Bioactive profiling and therapeutic potential of mushroom (*Pleurotus tuberregium*) extract on Wistar albino rats (*Ratus norvegicus*) exposed to arsenic and chromium toxicity

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ABSTRACT

Mushroom species are valued in gourmet traditions around the world for their unique taste, aroma, nutritional value and medicinal potentials. The bioactive profiling of *P. tuberregium* mushroom was evaluated to determine its therapeutic effect on Wistar albino rats exposed to arsenic (As) and chromium (Cr) toxicity. Proximate analysis of *P. tuberregium* showed high composition of carbohydrate (80.24) followed by moisture (21.16), protein (11.46), ash (3.03) and fibre (0.25) content. Phytochemical analysis revealed the presence of polyphenols (2.58), alkaloid (2.46), oxalate (4.25), flavonoid (1.68), tannin (0.38) and Saponin (trace) in trace amount. Mineral analysis yielded variable amounts of Na, Mg, K and Ca. Therapeutics assessment of *P. tuberregium* to Wistar albino rats exposed to As-Cr toxicity showed improved feed and water intake during the exposure duration. Haematological indices revealed significant increase in platelet (PLT), granulocytes and monocytes while lymphocyte (LY) and red cell distribution width (RDW) were low. Biochemical and redox marker of liver and kidney profiles showed decrease in alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) in the liver. Creatinine and urea in the kidney also decrease while total protein increased significantly. Malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione S-transferase (GST) decrease in the liver and kidney of the therapeutic group when compared with As-Cr treated rats. The presence of alkaloids and flavonoids in significant amount may have contributed in the therapeutic changes observed in all the parameters. Therefore, our findings conclude that *P. tuberregium* possessed remarkable effect against As-Cr induced toxicity in albino rats and may be useful in metal toxicity treatment in man and may be concluded that they are therapeutically effective.

1. Introduction

Africa population is highly vulnerable to frequent exposure to heavy metal pollution because of rapid industrialization and urbanization. Heavy metals are significant environmental pollutants and have become a major public health threat because they are persistent in the environment and can lead to serious wildlife and human health effects and even death. Two recent incidents that highlighted the vulnerability of African population to large scale metal exposure is the lead (Pb) poisoning that occurred in Zamfara State, Nigeria in 2010 from informal artisanal gold mining activities [69] and Dakar, Senegal between November 2007 and March 2008 from informal lead-acid battery dismantling and recycling [44]. Heavy metals enter the environment through natural and anthropogenic means. Anthropogenic activity has contributed significantly to the elevated environmental concentrations of heavy metals in Africa. As and Cr are predominantly products of anthropogenic insults that have become ubiquitous in natural ecosystems. They affect global health due to their toxicity and carcinogenicity [9,19,20,48,49,65,68,106]. As and Cr exist in the environment at low concentrations in soil, water, air, and food such that humans are constantly exposed to this contaminant through the food chain.

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Arsenic is a toxic metalloid which represents a global environmental health threat. It is the twentieth most abundant element on earth and can exist in organic and inorganic forms with different oxidation or valence states [111]. The valence states of arsenic compounds relevant to human health are the trivalent (As III) and pentavalent (As V) states [111]. The inorganic forms such as arsenite and arsenate compounds are predominantly lethal to living organisms. As is released into the terrestrial and aquatic ecosystems through a combination of natural and anthropogenic processes such as mining and processing of ores and humans may be exposed by natural, industrial, or from unintended sources [53]. Accidental consumption of Arsenic through food chain may result in acute and chronic arsenicosis typically defined by classical dermal stigmata and internal disorders.

Chromium also is one of the most toxic heavy metals and 7th most abundantly element in the environment [76,82,93]. It also occurs in several oxidation states in the form of divalent Cr (II), trivalent (Cr III) and hexavalent (Cr VI) [94,108]. The most commonly occurring forms are Cr (III) and Cr (VI), with both states highly toxic to animals, humans and plants [76] due to their ability to accumulate in tissues of organisms. Cr occurs naturally by the burning of oil and coal, petroleum from ferro chrome refractory material, pigment oxidants, catalyst, chromium steel, fertilizers, oil well drilling and metal plating tanneries. Anthropogenically, Cr is released into the environment through sewage and fertilizers [38,108]. Cr (III) is immobile in its reduced form and is insoluble in water whereas Cr (VI) in its oxidized state is highly soluble in water and thus mobile [116]. The health hazards associated with Cr exposure are dependent on its oxidation state, ranging from the low to high toxicity of the hexavalent form which has been classified as a carcinogen possessing mutagenic and teratogenic properties [108,112]. Cr has nutritive importance [57] in very small amount and plays an important role in glucose metabolism by serving as a cofactor for insulin action.

