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Protective Effect of *Pleurotus tuberregium* Polysaccharide (PTP) and *Ganoderma lucidum* Polysaccharide (GLP) on Lead-Induced Testicular Derangement in Rats

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ABSTRACT

Background and Purpose: Lead poisoning is of global public health concern because of its several organ-related toxicities including neurotoxicity, nephrotoxicity, and gonadotoxicity, among others. Polysaccharides from *Ganoderma lucidum* (GLP) and *Pleurotus tuberregium* (PTP), edible mushrooms, were investigated for their ameliorative and/or protective effects against lead-induced testicular derangement in rats.

Methods: Forty-eight (48) male Wistar rats were randomly assigned to six groups (n=8). Group 1 served as control, and received distilled water; group 2 received lead acetate (25 mg/kg); group 3 was administered lead acetate (25 mg/kg) plus GLP (100 mg/kg); group 4 received lead acetate (25 mg/kg) plus PTP (100 mg/kg); group 5 was administered lead acetate (25 mg/kg) plus dimercaptosuccinic acid (50 mg/kg); and group 6 received lead acetate (25 mg/kg) plus Penicillamine (30 mg/kg) for 28 days. On the 28th day, the rats were anaesthetized using ketamine. Semen, testicular antioxidant parameters, inflammatory markers, immunohistochemistry and histopathology were evaluated.

Results: The results showed that GLP and PTP treatment protected against lead-induced testicular impairment, enhanced sperm motility and sperm viability, and increased antioxidant level. Expression of pro-inflammatory enzymes in the testes were significantly reduced (P<0.05) in the GLP and PTP treated groups. Histology of the testes of rats administered with lead only, revealed seminiferous tubules with maturation arrest, while the GLP and PTP treated groups showed normal seminiferous tubes containing spermatozoa.

Conclusion: This study suggests that GLP and PTP could protect against lead-induced testicular impairment by modulating antioxidant status and inflammatory markers.

INTRODUCTION

Lead (Pb) poisoning is a growing global public health concern (Flora *et al*., 2012; La-Llave-León *et al.,* 2016). Lead is useful in different production factories, such as paint, plastic, ceramic, cosmetic and some parts of automobiles (Flora *et al*., 2012). Lead may be found in soil, paints, brass plumbing fitting, bullets and in water (Wani A *et al*., 2015; WHO, 2024). Lead can be toxic to different organs of the body including the brain, liver, blood, kidneys and reproductive systems via several mechanisms. In the male reproductive organ, lead induced toxicity mainly affects the hypothalamic-pituitary-testicular axis resulting in reduction in sperm count and sperm motility, which have been implicated in the aetiology of male infertility (Ait-Hamadouche *et al*., 2013). Increase in abnormally formed spermatozoa, spermatocytes degradation, as well as spermatogonia necrosis, resulting from decrease in androgen concentration has been linked to lead toxicity (Allouche *et al*., 2009; Anjum and Reddy, 2015). The synthesis and breakdown of follicle stimulating hormones (FSH), luteinising hormone (LH) and testosterone are affected by exposure to lead. It also affects the development of spermatozoa. Lead-induced testicular dysfunction also occurs as a result of testicular oxido–inflammatory stress and apoptosis (Patrick, 2006; Adibmoradi *et al.,* 2015).

The main cause of lead poisoning in the body is the increased production of reactive oxygen species (ROS). Lead produces ROS which include hydrogen peroxide, and reactive nitrogen species (RNS) (Hsu *et al*., 1997; Patrick, 2006). Reactive oxygen species are controlled by glutathione in the body (Su *et al.,* 2019). Lead equally has effects on other antioxidant enzymes, including superoxide dismutase and catalase (Elgawisha and Abdelrazek, 2014). Convectional long established chelating agents such as 2, 3-dimercaptosuccinic acid (DMSA) and penicillamine (PENN) are toxic to the testes (Flora and Pachauri, 2010; Sisombath *et al.*, 2014). Natural products or medicinal mushrooms which are rich in antioxidants have been reported to attenuate free radical-induced tissue damage (Li *et al*., 2022). Antioxidants present in mushrooms such as *Ganoderma lucidum* have been shown to inhibit or reduce ROS in tissues and cells and to abrogate their harmful effects (Li *et al.,* 2022; Zhang *et al.,* 2024). Prevention of the formation of free radicals is very important in arresting lead-induced testicular toxicity. This study was designed to evaluate the protective effects of *Pleurotus tuberregium* polysaccharide (PTP) and *Ganoderma lucidum* polysaccharide (GLP) against lead acetate-induced testicular derangement in Wistar rats.

MATERIALS AND METHODS

Chemicals and Solvents

Dimercaptosuccinic acid (DMSA), penicillamine (PENN), hydrogen peroxide, thiobarbituric acid, Ellman reagent, and trichloroacetic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Other chemicals and reagents were of analytical grade.

Extraction of Polysaccharides from *P. tuberregium and G. lucidum*

Pleurotus tuberregium and *Ganoderma lucidum* were obtained from an open forest in Iwaro Oka-Akoko, Ondo State, Nigeria. The mushrooms were identified and authenticated by a botanist, Dr. Ademola Olatokunbo of the Department of Plant Science and Biotechnology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State. The dried powdered (500 g) each of *G. lucidum* and *P. tuberregium* fruiting bodies were added separately to 2 L of water and boiled for 1 h at 60ºC separately. The mixtures were then filtered using Whatman filter paper #1 to obtain the filtrate and residue. The filtrate was centrifuged at 4000 rpm for 10 min at room temperature to obtain the supernatants. The supernatants were concentrated in a water bath set at 55ºC. The concentrates were precipitated with four volumes of absolute ethanol (i.e., in ratio 1:4) and centrifuged to separate the precipitates. The precipitates were dissolved in small volumes of distilled water and concentrated in the water bath to obtain *Pleurotus tuberregium* polysaccharide (PTP) and *Ganoderma lucidum* polysaccharide (GLP) extracts. The extracts were stored in an opaque container in a refrigerator at 4°C and were reconstituted in distilled water before being administered to the rats, according to the experimental protocol.

