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# Efficiency of *Pleurotus florida* dietary supplement in reducing Cadmium toxicity in Albino Rat (*Rattus norvegicus*)

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**ABSTRACT**

*Pleurotus florida* was investigated to reduce cadmium toxicity via haematological and histopathological analysis in Wistar albino rats. A total number of 28 *Rattus norvegicus* were divided into 7 groups (2 male and 2 female) different feeding concentration of cadmium and mushroom. Clinical signs observed during the 16days dosing period include loss of appetite, weight loss, diarrhoea, sluggishness and coughing, for the treated groups (B -G). Haematology results showed that there were significant differences ($p<0.05$) in WBC, PLT, MCH and MCHC but they were no significant differences ($p>0.05$) in RBC, MCV, MPV, LY and Hgb of the treated groups. The histopathological alterations in sleep were time and sex dependent ranging from slightly atrophied follicle to moderate atrophied follicle in both male and female rat, but more severe in female. Abatement of Cadmium toxicity from this experiment using *P. florida* was successful although was dependent on the concentration of the exposed metal.

**Key words**: *Pleurotus florida, Rattus norvegicus,* Cadmium toxicity, Histopathology.

## INTRODUCTION

Cadmium (Cd) is a very toxic heavy metal and an important environmental pollutant which is present in the soil, water, air, food and in cigarette smoke. Cadmium has been released into the environment through human activities and is routinely found as a contaminant in tissues collected from the human population throughout the world (Michael *et al*., 2002; Nawal *et al.,* 2015). Acute administration of Cd often induces lethal toxicity in mice and rats (Jung *et al., 2001*) and like other endocrine disruptors, it is able to modify the basal activity of the testes (Anunciación *et al*., 2001). Cadmium accelerates lipid peroxidation by stimulating the peroxidation chain reaction in the target organs, resulting in the generation of ROS and consequently the induction of cytotoxicity (Oteiza *et al.,* 1999). Cadmium causes anaemia (Kostić *et al.,* 2003; Horiguchi 2011) and induces immunological effects (Blakley, 1985, Hounkpatin *et al.,* 2012). Haematological and immunological effects induced by cadmium and mercury were verified by Guédénon *et al*. (2012). Several studies have demonstrated the effect of cadmium (Cd) on various organ-systems in the body. It has been reported that Cd-induced nephrotoxicity, testicular damage, lung damage, hepatotoxicity, and body weight loss (Ige *et al.*, 2011). The alterations in haematological changes serve as the earliest indicators of toxic effects on the tissue (Paprikar, 2003). Blood is the most important tissue, in which changes in metabolic processes are reflected, therefore, abnormal alteration in blood parameters are the reliable indicator of toxic effects of drugs, chemicals and diseases (Lodia and Kansala, 2012; Hounkpatin *et al.,* 2013). However, the toxicity of many chemicals may be prevented or ameliorated by traditional medicinal plants, as they have been severally reported to have multiple biological activities including free radical scavenging activity (Fakurazi *et al*., 2012). Mansour *et al*. (2014) investigated phytochemical properties of crude extracts of medicinal plants and reported the presence of bioactive antioxidant compounds for myriads of nutritional and medicinal benefits and this has contributed immensely to the ongoing growing recognition and use of medicinal plants as complimentary or alternative remedies (Kumbhare *et al*., 2012). Enagboma *et al.* (2015) reported the importance of tropical sclerotial mushroom (*Pleurotus species*) with several medicinal properties in extracts, such properties include antioxidant, anti-tumour, antigenotoxic, bio antimutagenic, anti-inflammatory, anti-lipidaemic, antihypertensive, and antihyperglycemic, antibacterial and antifungal activities (Fillipie and Umek, 2002; Hu *et al.,* 2006; Ngai and Ng, 2006). This study