Most heavy metal poisoning treatment has primarily been by chelation therapy. The chelating agents bind to toxic metal ions to form complex structures which are easily excreted in urine and faeces from the body [32]. However, the use of natural products as alternative chelating agents in heavy metal toxicity treatment has not been fully explored. Mushrooms a source of biologically active compounds of medicinal and therapeutic values occur naturally in Nigeria during the early and late rainy seasons [35] and are usually found in forests, grasslands, damp rotten logs etc. P. tuberregium are common species widely consumed for their nutritional value, taste, aroma and nutraceuticals/medicinal properties [66,113]. Nearly 80% of the world’s population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [98]. A variety of compounds with important pharmacological properties have been isolated from mushroom, which include polysaccharides, polysaccharopeptides, polysaccharide-protein with immuno-enhancing and anticancer properties [114]. Also, other isolates have shown potential antiviral, anti-bacterial, antiparasitic, anti-inflammatory, and anti-diabetic properties in preliminary studies [70]. Various researches have shown that mushroom lectin is a powerful scavenger of the superoxide anion, the hydroxyl radical and nitrogen dioxide [56]. Pleurotus spp. has been proven to have anticholesterolemic and antioxidant properties [4], blood lipid lowering effects [46,90], antihepatoma and antisarcoma activities [113]. Effects of heavy metals on several biochemical and haematological parameters have been studied by many researchers [47,51,81,95,102,110] however therapeutic role of mushrooms in heavy metal toxicity remain very scanty in literatures. Therefore, this study aims to evaluate the therapeutic application of P. tuberregium against arsenic and chromium induced toxicity using a battery of biochemical and haematological tests as valuable biological indicator.

2. Materials and methods

2.1. Experimental animals

Eighteen (18) healthy male albino rats of Wistar strain (Rattus norvegicus) of 8–10 weeks old weighing approximately 170–200 g were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The study protocol followed the principles and Guide for the Care and Use of Laboratory animals of the National Institute of Health (NIH) [83]. The rats were acclimatized for 21 days in plastic cages at temperature (22 ± 2 °C), humidity (40 ± 10%) and 12 h light-dark cycle and given laboratory chow and tap water ad libitum before the experiment.

2.2. Chemicals

All chemicals used were of the analytical grade. Potassium dichromate (K₂Cr₂O₇) (Mol. wt., 294.18, CAs No. 7778-50-9) and Sodium arsenite (Na₂AsO₂, Mol. Wt., 129.9 As 57.6% CAS No 7784-46-5) were purchased from Rovet Scientific Limited Benin City, Nigeria.

2.3. Collection of fungi

Fresh fruiting bodies of P. tuberregium were collected from Enwan community in Akoko-Edo Local Government area of Edo State. They were identified in the Department of Plant Biology and Biotechnology, University of Benin. Samples were taken to the laboratory of Ecotoxicology and Environmental Forensics for preparation of extract, determination of phytochemical properties and therapeutic bioassay test.

2.4. Preparation of extract

The methanol extract of P. tuberregium was prepared according to the method described by Ref. [107] with slight modifications. Briefly, freshly harvested whole matured mushrooms were thoroughly washed with cold sterile water and disinfected by treating with Mercury (II) chloride (HgCl₂) and washed again. The edible portions were carefully removed, cut into small piece using a stainless-steel blade and then air dried in shade under room temperature for seven days. Samples free of moisture and had crunchy appearance were separately crushed into fine powders using a blender. 100 g of crushed samples were mixed with 200 ml of pure methanol and were kept in a shaker (IKA400i, Germany) at 120 rpm and 30 °C for 48 h. The liquid extracts were filtered using Whatman No. 4 filter paper. The residue was then extracted with two additional 200 ml portions of methanol, as described earlier. The combined methanolic extracts were evaporated using a rotary evaporator (R-215, BUCHI, Switzerland) under a reduced pressure (100 psi) at a controlled temperature (40 °C) to remove the solvent and obtain the soluble components of the samples in a paste form. Each extract (the concentrated) (47.51%) extract (mass of extract × 100/mass of powder) was stored in a sterile container and preserved in a refrigerator at 4 °C for further use.

2.5. Chemical and bioactive profiling of mushroom

2.5.1. Proximate and mineral analysis

P. tuberregium edible mushroom species was analysed for food composition according to the method of Association of Official Analytical Chemists [5]. These include the determination of crude protein, fat, moisture content, ash, fibre, carbohydrate and minerals. Values for heavy metals were determined using Atomic Absorption Spectrophotometer (AAS). The percentage of all the fractions (crude protein, crude fat, minerals and ash) were added together and subtracted from 100 to obtain the total carbohydrate percentage.
2.5.2. Phytochemical screening

The prepared liquid extracts of the *P. tuberregium* sample were chemically tested for the presence of bioactive chemical constituents using standard procedures [25,105]. The extracts were then screened for alkaloids, flavonoids, tannins, saponins, Oxalate reducing compounds, polyphenol, Hydrogen cyanide, Hydroxymethylphlobatannins and anthraquinones.

2.6. Bioassay procedure

Wistar albino rats were randomly selected and divided into four groups with two animals in each group. Each rat was marked to permit individual identification and was kept in the cage prior to dose administration.