Experimental Animals

Male Wistar rats (180-230 g) were purchased from McTenny Farms, a commercial private colony near Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria, and housed under natural light and temperature conditions. Pelletized rat chow and water were provided *ad libitum*. Procedures involving animal handling followed the guidelines published by the National Institute of Health (Clark *et al*., 1997) and the study was reviewed by the University of Ilorin Ethical Review Committee (UIERC) and given an approval number UERC/ASN/2023/2572.

Experimental Design

Adult male rats (48) were randomly assigned to six groups (n=8). Group 1 received distilled water and served as control; group 2 received lead acetate (Pb) only (25 mg/kg); group 3 received Pb (25 mg/kg) + PTP (100 mg/kg); group 4 received Pb (25 mg/kg) + GLP (100 mg/kg); group 5 received Pb (25 mg/kg) + DMSA (50 mg/kg); and group 6 received Pb (25 mg/kg) + PENN (30 mg/kg) concurrently. Treatments were administered via the oral route using an orogastric tube for 28 days. At the conclusion of treatment procedures with drugs and extracts, animals were sedated with ketamine/xylazine (75/10 mg/kg, i.p.) and 4 mL of blood was drawn via cardiac puncture into plain tubes for hormonal assays. Two testes were harvested and rinsed free from blood and fat. One testis from each rat was prepared

for antioxidant and inflammatory marker enzymes assays while the other was fixed in Boulin's fluid for histological and immunohistochemical evaluations.

Sperm Parameters

Sperm motility

Sperm motility was evaluated immediately after the collection of epididymal fluid following the classical method described by Zemjanis (1970) and *Oghenetega et al*. (2024). Briefly, a small aliquot of the fluid was placed on a clean glass slide to which two drops of warm 2.9% sodium citrate solution were added. The preparation was then covered with a cover slip and examined under a light microscope (Leica DM 750) with a heated 37ºC stage, using a 40× objective lens under reduced illumination, to assess the proportion of motile sperm cells.

Sperm count

The caudal epididymis was dissected and homogenized in 5 mL of normal saline, after which the total homogenate volume was recorded. Sperm concentration was assessed using an improved Neubauer haemocytometer, and the results were expressed as the number of spermatozoa ($\times 10^6$) per millilitre of suspension, as previously described by Zemjanis (1970) and Oghenetega *et al*. (2024).

Sperm viability

Sperm viability was determined using the Eosin-Nigrosin staining technique, as outlined by Zemjanis (1970) and Oghenetega *et al*. (2024). The coverslip from the motility preparation was carefully removed, and two drops of the Eosin-Nigrosin stain were mixed with the specimen to create a smear. The smear was air-dried and subsequently examined under a 40× objective lens. Live sperm cells excluded the Eosin stain and thus appeared unstained, whereas dead sperm cells absorbed the stain and appeared pink or red. Viability was expressed as a percentage, calculated by counting 100 sperm cells per slide and determining the live-to-dead cell ratio.

Preparation of Testicular Homogenates

Phosphate buffered saline was used to homogenize the testes. The homogenate was centrifuged at 4ºC for 15 min at 3000 rpm. The supernatant was collected and used for biochemical assays including the measurement of antioxidant parameters and quantification of inflammatory marker enzymes. The other testis was sectioned for histopathologic and immunohistochemical assessments.

Serum Levels of FSH, LH and Testosterone

Serum was obtained after centrifuging blood samples at 4ºC for 15 min at 3000 rpm. The concentrations of LH, FSH and testosterone were determined in the serum using ELISA kits (Bioasssay systems, Hayward, CA, USA) according to the manufacturer's instructions.

Antioxidant Assays

The reduced glutathione (GSH) levels in supernatant of testicular homogenates, were determined using Ellman's reagent according to the protocol of Jollow *et al*. (1974). Malondialdehyde (MDA) level was determined using thiobarbituric acid reacting substance according to the method of Nagababu *et al*. (2010). The assay of nitrite concentration in the samples was measured using Griess reaction (Green *et al*. 1982). Catalase (CAT) activity was measured using colorimetric assay based on the yellow complex with molybdate and H_2O_2, which was described by Goth *et al*., (1991). Superoxide dismutase (SOD) activity was determined kinetically using the adrenaline autooxidation method (Misra and Fridovich, 1972).

Determination of Pro-inflammatory Cytokines and Myeloperoxidase Activity

Enzyme linked immunosorbent assay (ELISA) kit (ThermoFisher Scientific, USA) was used to measure interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α) in testicular homogenates supernatant according to manufacturer's instruction. The levels of cytokines were estimated by interpolation from standard curves by colorimetric measurements at 450 nm in an LT4500 ELISA plate reader (Labtech, UK).

