical difference between compared data.

RESULTS

Effect of *G. lucidum* Extract on Body Weight and Relative Organ Weights

Daily administration of the various treatments for 28 consecutive days did not significantly alter the weight of the animals when compared with vehicle-treated controls. Animals treated with Pb only, Pb plus 100 mg/kg of extract, Pb plus 200 mg/kg of extract had mean percentage weight loss that was not significantly different from the other groups.

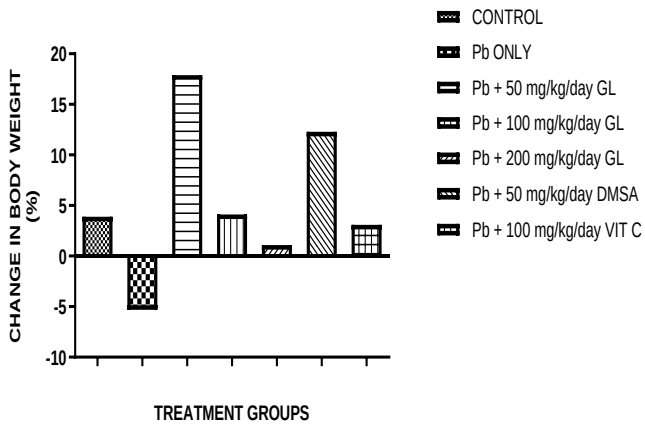

Figure 1: Percentage change in whole-body weight following concurrent administration of Pb and doses of *G. lucidum* aqueous extract for 28, n=5. Pb: lead acetate, DMSA: 2,3-dimercaptosuccinic acid, Vit C: Vitamin C.

In comparison with Pb-only treated rats, the relative organ weights of spleen, lungs and kidney were not significantly different following the concurrent daily oral administration of GL, DMSA or vitamin. The relative weight of the liver although reduced in the Pb-only group but not significant when compared with the control.

Table 1: Effects of concurrent administration of *G. lucidum* and Pb for 28 days on relative organ weight of rats

Parameters	Control	Pb	Pb + 50 mg/kg/ day GL	Pb + 100 mg/kg/ day GL	Pb + 200 mg/kg/ day GL	Pb + 50 mg/kg/ day DMSA	Pb + 100 mg/kg day Vit C
LIVER	6.8±0.4	6.1±0.6	6.5±0.8	7.5±0.3	6.6±0.4	5.6±0.6	7.1±0.3
HEART	0.7±0.3	0.6±0.2	0.5±0.5	0.7±0.5	0.6±0.4	0.7±0.1	0.7±0.1
SPLEEN	0.7±0.4	0.9±0.1	0.8±0.1	0.9±0.1	0.9±0.1	0.5±0.1	0.9±0.2
LUNGS	1.3±0.1	1.5±0.1	1.0±0.1	1.3±0.2	1.4±0.1	1.7±0.6	1.4±0.1
KIDNEY	1.0±0.1	1.3±0.9	1.4±0.1	1.3±0.1	1.2±0.1	1.5±0.1	1.2±0.1

Values are not significantly different from control. Values are expressed as Mean ± SEM, n=5. Pb: Pb acetate; DMSA: 2,3-dimercaptosuccinic acid, Vit C: Vitamin C.

Effect of *G. lucidum* Extract on Liver Lead Levels

Following the daily administration of Pb to the Wistar rats, the Pb content in the serum was significantly (P<0.001) reduced in the group treated with 100 mg/kg of the aqueous extract of *G. lucidum*. The dose of 50 mg/kg of 2,3-dmecaptosuccinic acid (DMSA) and 100 mg/kg of vitamin C also significantly (P<0.001) reduced the serum Pb content in the rats in comparison with Pb-only group

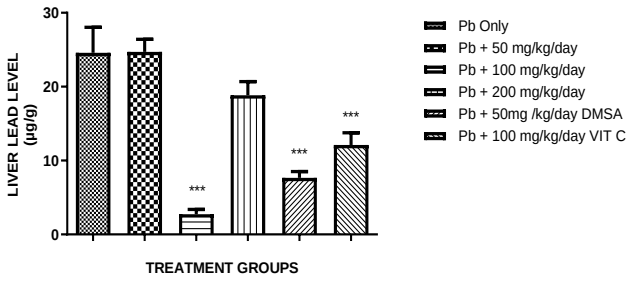

Figure 2: Effects of daily concurrent administration of Pb and *G. lucidum* aqueous extract on liver Pb levels after 28 days. ***P<0.05 when compared with Pb-only group. Values are expressed as Mean ± SEM n=5. Pb: Lead acetate, DMSA: 2,3-dimercaptosuccinic acid, Vit C: Vitamin C.