- **Group A:** Animals received normal diets + 150 mg/kg *P. tuberregium* mushroom extract dissolved in saline water (treated group).
- **Group B:** Animals received normal diets + 10 mg/kg Sodium arsenite (NaAsO₂) and Potassium dichromate (K₂Cr₂O₇) (treated group).
- **Group C:** Animals received normal diets + 10 mg/kg of Sodium arsenite (NaAsO₂) and Potassium dichromate (K₂Cr₂O₇) + 150 mg/kg of *P. tuberregium* mushroom extract dissolved in saline water (therapeutic group).
- **Group D:** Animals received normal diets and drinking water ad libitum (normal healthy control).

All other reagents and chemicals used were of analytical grade. Doses of *P. tuberregium* extract were administered orally by oral feeding-gavage method to Wistar albino rats five times a week throughout the duration of the study. During exposure, As-Cr concentrations in exposure water were determined by atomic absorption spectrometer (AAS). The animals were observed for gross behaviour, changes in general appearance, weight gained and toxicity as described in OECD guidelines 423 [89]. Also, each rat was weighed and food intake was determined. Feed efficiency was calculated according to the equation below.

Feed efficiency = \( \frac{\text{Body Weight Gain}}{\text{Food Intake}} \)

Relative organ weight = \( \frac{\text{Absolute organ weight (g)}}{\text{Bodyweight at sacrificed day (g)}} \times 100 \)

Concentration (mg/ml) = \( \frac{\text{Dose rate (mg/kg body weight) x Body Weight (kg)}}{\text{Volume (ml)}} \)

2.6.1. Blood collection

At the end of the 28 days experimental period, rats were starved overnight and anaesthetized using diethyl ether. The rats were dissected and 10 ml of blood samples were collected from the abdominal aorta with disposable heparinized syringe and subsequently divided into 5 ml and 10 ml of blood samples were collected from the abdominal aorta overnight and anaesthetized using diethyl ether. The rat was dissected and anthraquinones.

2.6.2. Preparation of serum

The procedure described by Ref. [117] was employed in the preparation of the serum. Briefly, 5 ml of the blood was allowed to clot for 10 mins at room temperature and then centrifuged at 1282g \( \times 5 \) mins using Hermle Bench Top Centrifuge (Model Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 h for different biochemical analysis.

2.6.3. Body and organ weights

The body weight of each rat group was measured just before and after completion of the treatments. Liver and kidney weights of all rats were measured after post treatment sacrifice.

2.6.4. Haematological parameters

Haematological parameters such as white blood cells (WBCs), neutrophils, lymphocytes, monocytes, eosinophils, red blood cells (RBCs), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), red cell distribution width-standard deviation (RDWSD), red cell distribution width-coefficient of variation (RDW-CV), platelets distribution width (PDW) and mean platelets volume (MPV) were analysed using an automated haematology analyser (Sysmex KX–21N (Sysmex Corporation, Kobe, Japan)).

2.6.5. Biochemical analysis of redox markers

To measure the activity of antioxidant enzymes as well as the oxidation of lipids and proteins, liver and kidney tissue samples were homogenized (100 g for 1 min, 1:10 w/v; Pobel 245432, Madrid, Spain) in a sucrose buffer (15 mM Tris/HCl, pH 7.4, 250 mM sucrose, 1 mM EDTA, and 1 mM dithiothreitol) in an ice bath. The homogenate was centrifuged at 900 g for 10 min at 4 °C. The resulting supernatant was used for the biochemical assay.

2.6.5.1. Glutathione peroxidase (GPx).

Activity of GPx was determined and expressed in terms of μmole in which GPx catalyzes the oxidation of GSH by hydrogen peroxide. GSH consumed/min/mg protein was measured at 420 nm on Spectrophotometer Genesys 10 UV [31].

2.6.5.2. Catalase (CAT).

The CAT activity was determined by the method of Aebi [119]. Catalase degrades H₂O₂, which can be measured directly by the decrease in the absorbance at 240 nm. In this method, 50 μl of liver homogenate diluted 50-fold was added to 650 μl of 50 mM phosphate buffer, pH 7.0. The reaction was initiated by addition of 300 μl of 54 Mm H₂O₂ and the decrease in absorbance was measured for 1 min at 25 °C. A unit of catalase activity was defined as such amount of the enzyme which decomposed 1 μmol H₂O₂ per minute.

2.6.5.3. Determination of glutathione (GSH) content.

GSH level was determined using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) for colour development and reading was taken at 412 nm. Briefly, 100 μl of supernatant of tissue homogenate was added to an equal volume of the metaphosphoric acid and then centrifuged at 2000g for 2 min to remove protein. Then 50 μl of 4 M triethanolamine per ml of the supernatant was added to increase the pH of the sample. For total GSH assay, 50 μl of sample was added to 150 μl of a reaction mixture containing 0.4 M 2-(N-Morpholino) ethane sulfonic acid, 0.1 Mm 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB) and 0.1 unit GR. The absorbance was measured using a GBC Cintra 20 Spectrophotometer at 5 min intervals for 30 min. Reduced glutathione (GSH) concentration were expressed as μmol g⁻¹ of wet tissue and μmol ML⁻¹ of plasma. An standard curve using reduced glutathione was used for calibration [28].