Myeloperoxidase enzyme activity was determined in testicular tissues according to the method of Bradley et al. (1982). Testicular homogenate was suspended in 1 mL of 0.5% hexadecyltrimethyl ammonium bromide (HTAB) in KPO_4 buffer and processed through freeze-thaw cycles 3 times at -20ºC. The suspension was then kept at 4ºC for 20 min, centrifuged at 2,000 *g* for 10 min. The supernatant (50 µL) was taken into 96 well plate and 150 uL of test solution (0.17 mg/mL O-dianisidine dihydrochloride and 0.0005% H_2O_2) added to the wells. The reaction was monitored kinetically for 5 min at a wavelength of 405 nm in a microplate reader (LT 4500, Labtech, UK).

Histological Examination of the Testes

The Bouin's fixed testicular specimens were dehydrated in alcohol before embedding in paraffin. Sections (5 µm) thick were cut, deparaffinized and stained with haematoxylin and eosin. The stained slides were examined with a light microscope (Olympus BX63 model) at ×400 magnification, and the resulting photomicrographs were used to evaluate histoarchitecture of the testes.

Immunohistochemistry

The fixed tissues embedded in paraffin were sectioned at 5 µm thickness using a microtome. The slides were floated on glass slides, dewaxed and the antigens retrieved using heat retrieval method. Immunohistochemical staining of nuclear reactive factor-2 (NRF-2) and nuclear factor κβ (NF-κβ) was carried out following standard procedures and the expression measured using Fiji-image J software (NIH, USA) according to the techniques described by Hsu *et al.* (1981).

Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test (GraphPad Prism 5.0 Software, San Diego, USA) and the resulting data were expressed as mean ± standard error of mean (SEM). The level of significance was fixed at $P< 0.05$.

RESULTS

Effects of Treatment on Sperm Parameters

Treatment with Pb only caused significant ($P<0.05$) reduction in the level of sperm morphology, sperm motility, sperm viability and sperm count when compared with the control group. However, treatment with GLP significantly ($P<0.001$) increased the level of sperm morphology (Figure 1A). Concurrent treatment with Pb and PTP or GLP significantly increased the level of sperm motility ($P<0.001$) as shown in Figure 1B, and sperm viability ($P<0.001$) (Figure 1C), when compared with the Pb-only group. Similar results were obtained with concurrent treatment of rats with Pb and DMSA or Pb and PENN. In Figure 1D, treatment with Pb caused a significant ($P<0.05$) decrease in sperm count when compared with control rats. Simultaneous treatment with Pb plus PTP or GLP or DMSA did not increase the level of sperm count when compared with the Pb only. Treatment with Pb plus PENN increased the level of sperm count albeit insignificantly (Figures 1A – 1D).

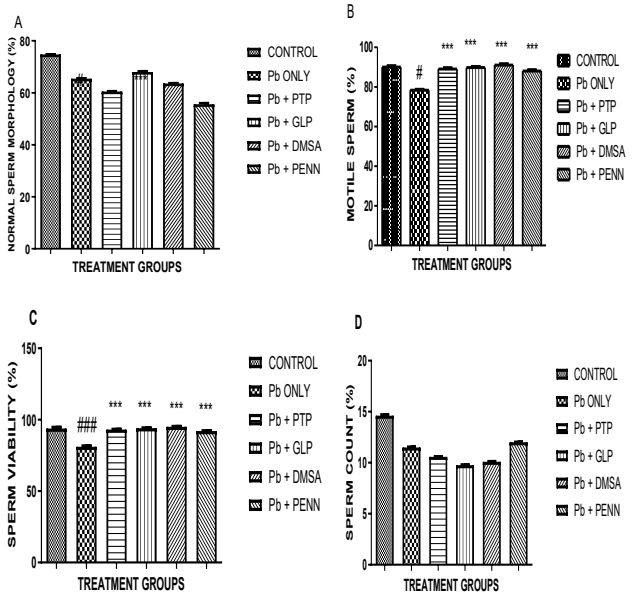

Figure 1: Effects of concurrent administration of lead acetate (Pb) with *Pleurotus tuberregium* polysaccharide (PTP) or *Ganoderma lucidum* polysaccharide (GLP) for 28 days on sperm morphology (A), sperm motility (B), sperm viability (C), and sperm count (D). $^{\#}P<0.05$, $^{\#\#\#}P<0.001$ compared with control and ***p<0.001 compared with Pb-only group. Values are represented as Mean ± SEM (n=5). DMSA: 2, 3-dimercaptosuccinic acid; PENN: penicillamine.

Antioxidative Effects of PTP and GLP

The group that received Pb-only treatment There was a significant decrease ($P< 0.05$) in the level of GSH, CAT, SOD and GST in the Pb-only group when compared with the control group (Figure 2). However, concurrent treatment with Pb and PTP or GLP or DMSA or PENN increased significantly ($P<0.05$) the level of GSH (Figure 2A). Treatment with Pb-only resulted in a decrease in the level of GST while the various treatment groups showed an insignificant increase in the level of GST (Figure 2B). While concurrent treatment with Pb and PTP or DMSA resulted in a significant ($P<0.001$) increase in the levels of catalase enzyme, treatment with GLP or PENN did not reverse the Pb-induced decrease in catalase enzyme level (Figure 2C). Concurrent administration of Pb with PENN resulted in a significant ($P<0.05$) increase in SOD while treatment of Pb with PTP or GLP or DMSA caused an insignificant increase in SOD (Figure 2D).