Effect of *G. lucidum* Extract on Serum Protein Levels

Figure 3 shows the serum level of albumin and total protein in the serum of the rats. The level of albumin was significantly (P<0.05) reduced in the Pb-only group when compared with the control. Doses of GL at 50, 100 and 200 mg/kg significantly (P<0.001) reversed the Pb-induced reduction in albumin level but neither DMSA nor vitamin C had this reversal effects.

The mean total protein concentration in the serum of the rats was significantly (P<0.05) reduced following Pb administration in comparison with control but doses of GL did not significantly reverse the effects of Pb. However, DMSA and vitamin C significantly (P<0.001) increased the level of total protein when compared with the Pb-only group.

Effect of *G. lucidum* Extract on Serum Lipid Levels

In Figure 4, doses of 50 and 200 mg/kg/day of GL concurrently administered with Pb resulted in a significant (P<0.05**)** decrease in serum total cholesterol (TC) level when compared with Pb-only group. The standard chelator, 2,3-dimercaptosuccinic acid (DMSA) or vitamin C administered in combination with Pb for 28 days significantly (P<0.01**,** P<0.001, respectively) reversed the elevation level of Pb when compared with Pb only group.

The administration of Pb resulted in significant (P<0.05**)** elevation of triglyceride (TG) level in the serum of rats. In groups treated with GL, the levels of TG were reduced significantly (P<0.01**)** when compared with Pb-only group. DMSA or vitamin C concurrently administered with Pb also significantly (P<0.001**)** reduced the activities of TG.

The level of high-density lipoprotein cholesterol (HDL-cholesterol) was significantly (P<0.05**)** reduced in the Pb-only group when compared with the control group. Doses of GL at 100 and 200 mg/kg/day significantly (P<0.001, P<0.05**)** increased the level of HDL when compared with the Pb-only group. DMSA or vitamin C administered concurrently with Pb increased the level of HDL but not statistically significant in comparison with Pb-only group.

Administration of Pb resulted in a significant (P<0.05**)** elevation of low-density lipoprotein cholesterol (LDL-cholesterol) level in the serum of rats. Treatment with 50 and 200 mg/kg/day of GL reduced the level of LDL-cholesterol significantly (P<0.001**)** when compared with the Pb-only group. DMSA but not vitamin C could significantly reduce the level of LDL when compared with Pb only group.