2.6.5.4. Glutathione reductase (GR).

GR activity was measured following the procedure [118] provided by the suppliers of the assay kit. GR activity was assayed spectrophotometrically by monitoring the oxidation of NADPH to NADP⁺ by GR at 340 nm. Briefly, 200 μl of reaction mixture contained 50 mM potassium phosphate (pH 7.5), 1 Mm EDTA, 1Mm GSSG and 0.1 mM NADPH. The reaction was initiated by addition of 20 μl of supernatant of tissue homogenate. The decrease in absorbance at 340 nm was recorded at 60 s intervals for 6 min. Each assay was performed in duplicate and enzyme units were reported as nmol NADPH oxidized/min/g of wet tissue of ml of plasma.
2.6.5.5. Malondialdehyde (MDA) level. The level of MDA as a measure of lipid peroxidation (LPO) was determined using the thiobarbituric acid (TBA) spectrophotometric assay with 1, 1, 3, 3-tetraethoxypropylene as a standard [85]. TBA reacts with some products of lipid peroxidation in acidic environment at increased temperature to form a pink compound. Briefly, 250 μl of a liver homogenate were added to 250 μl of distilled water, 500 μl of 15% trichloroacetic acid (TCA), and 500 μl of 0.37% TBA. TCA and TBA solutions were prepared in 0.25 M HCl. The samples were heated in a boiling water bath for 10 min. After cooling, the samples were centrifuged at 12,000g for 10 min. The absorbance of the supernatant was measured at 532 nm on GBC Cintra 20 Spectrophotometer.

2.6.5.6. Determination of superoxide dismutase (SOD) activity. SOD activity was measured by a colorimetric assay in the tissue homogenates using modified method of NADH-phenazinemethosulphate-nitritobenzene diamzonum inhibition reaction. Superoxide radical anion O2-, the substrate for SOD, was generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen. The pink oxidation product of epinephrine (adrenochrome) was measured spectrophotometrically at 485 nm [74]. Briefly, to 875 μl of 50 mM carbonate buffer, pH 10.2, 25 μl of liver homogenate diluted 100-fold and 100 μl of 10 mM epinephrine solution were added. The absorbance at 485 nm on Spectrophotometer Genesys 10 UV was measured for 3 min [59]. As a standard 25 μl of SOD solution with known activity was used.

2.6.5.7. GST activity. GST activity was assayed using 4-chloro, 1,3-dinitrobenzene (CDNB) as a substrate by the procedure of Ref. [109]. The assay was conducted by monitoring the appearance of the conjugated complex CDNB and GSH at 340 nm. The specific activity of this enzyme is calculated as OD/mg protein/min.

2.6.6. Measurement of biochemical parameter

Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total proteins, urea and creatinine were determined colorimetrically by using the standard ready-to-use kits and methods of Randox laboratories. The manufacturer’s instructions for each biochemical parameter were strictly followed during the assessment. Serum sodium and potassium was estimated using ion selective electrode (an automated method).

2.7. Statistical analysis

Data analysis was carried out using IBM SPSS 21. Data were analyzed using general descriptive statistics, one Way Analysis of Variance (ANOVA) at 95% probability level of significant. Duncan’s multiple range tests were used to compare the different experimental groups.

3. Results and discussion

3.1. Phytochemical analysis of P. tuberregium

The result of the phytochemical analysis of ethanolic extract of P. tuberregium and distributions are presented in Tables 1 and 4. The result reveals the presence of alkaloids, saponins, flavonoids, tannins and polyphenols. The reducing compounds, anthraquinones and hydroxymethylanthraquinones were also determined and found to be totally absent in the sample. Alkaloids, oxalate, flavonoids and polyphenol compounds were present in excess while Saponins and tannin were in trace amount. Ethanolic extract showed strong reaction with the phytochemical parameters because ethanol is a well-known organic solvent which dissolves phytochemical metabolites more readily than water. Flavonoids in mushrooms have been found to exercise anti-oxidative activities, protection against allergies, inflammation, platelet aggregation, microbes, ulcers, hepatic toxicity, viruses and tumour [37]. Saponins have great potentials as fertility agents, increase and accelerate the body’s ability to absorb calcium and silicon, thus assisting in digestion. Levels < 10% are considered safe and non-toxic while high saponin levels have been associated with gastroenteritis, manifested by diarrhoea and dysentery [11]. Alkaloids are therapeutically efficient bioactive substances used as basic medicinal agents because of their analgesic, antispasmodic and bactericidal properties [3].