**Table 1.** Experimental groups and varying dose.

| Group | Concentration |
|---|---|
| Group A ( Control) | Grower mash and Water |
| Group B | Treated with 7% Cadmium sulfate and mushroom extract |
| Group C | Treated with 3.5% Cadmium sulfate and mushroom extract |
| Group D | Treated with 1.75% Cadmium sulfate and mushroom extract |
| Group E | Treated with 7% Cadmium sulfate |
| Group F | Treated with 3.5% Cadmium sulfate |
| Group G | Treated with 1.75% Cadmium sulfate |

was aimed at investigating the ameliorative effect of different concentration of mushroom (*Pleurotus florida*) on haematological and histopathological changes induced by cadmium toxicity in blood and spleen of Wistar albino rats.

## MATERIALS AND METHODS

Fourteen male and female Wistar albino rats (twenty-eight rats) weighing 150-200g were used for the study. They were randomly distributed into one control group (A) and six treated groups, (B, C, D, E, F and G) containing four animals (2 male and 2 female) per group and were on the standard normal diet provided with water ad libitum. The rats were kept in properly numbered large wooden cages with stainless steel top grill having facilities for the meal. The animals were maintained in 12 hours light and dark cycle at 28ºC ± 2ºC in a well-ventilated animal house under natural conditions in large wooden cages and they were acclimatised to laboratory conditions for 14 days prior to the commencement of the experiment. The six treated groups were divided into two sets (3 groups each). The two sets of treated groups received orally varying doses, group B, C, and D were designed to receive Cadmium Sulfate and mushroom extract at varying doses (7%, 3.5% and 1.75% concentrations) at a rate of 0.5ml /rat/day of Cadmium Sulfate and at a rate of 1.0ml / rat/day of mushroom extract and the second set of treated groups (E, F and G) received orally varying doses (7%, 3.5% and 1.75% ) of Cadmium Sulfate only at a rate of 0.5ml /rat/day for 16days (Table 1). Control group animals were fed with growers mash pelleted food and water only. They were continuously observed for 42hours to detect any changes in autonomic or behavioural responses; Spontaneous activity, irritability, corneal reflex, urination and salivation. The manufacturer's specifications for the food were 20.0% protein, 4.0% fat, 7.0% fiber, 5.0% ash, and 1.0% sodium (K.A. Oshomagbe farm services, Nigeria). The animal care and handling in this research was approved by the University-Institutional Animal Care and Use Committee (UNIBEN-IACUC). This research followed the animal care and use and this includes the use, care and transportation of rat for toxic pathological testing.

### Mushroom Extracts Preparation

Four hundred and forty gram of fresh mushroom (*Pleurotus florida*) was weighed into a moisture box, washed and grinded using a electric grinding machine (model MX 491N, National) mixed with 1000ml of distilled water into a thick liquid paste. The blended mushroom (supernatant) was left to soak for 72hours and stored in an airtight plastic container at 5ºC in a refrigerator, then filtered with Whatman No. 1 filter paper to obtain the filtrate (mushroom extract),then the filtrate was stored in an airtight plastic container at $5^{o}$C in a refrigerator until required for use, the residue was disposed off. The mushroom solution containing 10.0 mg of extract per ml (Barros *et al.*, 2007; Vamanu, 2012).The mushroom extract (filtrate) was administered orally to experimental rats using oralgastric tube (gavage).

## HISTOMETRICAL ASSAY

### Relative organ weight

Every four days, one animal each were taken from each group and were anaesthetized under light chloroform anaesthesia. Organ**,** spleen were carefully dissected out and weighed in grams. The relative organ weight of each animal was then calculated as follows,

## COLLECTION OF HAEMATOLOGICAL AND HISTOPATHOLOGICAL ASSAY

Every four days, one rat each were randomly selected from each treatment and control groups and 1ml of blood was collected using 2ml syringe, the blood samples were collected from inferior vena cava under mild chloroform anesthesia for the estimation of hematological parameters like haemoglobin concentration, RBC, WBC,