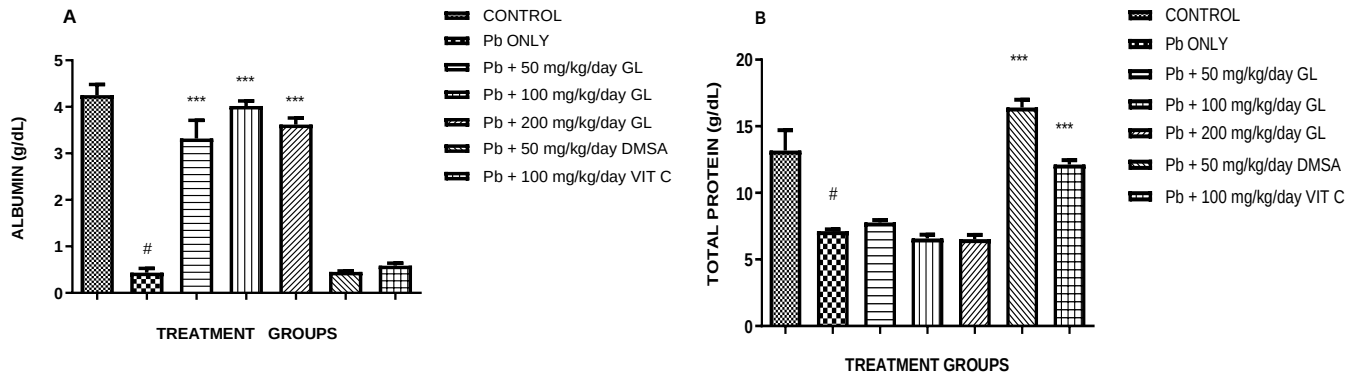

Figure 3: Effects of concurrent administration aqueous extract of *G. lucidum* (GL) and Pb on albumin and total protein of rats after 28 days. #*P*<0.05 compared with control; **P*<0.05, ****P*<0.001 compared with Pb-only group. Values are expressed as Mean ± SEM, n=5. Pb: Lead acetate, DMSA: 2,3-dimercaptosuccinic acid, VIT C: Vitamin C.