Administration of Pb caused a significant increase ($P<0.05$) in nitrite level when compared to the control group. Concurrent administration of Pb with PTP or PENN resulted in a significant ($P<0.05$) reduction in nitrite level

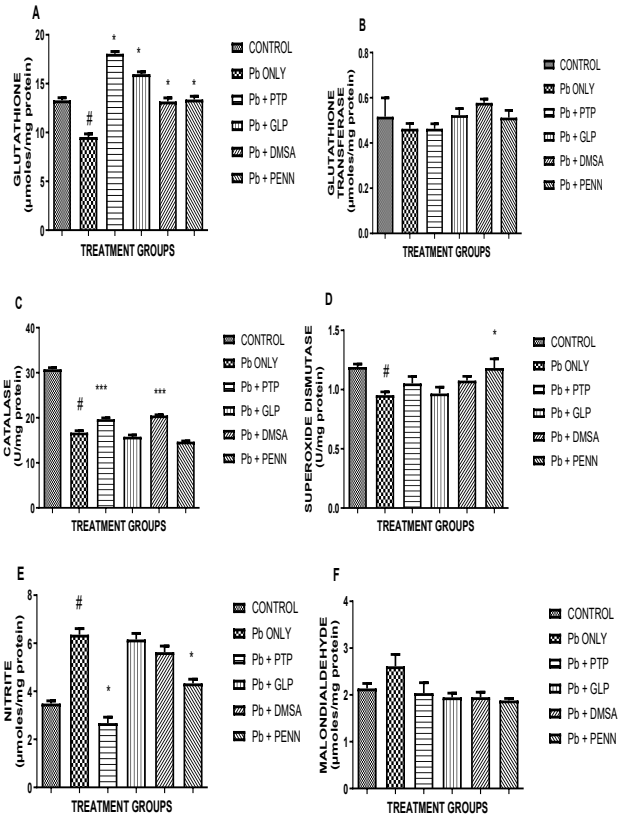

Figure 2: The ameliorative effect of PTP and GLP extracts in PbA-induced oxidative response. (A) GSH, (B) GST, (C) CAT, (D) SOD, (E) NITRITE, (F) MDA. Values are represented as Mean ± SEM (n=5). $^{\#}P<0.05$ compared with control and $*P<0.05$, $***P<0.001$ compared with Pb-only group. Pb: Lead acetate; PTP: *Pleurotus tuberregium* polysaccharide; GLP: *Ganoderma lucidum* polysaccharide; DMSA: 2,3-dimercaptosuccinic acid; PENN: Penicillamine.

while concurrent administration of Pb with either GLP or DMSA insignificantly reduced the Pb-induced increase in nitrite (Figure 2E). Administration of Pb caused an insignificant increase in the MDA levels but concurrent administration with either PTP or GLP or DMSA or PENN insignificantly reduced the Pb-induced oxidant levels of MDA (Figures 2F).

Effect on Markers of Testicular inflammation

Administration of Pb resulted in the significant (P<0.05) elevation of TNF-α and IL-6, two important pro-inflammatory markers. Concurrent administration of PTP or GLP or DMSA or PENN with Pb resulted in significant (P<0.001) reduction of Pb-induced increase in TNF-α levels. Significant reduction (P<0.001) in Pb-induced elevation of IL-6 levels was also obtained with concurrent administration of Pb with PTP or GLP or DMSA (Figures 3A and 3B).

Levels of myeloperoxidase, an oxidative enzyme, were insignificantly elevated by treatment with Pb compared to the control group. Concurrent administration of Pb with PTP caused a significant (P< 0.05) decrease in Pb-induced increase in this oxidative enzyme (Figure 3C).

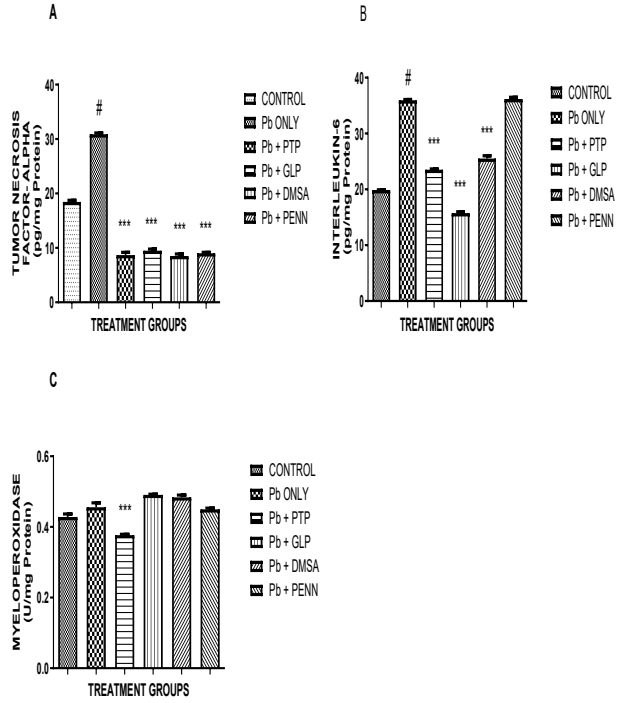

Figure 3: PTP and GLP ameliorated Pb-induced testicular derangement. (A) Tumor necrosis-alpha (TNF-α), (B) Interleukin-6 (IL – 6), (C) Myeloperoxidase (MPO). Values are represented as Mean ± SEM (n=5). #P<0.05 compared with control and ***P<0.001 compared with Pb-only group. Pb: Lead acetate; PTP: *Pleurotus tuberregium* polysaccharide; GLP: *Ganoderma lucidum* polysaccharide; DMSA: 2,3-dimercaptosuccinic acid; PENN: Penicillamine.