| Mushroom                      | Yoruba (West) | Igbeto (East) | Hausa (North) |
|-------------------------------|---------------|---------------|---------------|
| Auricularia auricular Judae (Bull.) Quel. | +             | +             | +             |
| Calvatia cyathiformis (Bosc.) Morg. | +             | -             | +             |
| Chlorophyllum molybdites (Mayer ex. Fr.) Masssee | *             | +             | +             |
| Coprinus picaceus (Bull. ex Fr.) | -             | +             | -             |
| Coprinus sordidus Berk. and Br. | -             | -             | -             |
| Cortinarius tramenterius Ulje and Bas. | -             | -             | -             |
| Cortinarius mellisiens Fries | +             | +             | *             |
| Daldinia concentrica (Biol. ex Fr.) Ces. and DeNot | *             | +             | *             |
| Lentirinus subabulis Berk | *             | +             | -             |
| Pleurotus ostreatus Jacq. | +             | +             | +             |
| Pleurotus palmonarius (Fr.) Quel | +             | -             | -             |
| Pleurotus squarrosulus (Mont.) Singer | +             | +             | +             |
| Psathyrella aereomagnosa Pegler | +             | +             | +             |
| Schizophyllum commune Fr. | +             | +             | +             |
| Termiformys clypeatus Heim | +             | +             | +             |
| Termiformys globules Heim and Goos | +             | -             | -             |
| Termiformys microcarpus (Berk. and Br.) Heim | -             | +             | -             |
| Volvariella volvacea (Bull.) Singer | +             | +             | +             |
| Volvariella esculenta (Mass) Singer | +             | +             | +             |
| Termiformys robustus (Beelli) Heim | +             | -             | -             |

Significance of values provided as: + = edible, +* = edible and medicinal, * = medicinal, - = uses unknown.

3.2. Proximate analysis

The proximate compositions of P. tuberregium are presented in Table 4. The results showed that they contain high quality carbohydrate and an appreciable amount of protein, crude fibre, crude fat and ash. This result showed that P. tuberregium is significantly rich in carbohydrate which conform with [34] that carbohydrates are the second major nutrient component of mushroom. The carbohydrate (80.24%) content recorded in this study compares favourably with 62.06–80.01% in mushrooms analysed by Ref. [88]. The value is however higher than the range (4.24–6.37%) reported by Ref. [62] in some edible mushroom. Mushrooms are valuable sources of dietary fibre [22] low in fat, no cholesterol and free fatty acids [17]. In view of the low-fat content recorded in this study, the mushroom, P. tuberregium is suitable as a component of weight restricted diet. The crude fibre content obtained in this study was 0.26% which is relatively lower when compared with previous works by Refs. [30,61,1]. Fibre is a good nutritional source in human food that prevents diverticulosis and aids absorption of trace elements in the gut as well as helps in the elimination of undigested food materials through the bowel [24]. Fibre content in food helps in speeding up the passage of faeces from the body, preventing them from sitting in the bowel for too long which could lead to several diseases like colon cancer and coronary heart disease [96]. The ash content was also low and have an appreciable amount of minerals. This finding showed that edible mushrooms P. tuberregium compared favourably with values reported for most legumes. In comparison, previous studies by Refs. [15,30] recorded higher values against the value of ash (1.45)
obtained in this study. Mushrooms are a good source of vitamins like B complex vitamins including riboflavin (B₂), niacin, pantothenic acid, thiamine (B₁), biotin, folate, vitamin B₁₂, vitamin D and the essential minerals, selenium, copper and potassium. The results also showed high moisture content (21.16%) which was lower than the values (60.70% and 88.40%) reported by Refs. [50,62] respectively in some mushrooms collected in Nigeria. It has been reported that most mushrooms obtained from literature had their protein content ranging from 19 to 39 mg/100 g on dry matter basis [61]. Generally, the nutritional value of mushrooms lies in their higher protein value than those of green vegetables [21].

P. tuberregium extract possessed antioxidant properties due to the presence of relatively high concentration of bioactive chemicals like flavonoids, tannins, alkaloids and phenolics. Phenolics and flavonoids have antioxidant activity due to their redox properties, which allows them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators [60]. Alkaloids cause antioxidizing effects by reduction of nitrate generation, which involves protein synthesis [26,52]. Tannins inhibit free radical-mediated lipid peroxidation by blocking the propagation of free radicals and inhibiting 3-hydroxy-3-methyl CoA reductase [10,23,27].