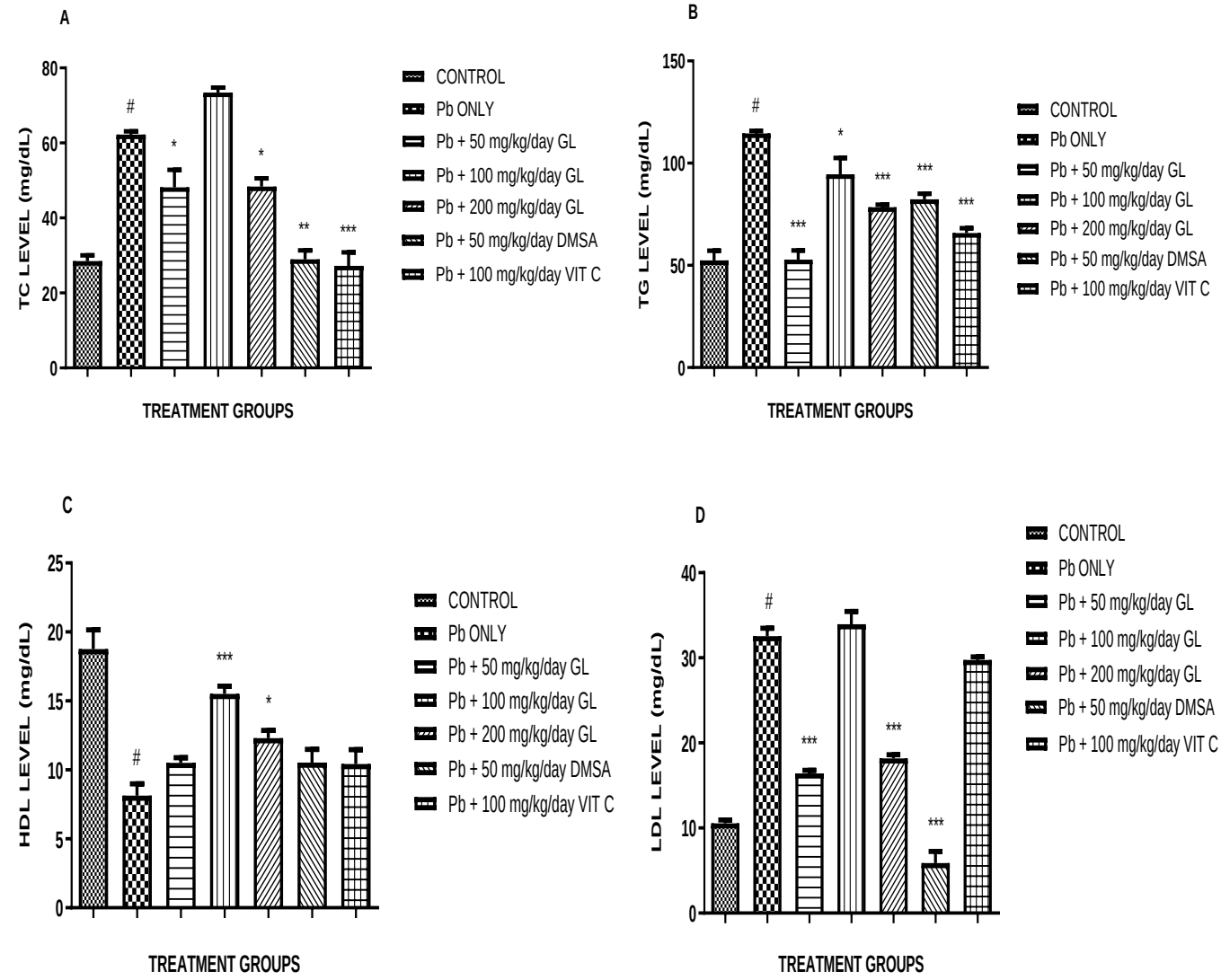

Figure 4: Effects of concurrent daily administration of *G. lucidum* (GL) and Pb for 28 days on serum lipids. (A) Total Cholesterol (TC) (B) Triglycerides (TG) (C) High Density Lipoprotein (HDL) (D) Low Density Lipoprotein (LDL). # *P*<0.05 compared with control and *P*<0.05, *P*<0.01 *P*<0.001 when compared with Pb-only group. Values are expressed as Mean ± SEM (n=5). Pb – Lead acetate, DMSA - 2, 3-dimercaptosuccinic acid, Vit C- Vitamin C.

Effect of *G. lucidum* Extract on Level of Liver Function Enzymes

Figure 5 shows that Pb significantly (*P*<0.05**)** increased the serum activity of AST in the Pb-only group when compared with the control group. All doses of GL significantly (*P*<0.001**)** reduced the serum level of AST in Pb administered Wistar rats when compared with Pb-only group. However, neither DMSA nor Vit C could reduce AST levels when compared with Pb only group.

Daily Pb administration for 28 consecutive days significantly (*P*<0.05**)** increased the serum activity of ALP in Pb-only group when compared with control group. However, the extract treated groups at doses of 50 and 200 mg/kg/day caused a significant (*P*<0.05**)** reduction in the activity of ALP. Also, DMSA or vitamin C in combination with Pb significantly (*P*<0.01, *P*<0.05 respectively) reduced the level of ALP.

Administration of Pb for 28 consecutive days significantly (*P*<0.05**)** increased the activity of ALT in the group treated with Pb only when compared with the control group. All doses of GL significantly (*P*<0.001**)** decreased the level of ALT in comparison with the Pb-only group. Also, DMSA or vitamin C significantly (*P*<0.001**)** reduced the level of ALT when compared with Pb-only group