**Table 2**. Haematological indices of wistar albino rat (*Rattus norvegicus*) given varying doses

| Haematological parameters | Group A | Group | Group C | Group D | Group E | Group F | Group G |
|---|---|---|---|---|---|---|---|
| WBC ($10^3$/µl) | 16.55±2.75[a] | 12.75± 0.85[c] | 8.61 ± 3.19[c] | 9.25± 3.15[c] | 11.10 ± 3.20[c] | 6.65 ± 1.55[d] | 10.60 ± 1.00[c] |
| RBC ($10^6$/µl) | 7.56± 1.94 | 9.55± 0.83 | 7.86 ± 0.36 | 8.66 ± 1.07 | 8.73 ± 1.085 | 9.065 ± 0.825 | 9.375± 0.585 |
| PLT ($10^3$/µl) | 472± 120.00[a] | 599.50±32.50[a] | 644.50 ± 85.50[a] | 1098.0±3825[b] | 547.50±83.50[a] | 822.50 ± 106.500[b] | 598.00 ± 3.00[a] |
| MCV (fl) | 54.50±5.10 | 56.95± 32.50 | 58.30 ± 0.90 | 57.80± 0.00[a] | 57.75± 4.95 | 53.50± 7.80 | 56.75± 3.15 |
| MPV (fl) | 5.60± 0.70 | 5.45±0.55 | 6.55 ± 0.25 | 5.550± 0.950 | 6.050± 0.550 | 5.850± 0.550 | 5.750± 0.650 |
| LY ($10^3$/µl) | 6.90 ± 0.70 | 5.55 ± 1.45 | 7.75 ± 2.15 | 5.65± 1.15 | 6.70± 4.10 | 3.90± 0.80 | 7.35± 3.55 |
| MCH (pg) | 17.25± 0.95[a] | 16.15± 0.15[a] | 18.60 ± 1.60[b] | 19.05± 0.75[b] | 18.60± 0.50[b] | 17.65±0.75[a] | 19.00±0.30[b] |
| Hgb (g/dl) | 13.25±4.05 | 15.54± 1.15 | 15.35± 1.55 | 16.50± 1.40 | 16.350± 2.450 | 16.100±2.100 | 17.850±0.850 |
| MCHC (g/dl) | 31.75±1.25[a] | 28.40± 0.10[b] | 32.45± 2.75[a] | 33.00± 1.30[a] | 32.350± 1.950 | 33.500±3.600 | 33.650±2.350[a] |

Figures on the same row having the same superscript are not significantly different (p>0.05)

MCV, MCH, MCHC, MPV, LY and PLT (Kjeldsberg, 1998; Moura, 1982; Smith, 1995). Blood samples were collected in 10% EDTA/ saline of pH 7.2. The spleens were excised immediately and thoroughly washed in ice-cold saline and weights were recorded. The spleen samples were collected and placed in 10% formalin for preservation in universal bottles for tissue concentration, the histological sections were placed onto glass slides; processed histology slides are stained using hematoxylin and Eosin (H&E) for histopathological assay.