### 3.3. Mineral analysis

Mineral analysis of P. tuberregium is in variable amounts of K, Na, Mg, P and Ca. This study showed that P. tuberregium contained 0.08% Phosphorus, 0.07% of Magnesium, 0.28% of Sodium, 0.20% of Calcium and 0.15% of Potassium. Ca in this study is second highest value with 0.2% which is important in the formation and maintenance of bones and teeth. They are also required in blood clotting and muscle contraction [115]. Mg in this study is low compare to other parameters. Mg is important and needed in over 300 enzymes and contributes to DNA and RNA synthesis during cell proliferation. It is also required for nerve and heart function as well as release of insulin and ultimate insulin action on cells. It decreases blood pressure by dilating arteries and preventing abnormal heart rhythm [115]. The presence of Ca, Mg and K in P. tuberregium is known to reduce hypertension and blood pressure therefore used in the prevention and treatment of high blood pressure [115]. Potassium is needed for glycogen and protein synthesis, and the metabolic breakdown of glucose. Phosphorous is an important component of phospholipids, nucleic acids and many key enzymes which play important role in energy and cell metabolism. Generally, minerals are essential for growth, development, maintenance and repair of the body. These minerals play a key role in the maintenance of osmotic pressure, and thus regulate the exchange of water and solutes within the body. They help in transmission of nerve impulses and muscle contraction and play a vital role in the acid-base equilibrium of the body. Therefore, their presence in P. tuberregium gives a positive weight to the nutritional importance of the plant.
Table 5

| Treatment                          | Liver Weight Gain (%) | Kidney Weight Gain (%) |
|-----------------------------------|-----------------------|------------------------|
| Control                           | 163 ± 9               | 0.164 ± 0.017          |
| Normal diets + Sodium arsenite    | 90 ± 5                | 0.194 ± 0.016          |
| Normal diets + Sodium arsenite +  | 155 ± 6               | 0.222 ± 0.014          |
| Potassium dichromate (K2Cr2O7)    | 150 ± 6               | 0.222 ± 0.014          |
| Normal diets + Mushroom           | 150 ± 6               | 0.222 ± 0.014          |

Data are expressed as mean ± standard error mean, n = 8. *Significant difference at P ≤ 0.05.

3.4. Body weight and behavioural alteration

All the Wistar albino rats of group B (Feed + Sodium arsenite (NaAsO2) + Potassium dichromate (K2Cr2O7)) showed more prominent signs (+ + + +) of toxicity as compared to control. Clinical signs observed in the present study were reduced feeding, low water intake and decreased body weight gain (Tables 3 and 5). The rats were dull and depressed (Table 2). Feed intake and live body weight decreased significantly (p < 0.05) in Sodium arsenite (NaAsO2) and Potassium dichromate (K2Cr2O7) treated rats (Group B). However, therapeutic groups treated with mushroom showed significant (p < 0.05) increase in feed utilization and live body weight. The clinical signs observed in this study could be compared with results obtained in Cr-treated broiler chicks and rats by Refs. [75,100] who reported dull, depressed and significant reduction in body weight. Decrease in feed intake could be due to inhibition of satiety centre by Cr and As resulting in loss of interest in the feed [8].

Increase or decrease in body and organ weight in animals due to exposure to chemicals can be achieved through a number of mechanisms. Organ weight data can provide sensitive indices of toxicologic change. In this study, decrease in body weight could be due to decrease in physiological internal activity which may damage health and subsequently lead to impaired weight gain [72]. Another reason for decreased body weight could be due to irregularities in metabolism mediated by Cr-AS induced liver damage [99]. This study demonstrates significant relationship between decreases in organ weight and heavy toxicity. Therefore, the reasons for organ mass decrease and associated functional implications are worthy of future studies.

3.5. Haematological studies

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. Haematological parameters (RBC, WBC, HB, PCV, ESR) showed significant (p < 0.05) decrease in Cr-As treated group as compared to control (group A). The haematological results Fig. 1 showed significant increase in the PLT counts when compared with control. The high platelet count which is referred to as thrombocytosis may be because of the harmful effects of As and Cr in bone marrow and hematopoietic organs. Decreased RBC count may have been associated with a decreased rate of erythropoiesis which eventually results in lower Hb level because of negative effect of As and Cr on erythropoiesis. The possible reason of inhibited erythropoiesis might be the action of arsenic on RBC membrane. Similar decrease in RBC number in animal model as well as in human population due to toxicant exposure has been reported by Ref. [97]. Another possible reason could be that the Cr can cross the red blood cell membrane easily and accumulate in it, thereby leading to DNA protein crosslinking and thus occurrence of anaemia. This is because Cr has the ability to bind to beta chain of haemoglobin so no haemoglobin available for heme synthesis leading to anaemia [2].

A decrease in nonspecific immunity of the subject exposed to arsenic may be associated with decreased WBC count. The significant decrease of WBC in As-Cr exposed groups might be due to hindered maturation. This result is in accordance with that obtained in previous studies by Refs. [43,97]. Decrease in haematological parameters following Cr administration in broiler [64], rats [13,63] and fish [101]. One of the most important findings of this study is that As and Cr-induced haematological changes but showed improvement in P. therueguem treatment against As and Cr-induced changes and can be used to alleviate the adverse effects of As and Cr toxicity in man.