Ameliorative Effect of PTP and GLP Pb-induced Decrease in Testicular Hormones

As shown in Figures 4A and 4B, rats treated with Pb only had decreased levels of serum and testicular testosterone when compared to the control. However, concurrent administration with PTP or GLP or DMSA or PENN, resulted in insignificant increase in the level of this androgen. The treatment of rats with Pb resulted in an increase in the level of LH, which was significantly reduced (P<0.001) when Pb and PTP were concurrently administered. Concurrent administration of Pb with GLP or DMSA or PENN, however, caused an insignificant reduction in Pb-induced elevation of LH (Figure 4C). Pb treatment resulted in an insignificant decrease in testicular FSH and when concurrently administered with either PTP or GLP or DMSA had little or no effects on Pb-induced reduction in FSH levels, while PENN insignificantly reversed Pb-induced decrease in FSH level.

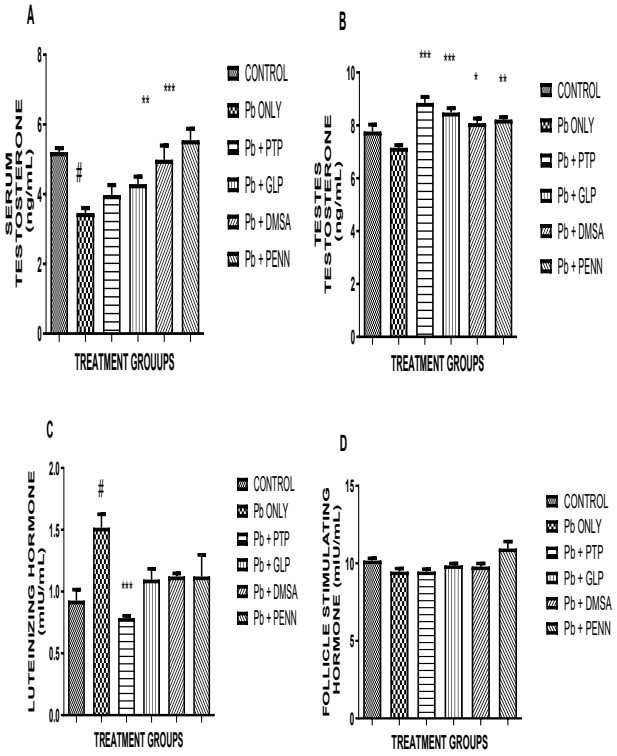

Figure 4: PTP and GLP ameliorated Pb-induced testicular derangement. (A) Serum testosterone, (B) Testicular testosterone, (C) Luteinizing hormone, and (D) Follicle stimulating hormone. Values are represented as Mean ± SEM (n=5). #P<0.05 compared with control and **P<0.01, ***p<0.001 compared with Pb-only group. Pb: Lead acetate; PTP: *Pleurotus tuberregium* polysaccharide; GLP: *Ganoderma lucidum* polysaccharide; DMSA: 2,3-dimercaptosuccinic acid; PENN: Penicillamine.

Reversal Effect of PTP and GLP Pb-induced Histoarchitectural Alteration in Testicular Dysfunction

Figures 5A – 5F are histological sections of the testis of rats. Exposure to Pb only (Figure 5B) altered the histoarchitecture of the tissues, which also containns seminiferous tubules with maturation arrest (black arrow).

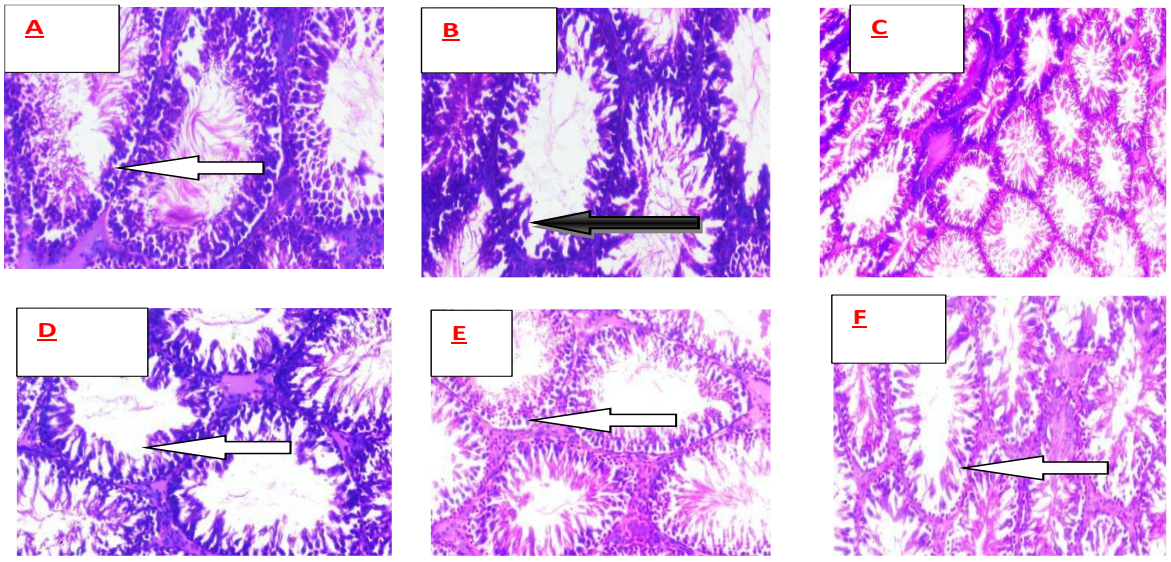

Figure 5: Photomicrographs of the testes of rats administered lead acetate (Pb) and *Pleurotus tuberregium* polysaccharide (PLP) or *Ganoderma lucidum* polysaccharide (GLP). 2,3-dimecarptosuccinic acid (DMSA), Penicillamine (PENN). (A) Control, (B) Pb-Only, (C) Pb + PTP, (D) Pb + GLP, (E) Pb + DMSA, (F) Pb + PENN (H&E, ×400).