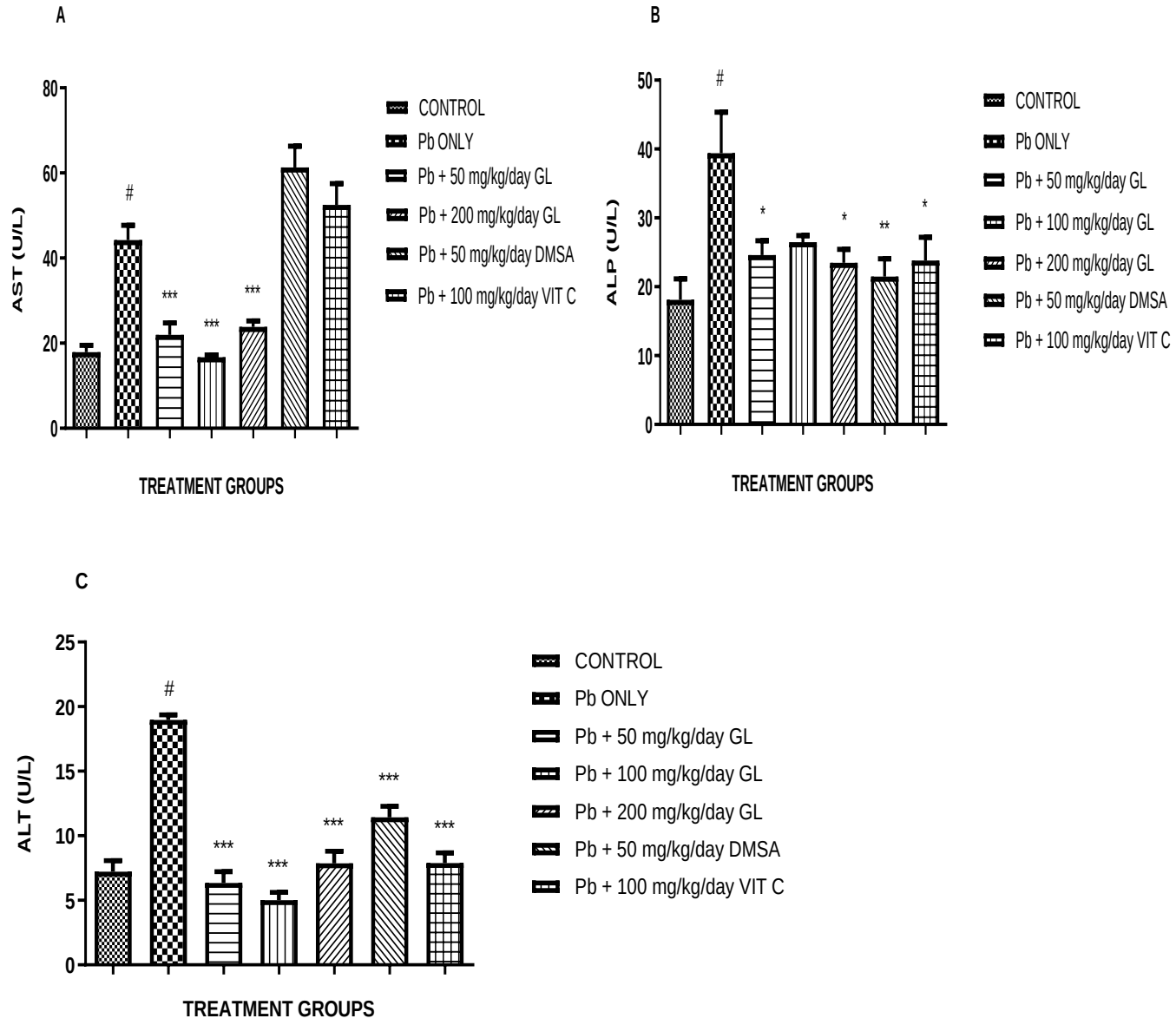

Figure 5: Effects of concurrent administration of *G. lucidum* and Pb on liver function enzymes of rats after 28 days. (A) Aspartate aminotransaminase (AST); (B) Alkaline Phosphatase (ALP); (C) Alanine aminotransferase (ALT). #*P*<0.05 compared with control; **P*<0.05, ***P*<0.01 ****P*<0.001 compared with Pb-only group. Values are expressed as Mean ± SEM, n=5. Pb: Lead acetate, DMSA: 2,3-dimercaptosuccinic acid, VIT C: Vitamin C.

DISCUSSION

This study shows the effect of *Ganoderma lucidum (GL)* extract on Pb-induced toxicity in Wistar rats. Aqueous extract of GL significantly ameliorated the increase in ALP, ALT and AST by Pb administration. The mushroom extract also reversed Pb-induced albumin and total protein reduction. The study shows that aqueous extract of GL did not have effects on whole body weights and relative organ weight of the rats.

Serum albumin levels of Pb-treated group were decreased. Albumin might have been consumed in the process of combating oxidative stress (Roche *et al*, 2008) evoked by Pb, thereby contributing to its low concentration Pb-only treated group. However, administration of GL increased the level of albumin significantly at all doses showing the potential ameliorative effect of aqueous extract of GL. Although not statistically significant, the administration of GL increased the level of total protein. Treatment with DMSA, a Pb chelating agent and vitamin C have been shown in the present study to mitigate hypoproteinemia associated with exposure to Pb. This amelioration of hypoproteinemia by DMSA and vitamin C might be due to protection of the liver from oxidative damage induced by Pb thereby encouraging the synthesis of proteins involved in detoxification, such as cytochrome P450 enzymes, which help to remove toxins and xenobiotics from the body (Flora et al., 2008; El-Neweshy and El-Sayed, 2011). Vitamin C, being an antioxidant, effectively neutralized the free radicals generated by Pb.