## RESULTS

Clinical signs observed during the 16days dosing period include loss of appetite, weight loss, diarrhoea, sluggishness, coughing, for the treated groups (B -G) while weight again and agility characterised the control (group A). In Table 2, histometrical analysis (% relative organ weight) revealed significant differences (p<0.05) between the treated groups (Group B, C, D, and G) and the control (Group A). Haematological indices of male and female Wistar Albino Rat (*Rattus norvegicus*) exposed to $CdSO_4$ and fresh aqueous mushroom extract (*Pleurotus florida*) as an abatement showed no significant differences (P>0.05) in RBC,MCV,MPV,LY and Hgb in the treated groups (B-G) when compare to the control group A (Negative control). However, there were significant differences (P<0.05) in WBC, PLT, MCH, and MCHC in the treated groups (B-G) when compare to the control group A. Normal histological patterns were observed in the sleep of (male and female) albino rat in the controls group A. There were slight to moderate differences in histopathological alterations observed for male and female sleep. Group B showed slight abatement after 4days, 8days and 12days, but there was moderate follicular activation in the male rat when compare to female rat with mild follicular activation which became pronounced after 16days. Group C exhibited moderate follicular activation in male rat, compare to female rat with mild follicular activation after 16days. Group D exhibited mild follicular activation in male rat, compare to female rat with mild follicular activation (but moderately abated) after 16days. Group E showed slight atrophied follicle after 4days, 8days, and 12days, but there was moderate atrophied follicle in the male rat when compared to female rat with severely atrophied follicle which became pronounced after 16days. Group F showed slightly atrophied follicle after 4days, 8days, and 12days, but there was moderate atrophied follicle in the male rat when compared to female rat with severed atrophied follicle which became pronounced after 16days. Group G showed slightly atrophied follicle after 4days, 8days, 12days, but there was moderate atrophied follicle in the male rat when compared to female rat with severely atrophied follicle which became pronounced after 16days.The atrophied follicle was much in Group E but less when compare to group G and group F respectively after 16days that is more histopathological changes (tissue damage) was observed as the graded dose increased from low dose to high dose. In the abatement groups (B-D), it was observed that group D) was moderately abated when compare to group F and group E respectively at varying doses of low dose to high dose after 16days.

**Table 3.** Histometrical indices of the sex comparison of Wistar Albino Rat (*Rattus norvegicus*) feed with varying doses of ($CdSO_4$ + extract) and ($CdSO_4$ Only) for 16days.

| Sex | Group A | Group B | Group C | Group D | Group E | Group F | Group G |
|---|---|---|---|---|---|---|---|
| Male | 0.5525± 0.1036 | 0.4290± 0.06 | 0.3145± 0.0365 | 0.504± 0.128 | 0.416± 0.128 | 0.299± 0.016 | 0.3965± 0.0215 |
| Female | 0.5540± 0.0240[a] | 0.2395± 0.0895[b] | 0.285± 0.023[b] | 0.4570± 0.00[c] | 0.439 ±0.064[c] | 0.3965± 0.0075[d] | 0.4305± 0.0265[c] |

Figures on the same row having the same superscript are not significantly different (p>0.05)

## DISCUSSION

Exposure to cadmium is well known to induce a variety of toxicity symptoms in both experimental animals and exposed populations (Asagba and Eriyamremu, 2007). The results of this study found that some haematological parameters vary widely depending on the batches of Wistar rats intoxicated with different concentration of cadmium. Results obtained in this study (Table 1) indicate that exposure of rats to cadmium was characterised by general fluctuations in blood cellular components. This reduction in blood cellular components could be as a result of toxicity and stress induced by cadmium on haematopoiesis in the rats (Oyewole *et al.,* 2016). Cadmium causes anaemia (Kostić et al., 2003; Horiguchi 2011) and induces immunological effects (Blakley 1985, Hounkpatin *et al.,* 2012). If the effect of environmental pollution on rat populations is to increase disease susceptibility, the mechanism may be acting via repression of the immune system (Bennett and Wolke, 1987). The alterations in the blood of exposed rat in this experiment agrees with the suggestion that Cadmium has been recognised as a biological toxicant (Hounkpatin *et al*., 2013). It is associated with several clinical complications like bone abnormalities, haematological alterations, renal and hepatic dysfunctions (Jarup *et al.,* 2000). This study on rats does not agree with other researches that shows significant decreases in the red blood cell (RBC) count and haemoglobin (Hb) concentrations, packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), leading to anemia, on cadmium exposure (Hounkpatin *et al*., 2013, Vinodini *et al.,* 2015) but rather revealed that cadmium exposure increased the red blood cell (RBC) count, haemoglobin (Hb) concentration significantly along with an increases in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), increase in platelets. The alteration in cellular components by cadmium could also be attributed to diminished production, redistribution from peripheral blood into the tissues or rapid destruction of blood cells (Hossain *et al*., 2002; Oyewole *et al.,* 2016).

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