Concurrent administration with PTP or GLP or DMSA or PENN resulted in normal and complete germinal cells, and seminiferous tubules that contained spermatozoa (Figures 5 C-5F).

Effect of PTP and GLP on Pb-induced Testicular Dysfunction and NF-kB Expression

Figures 6A – 6F show immunohistochemically stained slides and the intensity scores of testicular expressions of NF-kB. There was an increase in the expression of NF-kB following the administration of Pb in the group administered with Pb only. Concurrent treatment with Pb and PTP or GLP did not reverse this effect of Pb-induced testicular toxicity. However, there was an insignificant reduction in the level of NF-kB expression due to concurrent administration of PENN with Pb.

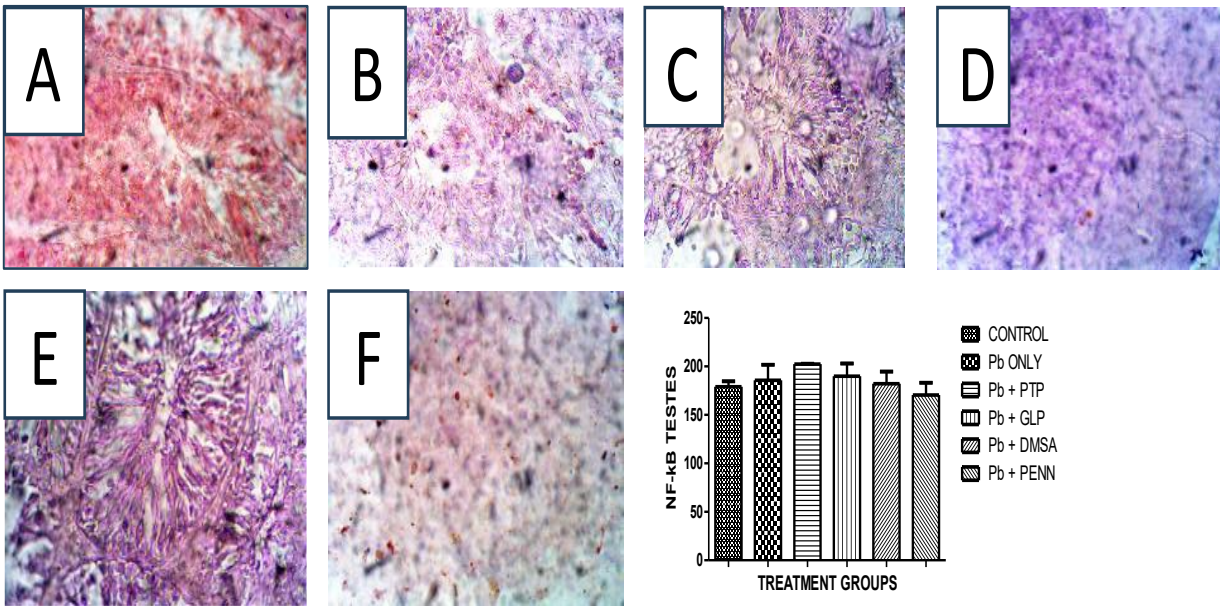

Figure 6: Micrographs of immunohistochemical staining and intensity score of testicular expression of NF-kB. (A) Control, (B) Pb-Only, (C) Pb + PTP, (d) Pb + GLP, (E) Pb + DMSA, (F) Pb + PENN. Values are represented as Mean ± SEM (n=5). Values are not statistically significant. Pb: Lead acetate; PTP: *Pleurotus tuberregium* polysaccharide; GLP: *Ganoderma lucidum* polysaccharide; DMSA: 2,3-dimercaptosuccinic acid; PENN: Penicillamine.

Effect of PTP and GLP on Pb-induced Testicular Dysfunction on NR-F2

Figures 7A-F show immunohistochemical stained slides and the intensity scores of testicular NR-F2 activities. Treatment with Pb resulted in significantly elevated NR-F2 expression compared to control rats. Coadministration of Pb plus PTP significantly (P<0.05) reduced the expression of testicular NR-F2 however coadministration with GLP had no effect. Concurrent administration of Pb with either DMSA (P<0.001); or PENN (P<0.01) significantly ameliorated Pb-induced elevation of NR-F2.

DISCUSSION

This study was designed to evaluate the protective effects of *Pleurotus tuberregium* polysaccharide (PTP) and *Ganoderma lucidum* polysaccharide (GLP) on lead-induced testicular toxicity which has been shown to suppress spermatogenesis and sperm development. Previous studies (Okolo *et al*., 2016; Okolo *et al*., 2017) demonstrated the protective effects of PTP on carbon-tetrachloride induced testicular injury in rats. Zhang *et al*.