3.5.1. Ameliorative potentials of P. tubерregium mushroom

Mushrooms have long been used not only as food but also for medicinal purpose in the treatment of various ailments. Herbal
to increased utilization of GSH by the cells which act as scavengers of free radicals or increased utilization of GSH for the activity of GPx forming oxidized GSH (GSSG) due to increased generation of ROS as reported by Ref. [67]. Significant depletion of hepatic GSH has also been reported by Ref. [54] in cadmium treated rats. In the liver, there was decreased utilization of GSH in As and Cr-treated rats which confirm with previous studies by Ref. [12,33,103]. Interestingly, P. tuberreguim extract in therapeutic treated rat markedly increased GSH level in liver and kidney. The protective effect of P. tuberreguim mushroom extract may be due to antioxidant and free radical scavenging activity of polyphenols. SOD, CAT and GPx constitute a mutually supportive team of antioxidant defense against reactive oxygen species. SOD is highly sensitive to contaminations stress. GST catalyzes the conjugation of xenobiotic electrophilic substances with GSH to form the corresponding GSH-S-conjugate. GR utilizes NADPH and maintains the GSH in a reduced form Ref. [77]. GR, GPx and GST play an important role in maintaining the intracellular redox balance. As shown in Tables 6 and 7.

GR, GPx and GST activities were significantly decreased in As and Cr-treated rat kidney. However, treatment with P. tuberreguim mushroom reversed the hepatic and kidney GR, GPx and GST activities to normal levels (P < 0.01) (Tables 6 and 7). Increase in hepatic and kidney ROS production supports this hypothesis. The concentration of TBARS is considered major oxidative stress markers. In this research, levels of ROS and TBARS were remarkably increased in As and Cr-exposure induced oxidative stress. Flavonoids in P. tuberreguim mushroom may act as free radical chain breakers which can inhibit ROS generation. Flavonoids can also prevent metal-catalyzed free radical formation by sequestering transition metals.

### Table 6

| Treatment                                      | MDA (nmol·mg⁻¹) | GSH (nmol·mg⁻¹) | SOD (nmol·mg⁻¹) | CAT (nmol·mg⁻¹) | GPx (nmol·mg⁻¹) | GR (nmol·mg⁻¹) | GST (nmol·mg⁻¹) |
|------------------------------------------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|----------------|
| Control                                        | 28.66 ± 2.949   | 1.77 ± 0.035    | 3.15 ± 0.071    | 52.5 ± 2.970    | 30.55 ± 1.485  | 0.675 ± 0.106  | 7.6 ± 0.424    |
| Normal diets + Sodium arsenite (NaAsO₂) + Potassium dichromate (K₂Cr₂O₇) | 48.37 ± 1.520   | 2.36 ± 0.092    | 3.82 ± 0.042    | 44.15 ± 1.344   | 35.7 ± 4.101   | 0.775 ± 0.035  | 9.05 ± 0.212   |
| Normal diets + Sodium arsenite (NaAsO₂) + Potassium dichromate (K₂Cr₂O₇) + Mushroom | 39.8 ± 0.566    | 1.94 ± 0.226    | 3.0 ± 0.141     | 50.77 ± 0.806   | 41.67 ± 0.042  | 0.625 ± 0.035  | 7.1 ± 0.141    |
| Normal diets + Mushroom                         | 34.13 ± 5.487   | 1.55 ± 0.212    | 2.215 ± 0.049   | 52.05 ± 0.354   | 30.55 ± 1.485  | 0.51 ± 0.042   | 5.17 ± 0.099   |

**Fig. 1.** Haematological parameters of induced Wistar albino rats after 28 days.

**Table 6**

Effects of Ar-Cr Toxicity and P. tuberreguim Extract Therapeutics on Oxidative Stress Indicators in the Liver Tissues.
Intake of *P. tuberregium* mushroom may be helpful in treating heavy metals toxicity. Long-duration epidemiological studies are required to determine the optimal doses of the dietary requirement for human to provide safe and effective dietary strategies against metal toxicity.

The oral administration of *P. tuberregium* species has been shown to significantly reduce As and Cr toxicity. This also conforms to the study of Refs. [6,36,55] on the ameliorative potentials of *Annona muricata* that significantly reduce arsenic mediated neurotoxicity. The extracts from a few other traditional medicinal plants such as *Moringa oleifera*, *Aloe Barbadensis* (Aloe Vera), and *Centella asiatica* have also been found to offer beneficial effects in protecting the vital organs of the body probably by reducing oxidative stress [40,41,42] and depletion of arsenic concentration in tissue [41] through the interactions of phytochemicals with cystine- and methionine-rich proteins [39,42,73]. Also, Ref. [87] reported the protective effects of *P. tuberregium* on carbon-tetrachloride induced testicular injury in Sprague dawley rats.