The belief that low density lipoprotein (LDL) cholesterol causes atherosclerosis and subsequent heart diseases is a fundamental precept of modern medicine (Colpo, 2005; Zhang *et al.* 2017). The primary function of high density lipoprotein (HDL) is to mobilize excess cholesterol to the liver for it to be metabolized to bile salts. This function of cholesterol removal from the tissues underlies the inverse relationship between the plasma concentration of HDLs and the incidence of heart diseases (Chalasani *et al.*, 2018). In this study, aqueous extract of *G. lucidum* significantly decreased the serum content of triglyceride and LDL-cholesterol showing the protective effect of the extract and markedly increased the level of serum high density lipoprotein (Liu *et al* 2017). The mean level of total cholesterol in the experimental groups treated with GL at all doses were much lower than those in Pb group. It has been reported that GL reduced lipids profile especially in total cholesterol and LDL-cholesterol (Liu *et al* 2017; Sobowale *et al.*, 2019).

In the present study, the activities of the enzymes of the liver function: alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) were increased in the Pb-only group. In a previous study carried out by Suleiman *et al*. (2013) showed an increase in liver enzyme activities in Pb-induced toxicity in rats. This increase in liver enzymes by Pb exposure could be due to the generation of reactive oxygen species (ROS), which produced oxidative damage to the cell membrane of the liver cells (Chen *et al.,* 2019; Cheng *et al.,* 2019). Serum ALT and AST are sensitive markers in the diagnosis of hepatic damage as they are cytoplasmic enzymes released into circulation upon hepatic cellular damage (Ait-Hamadouche *et al.,* 2012). However, serum ALT activity tends to be more specific in liver than AST (Ambali *et al.,* 2011). Thus, the significant increase in serum ALT activity witnessed in the rats that were administered only Pb was an indication of hepatic damage. Increase in AST and ALT activities indicates that prolonged exposure to Pb might have deleterious effect on hepatocytes. The increase in serum alkaline phosphatase (ALP) activity observed in the study showed that administration of Pb to the animals could also cause extra-hepatic damage. There could have been biliary cell membrane damage caused by Pb toxicity thereby eliciting elevated serum ALP activity (Kodavanti *et al*., 1989; Peng et al., 2022). Thus, increased ALP activity observed in the Pb-treated group of rats agrees with a previous finding (Suleiman, *et al*, 2013). However, elevation in the level of serum ALP activity could be due to increased permeability, damage, or/and necrosis of cells, which; in the present study, might have been caused by Pb toxicity (Ait-Hamadouche *et al.,* 2012). Thus, the study has established the disruptive effect of Pb as seen in the group treated with only Pb. The aqueous extract of *G. lucidum* at all doses were able to protect against the hepatoxicity of Pb. This may be due to the presence of polysaccharide (Sobowale *et al*., 2019) which was able to protect the immune cells from oxidative damage as equally reported by Ooi and Lui (2000). *Ganoderma lucidum* also significantly ameliorated the superoxide dismutase (Sun-Hee Jang *et al*., 2014). Results from this study showed that GL ameliorate increased in AST, ALT and ALP which is as a result of the presence of bioactive compounds, including polysaccharides and phenolic compounds, which possessed antioxidant properties. These compounds can neutralize free radicals, reducing oxidative stress and inflammation in the liver (Kim *et al.,* 1999; Wu and Xu, 2015; Sobowale *et al.,* 2019). Treatment with DMSA or vitamin C caused a decrease in the activities of ALP and ALT thereby ameliorating Pb-induced hepatic damage (El-Neweshy and El-sayed, 2011). This shows that vitamin C, an effective reducing agent, and DMSA a standard Pb chelator, both protect the organs from Pb-induced damage (Mishra and Acharya, 2004; Ait-Hamadouche *et al*, 2012).

CONCLUSION

This study has shown that lower doses of GL than were previously used by Sobowale *et al.* (2019) ameliorate Pb-

induced elevation of liver enzymes in rats. The lower doses also result in favourable lipid profiles. It indicates that the medicinal mushroom could be used adjunctively in the treatment of Pb toxicity.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION

MTS conceptualized the study. The experiments and manuscript drafting were carried out by both MTS and JAS.

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AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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