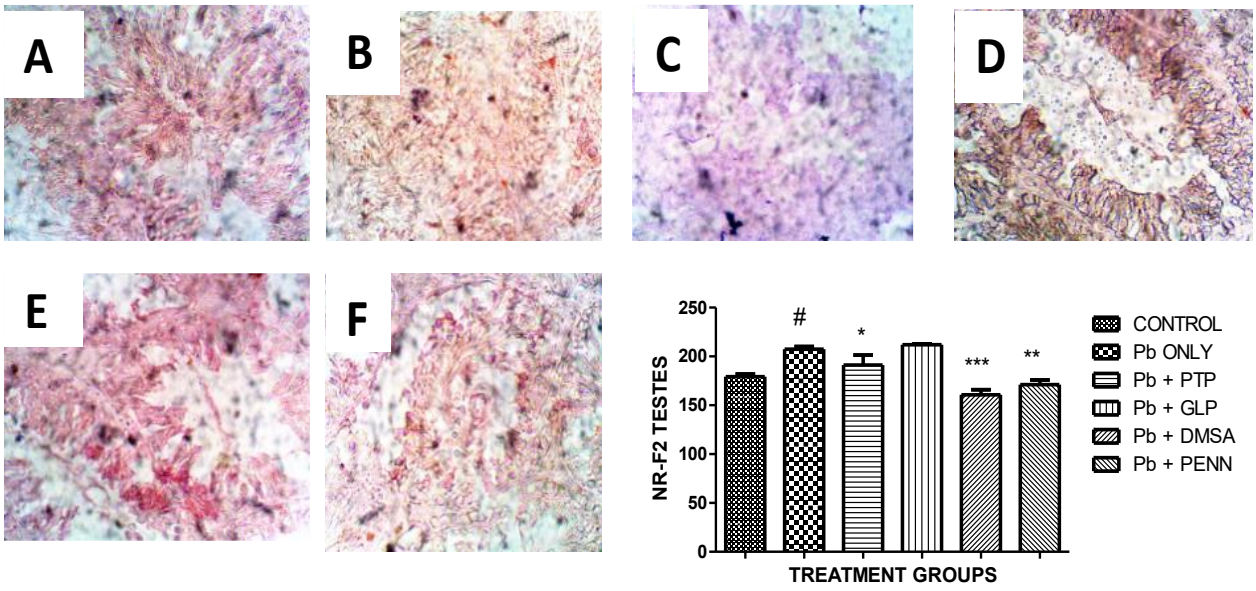

Figure 7: Micrographs of immunohistochemical staining and intensity score of testicular expression of NR-F2. (A) Control, (B) Pb-only, (C) Pb + PTP, (D) Pb + GLP, (E) Pb + DMSA, (F) Pb + PENN. Values are represented as Mean ± SEM (n=5). $^{\#}P$<0.05 compared with control and *P<0.05, **P<0.01, ***P<0.001 compared with Pb-only group. Pb: Lead acetate; PTP: *Pleurotus tuberregium* polysaccharide; GLP: *Ganoderma lucidum* polysaccharide; DMSA: 2,3-dimercaptosuccinic acid; PENN: Penicillamine.

induced testicular derangement in Wistar rats. Markers of sperm functions which were assessed via semen analysis were negatively affected after 28 days exposure to Pb. This is consistent with previous studies which reported that Pb caused degenerative changes in spermatids, inducing vacuolization and a decrease in the number of cytoplasmic organelles in Leydig cells (Acharya *et al*., 1997**;** Fahim *et al*., 2013). More specifically, we report that exposure to Pb, decreased sperm count, sperm motility and sperm viability (Al-Shaikh *et al*., 2013**;** Elgawish and Abdelrazek, 2014; Anjum and Reddy 2015; Sudjarwo *et al*., 2017; Massányi *et al*., 2020). The observation that Pb-treated mice have a lower sperm count has been well documented (Acharya et al., 2003). Reduction in sperm viability and sperm motility could be because of oxidative stress which was observed in the group of rats administered with Pb only. Exposure to Pb can induce oxidative damage, leading to the generation of harmful free radicals and depletion of the body's antioxidant defenses (Patrick, 2006) but concurrent treatment with PTP and GLP increased sperm viability and sperm motility. These findings suggest that PTP and GLP mitigate lead-

(2024) showed that GLP ameliorated cyclophosphamide-induced male reproductive injury in mice. Sokol *et al*. (1998) reported that exposure of male rats to Pb resulted in suppression of serum testosterone, intratesticular testosterone, and spermatogenesis, without a concomitant increase in gonadotropins, leading to the conclusion that a major site of lead toxicity is the hypothalamic-pituitary axis. Exposure to Pb is associated with hormonal imbalance causing reproductive impairment by disrupting the hypothalamic-pituitary control of FSH and LH secretion, resulting in impaired spermatogenesis and male fertility.

Exposure to Pb has been shown to cause oxidative stress by distorting the homeostatic balance between free radical production and antioxidant activities (Ercal *et al.*, 1996, Acharya *et al.*, 2003; Patrick, 2006), resulting in increased lipid peroxidation. The deleterious effects of increase in reactive oxygen species (ROS) production in tissues have been suggested as the main mechanism underlying morbidities associated with lead toxicity (Hsu *et al*., 1997). In our study, it was observed that there was disruption of