The measurement of enzyme activities in rat liver is significant in the investigation and diagnosis of liver injury. Levels of AST, ALT, and ALP biomarker enzymes in the liver are widely used as sensitive markers to evaluate toxicological effects in the liver [79]. Increased levels of ALT reflect hypertrophy and other serious conditions of the liver cells [45]. The biomarker AST, apart from being an indicator of liver toxicity, is also associated with heart and muscle disorders [91]. ALP is abundant in the cells lining of the biliary duct of the liver and are used in the diagnosis of biliary obstruction [18]. In the present study, the level of AST, ALT and ALP (Table 8) were significantly in As and Cr treated rats. This indicates abnormalities in liver cells [71]. Accordingly, therapeutically treated rats with mushroom significantly reduced the effects of As and Cr toxicity with gradual decrease in AST, ALT and ALP levels. These results therefore indicate that mushroom extract can ameliorate heavy metal toxicity significantly. Again, significant increase in antioxidant enzyme activities suggests an antioxidant defense response to oxidative stress-induced perturbations of liver and kidney functions to maintain redox balance.

Urea, total protein and creatinine are important biomarkers of kidney dysfunction [80]. Creatinine is considered as one of the most reliable indicators of the efficiency of renal function. Increased blood creatinine is strongly related to renal damage. In the present study, the level of creatinine and total protein showed significant increase in As and Cr treated rats. However, the oral administration of mushroom extract gradually reduces the level of creatinine. The regular consumption of mushroom may help to ameliorate the kidney function by reducing the creatinine values.

4. Conclusion

The results of this study suggest that the consumption of local edible mushroom, *P. tuberregium* could in addition to its high nutritive value, protect humans from the damage caused by heavy metal toxicity. The high fibre and carbohydrate content in addition to moderate protein and fat content indicate that mushroom holds tremendous promise in developing countries like Nigeria. Low dose (150 mg/kg bw) of *P. tuberregium* extract show ameliorative effects in treated groups. Therefore, eating mushrooms in moderation may provide health benefits, and help to reduce heavy toxicity risk. The protective efficacy of *P. tuberregium* extract may be due to the presence of several active components which may provoke the activity of free radical scavenging enzyme systems and renders protection against As-Cr induced effects. The metalloprotective role of *P. tuberregium* may be attributed to the presence of saponins, alkaloids and flavonoids.

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### Table 7

| Treatment | MDA (nmol mg⁻¹) | GSH (nmol mg⁻¹) | SOD (nmol mg⁻¹) | CAT (nmol mg⁻¹) | GPx (nmol mg⁻¹) | GR (nmol mg⁻¹) | GST (nmol mg⁻¹) |
|-----------|-----------------|-----------------|-----------------|-----------------|----------------|---------------|----------------|
| Control   | 35.15 ± 0.495   | 34.2 ± 1.344    | 17.695 ± 1.336  | 37.45 ± 1.061   | 32.795 ± 1.365 | 55.64 ± 1.725 | 2.15 ± 0.240   |
| Normal diets + Sodium arsenite (NaAsO₂) + Potassium dichromate (K₂Cr₂O₇) | 52.8 ± 0.849 | 29.695 ± 0.629 | 15.675 ± 0.601 | 34.325 ± 1.237 | 28.15 ± 0.990 | 40.06 ± 0.368 | 0.875 ± 0.035 |
| Normal diets + Sodium arsenite (NaAsO₂) + Potassium dichromate (K₂Cr₂O₇) + Mushroom | 49.09 ± 0.580 | 33.16 ± 0.028 | 13.515 ± 0.332 | 29.075 ± 0.955 | 31.19 ± 0.035 | 54.07 ± 0.382 | 1.825 ± 0.035 |
| Normal diets + Mushroom | 38.89 ± 0.863 | 31.975 ± 0.049 | 16.675 ± 0.035 | 33.1 ± 0.990 | 32.39 ± 0.792 | 51.125 ± 1.450 | 1.965 ± 0.049 |

### Table 8

| Treatment | ALT | ALP | AST | Total Protein | Creatinine | Urea |
|-----------|-----|-----|-----|---------------|------------|------|
| Control   | 27.65 ± 0.636 | 30.65 ± 0.778 | 30.59 ± 0.721 | 37.75 ± 0.636 | 10.65 ± 0.636 | 0.865 ± 0.120 |
| Normal diets + Sodium arsenite (NaAsO₂) + Potassium dichromate (K₂Cr₂O₇) | 41.44 ± 2.814 | 40 ± 1.273 | 40.02 ± 1.054 | 30.05 ± 0.212 | 15.55 ± 0.354 | 0.4 ± 0.141 |
| Normal diets + Sodium arsenite (NaAsO₂) + Potassium dichromate (K₂Cr₂O₇) + Mushroom | 38.7 ± 1.131 | 38.7 ± 0.566 | 42.39 ± 1.987 | 36.05 ± 0.212 | 12 ± 0.566 | 0.35 ± 0.071 |
| Normal diets + Mushroom | 32.85 ± 0.566 | 34.15 ± 1.485 | 37.86 ± 0.834 | 36.45 ± 0.495 | 12.1 ± 1.131 | 0.28 ± 0.028 |