spermatogenesis in the lumen of the seminiferous tubules of the testes of rats exposed to lead acetate. This may be attributed to oxidative stress (Asadpour *et al*., 2013). Treatment with Pb for 28 days in the present study resulted in decreased testicular SOD, CAT, GST and GSH activities which is indicative of testicular oxidative stress in the Pb-only group. There was also an increase in the levels of MDA, a product of lipid peroxidation, and nitrites. The increase in MDA and nitrite levels observed in the Pb-only group can be attributed to decrease in SOD, CAT, GST and GSH level, which resulted in lipid peroxidation. This result is supported by other previous studies (Marchlewicz *et al*., 2004, Mishra and Acharya, 2004; Elgawish and Abdelrazek, 2014; Sudjarwo *et al*., 2017). Excessive amounts of ROS affect redox balance and results in oxidative stress and attendant lipid peroxidation (Hsu *et al*., 1997; Aprioku, 2013; Patrick, 2006). Lead exposure has been shown to reduce the testicular levels of endogenous antioxidants which are important markers of oxidative stress, such as GSH, CAT and SOD in rats (Abdel Moniem *et al.*, 2010) and mice (Sharma *et al*., 2010; Zhang *et al*., 2024). It has been reported that because the testis readily succumbs to oxidative stress, it is probably not surprising that lead-induced oxidative stress is easily manifested in the testis (Tomascik-Cheeseman *et al*., 2004). In addition, sperm plasma membrane, being rich in polyunsaturated fatty acids, is highly susceptible to ROS attack (Asadpour *et al*., 2013). Lead accumulates in the reproductive system and has been implicated in the development of oxidative stress via induction of lipid peroxidation (Marchlewicz *et al*., 2007, Mishra and Acharya, 2004)**.** Treatment with PTP and GLP resulted in decreased NO and MDA levels, accompanied by a significant increase in SOD, CAT, GST and GSH, which showed that PTP and GLP boosted the antioxidant defence system. Also, lead-exposed rats treated with DMSA and PENN for 28 days showed significant increase in the level of SOD, CAT, GST and GSH when compared with Pb-only group. Flora and Pachauri (2010) and Sisombath *et al*. (2014) previously reported that DMSA and PENN bind to lead and enhance its excretion from the body.

Testicular levels of some inflammatory cytokines (TNF-α and IL-6) and MPO an inflammatory marker enzyme were investigated to establish the possibility of testicular inflammation after exposure to lead acetate. When compared with the control group, the levels of cytokines, TNF-α, IL-6 and MPO, in Pb-only group were significantly higher when compared to the untreated control, suggesting that Pb produced testicular inflammation. Treatment with PTP and GLP reduced the level of TNF-α, IL-6 and MPO. This showed that PTP and GLP may possess anti-inflammatory properties. Previous studies by Cai *et al.* (2018**)** support the findings from this study on the anti-inflammatory potentials of GLP. In addition, Wei *et al.* (2018) showed that polysaccharides from *Ganoderma lucidum* alleviated dextran sulfate sodium-induced colitis in mice and advocated for the use of GLP in the management of inflammatory diseases of the bowel.

Spermatogenesis is a concerted sequence of events from the development of spermatogonia to spermatozoa. This process involves differential gene expression and cell-cell interplay regulated by key endocrine stimuli. The testicular germinal cell which serves as a first step in sperm formation requires testosterone for cell division and maturation and subsequent release by the Leydig cell. FSH in concert with testosterone, affects the maturation, proliferation and function of the supporting Sertoli cells that produce regulatory signals and nutrients for the maintenance of developing sperm cells (Ramaswamy and Weinbauer, 2014; Smith and Walker, 2014). In males, FSH and LH work together to trigger their testes to begin producing testosterone. In our study administration of Pb resulted in decrease in the levels of follicle FSH and testosterone. Exposure to Pb causes decreased testosterone production (Anjum and Reddy, 2015). This explains the decrease in sperm count observed in Pb-only group. Reduction in sperm count and sperm motility in Pb-treated rats could be due to decrease in FSH. Both FSH and LH are crucial for regulating testosterone production and spermatogenesis and are essential for sperm development and function (Allouche *et al*., 2009). The anterior pituitary gland secrets LH which enables the Leydig cells to produce testosterone. Exposure to Pb causes direct testicular injury which results in decreased testosterone release from the Leydig cells.

In this study, immunohistochemical staining revealed that administration of lead acetate caused an insignificant increase in the level of expression of testicular nuclear factor kappa B (NF-kB) in the Pb-only group. However, treatment with GLP or PTP did not reverse the observed Pb-induced increase. Inflammation is a biological defence mechanism that is triggered in response to harmful insults such as pathogens, toxins, and injuries, which damage the cells.

The nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcriptional factor which regulates cellular resistance to oxidants. Nrf2 controls the basal and induced expression of an array of antioxidant response element-dependent genes to regulate the physiological and pathophysiological outcomes of oxidant exposure. Nrf2 plays a crucial role in inducing the body's antioxidant and anti-inflammatory responses, such as regulating redox balance, energy metabolism, drug metabolism and proteasomal degradation (Qiang, 2013; Ngo and Duennwald, 2022). Studies have shown that lead acetate exposure in rats leads to an up-regulation of Nrf2 at both the mRNA and protein levels in testicular tissue. This increase in Nrf2 expression is thought to be a protective mechanism against the oxidative stress caused by lead acetate (Wang *et al*., 2013). Immunohistochemical findings from the current study showed that PTP, as well as DMSA and PENN treatment of rats caused a decrease in Pb-induced elevation of Nrf2.

Histopathological alteration occurred in the testes because of Pb toxicity. In line with the previous studies by Fahim *et*

al. (2013) and Adibmoradi *et al*. (2015), our histopathological results showed that the group given Pb only had spermatozoa maturation arrest as the lumen contained no spermatozoa, and germinal cell development was incomplete. However, concurrent treatment with PTP or GLP reversed these alterations.

CONCLUSION

This study has explored the roles of PTP and GLP in enhancing spermatogenesis and their ability to protect against mechanisms related to testicular oxidative stress and inflammation associated with Pb toxicity. Our findings therefore suggest that PTP and GLP are natural substances with antioxidants and anti-inflammatory potentials capable of mitigating Pb-induced testicular toxicity.

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CONFLICT OF INTEREST

All the authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

OEO and MOS conceptualized the study. MOS carried out the experiment and wrote the manuscript. SOA contributed to the writing of the manuscript. JTA carried out the immunohistochemistry aspect. AMA and OEO supervised the entire work.

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All the authors declare no conflict of interest.

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