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RESEARCH ARTICLE

THE BIOREMEDIATION POTENTIAL OF DEAD BIOMASS OF *ASPERGILLUS FOETIDUS* MTCC 8876 AGAINST LEAD TOXICITY IN MALE SWISS ALBINO MICE

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ABSTRACT

Lead is the most ubiquitous, unessential and detrimental heavy metal even at very trivial level. Exposure of lead occurs through the soft tissue of different organ systems like gastrointestinal, excretory, nervous and reproductive tissue of animals. In this study male Swiss albino mice weighing 15–30 g (age 2-2.5 months) were randomly divided into different groups. In order to assess the cellular oxidative stress due to Pb (II) toxicity, LPO level, reduced glutathione content, and total protein level were measured in different tissues like brain, liver, testis and kidney of these animals. Assay of some antioxidant enzymes like catalase and glutathione s-transferase were carried out. These biochemical observations were supplemented with some histological examination of liver, brain, testis and kidney. Recovery from lead burden of intoxicated mice was also analyzed. In this study an attempt was made to evaluate the bioremediation efficacy of the dead biomass of the fungal strain, *Aspergillusfoetidus*MTCC8876 for the removal of lead from the different tissues of a lead treated male Swiss albino mice.

INTRODUCTION

Heavy metal toxicity is the major environmental concern of this era throughout the world. Due to different deleterious anthropogenic activity the earth has become the huge basin of toxic metals. Among other heavy metals lead is considered as a most non-essential heavy metals which is introduced to environment through different sources such as battery manufacturing plant, printing, pigments, fuels, photographic materials, fertilizers, pesticides, lead based paints and additives in pigments in paint industry and gasoline (Parvathi *et al*., 2007).

Lead is considered as a genotoxic substance which exerts irreversible conformational changes in nucleic acid and proteins, followed by DNA damage and apoptosis (Fracasso *et al*., 2002), produces reactive oxygen species (ROS) by auto-oxidation and Fenton reaction and interferes with oxidative phosphorylation and disturbs the osmotic balance of all biota (Bruins *et al*. 2000). Lead has many deleterious effects in animals, including neurological, behavioral, immunological, renal, hepatic and especially hematological dysfunctions (Bellinger, 2008; Rosenberg *et al*., 2007; De Marco *et al*., 2005). According to International Occupational Safety and Health Information Centre (1999) lead targets the prime organs of the body such as bones, brain, blood, kidneys, and thyroid gland and exerts deleterious effects onthe biochemical, histopathological, neuropsychological, fetotoxic, teratogenic, and reproductive parameters .

For this reason, release of Pb in the environment has become a major concern and requires a proper removal guideline. There are several physicochemical technologies have already been reported for the removal of lead, such as chemical precipitation, neutralization, activated carbon adsorption, ion exchange resins, reverse osmosis, solvent extraction and electrochemical technologies etc. (Kadirvelu *et al*., 2002). But these processes are not eco-freindly and cost effective and release secondary toxic substances. Most importantly all these procedures are only applicable for non-living matter. To detoxify lead from living organism is a more serious issue and all of these previously mentioned procedures are found to be impractical proposition. Treatment of lead intoxication in living organism has primarily relied on chelation therapy. Disodium ethylenediamine tetra acetic acid (Na2EDTA) (Flora *et al* 1994) and 2, 3-dimercaptosuccinic acid (DMSA), are the most common oral chelators that showed positive effects against metal poisoning but these chelators in turn are potentially hazardous for living organisms and often fail to remove the whole Pb burden from all body tissues.

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In order to address this problem, ecologically convenient therapies to promote chelation, detoxification and protection are gaining recognition because they have minimal side effects. In recent years the process of bioremediation has been found to be very promising. It has been reported that metals present even at very low concentrations could be removed by bioremediation techniques.

In this bioremediation procedure the metal binding efficiency of biological agents with high proficiency to remove heavy metals from contaminated sites has been utilized. It was reported that microbial biomass act as a metal sink by absorbing the metal through cell wall, pigments, extracellular polysaccharides, cysteine rich amino acid groups through intracellular accumulation or precipitation of metal ion in or around the cell in a rather non-toxic form (Vijayaraghavan& Yun, 2008). In these context filamentous fungi has been reported to be a better option for this purpose. Use of dead biomass could be a better option instead of the living cell. Because in case of dead biomass there is no requirement of growth media or energyrequirement for active intracellular metal transport or heavy metal toxicity on microbial growth or toxicity factors of the fungi itself (Baik *et al* 2002). It has also been observed that not only the biosorbedmetals can be easily adsorbed and recover, the regenerated biomass can be reused. So dead-biomass in bioremedial application offers certain advantages over live biomasses. There are several reports which have investigated the bioremedial potency of dead biomass of fungi (Tobin *et al*, 1990; Vasudeban 2003).

In the light of bioremediation efficacy of *Aspergillusfoetidus* MTCC 8876, this study was carried out to investigate the possible protective bioremedial mechanism of this strain on biochemical, hematological, histochemical and various oxidative stress and toxicity related biochemical parameters in liver kidney testis and brain of lead intoxicated male Swiss albino mice.

MATERIALS AND METHOD

Preparation of dead biomass of Aspergillusfoetidus MTCC 8876

The Pb resistant strain of *Aspergillusfoetidus* MTCC 8876 was grown at 32^0C in an orbital shaker at 175 rpm by the shake flask method in aerobic condition. Liquid CD broth was used for growth of inoculated fungal spores and pH of the medium was adjusted to 8.0 before autoclaving. Biomass was harvested after 96 h of growth period, filtered and washed with de-ionized water. To obtain the dead biomass the test strain was heat killed through autoclaving. The heat killed biomass was dried properly and refrigerated until use. This dead biomass of *Aspergillusfoetidu s*MTCC 8876 was administered to the animals with their normal food consumption at a dose of 150 & 250 mg kg^{-1} body weight per day for 30 days. Mention about control animals…

Chemicals

All the chemicals used in this study were of analytical reagent grade and purchased from Sisco Research Laboratories, MARK(India).The Kits used for histochemical analysis were purchased from Span diagnostic Limited (India)

Experimental animals

Male Swiss albino mice (*Musmusculus*L.) weighing approximately 25–30 g (Aged 2-2.5 months) were used. The mice were housed in polypropylene cages in an air-conditioned room with temperature maintained at 25^0 C±3.0 ^{0} C, relative humidity of 50% ± 5% and 12h alternating light and dark cycles. The mice were provided with a nutritionally adequate diet (Hindustan lever Limited, India) and drinking water *ad libitum*. The Animal Ethical Committee of Department of Zoology, University of Kalyani approved the study.

Experimental design

In the present study, 78 adult male Swiss albino mice (Musmusculus L.) weighing 25–30 g (aged 2-2.5 months old) were used for histochemical, biochemical, histological and metal analysis studies.

For biochemical and histochemical analysis, 30 mice were randomly divided into 6 groups of 5 mice each.

For metal analysis, 30 mice were divided into 6 groups (n=5)
For histological analysis rest of 18 mice were divided into 6 groups (n =3)

- ***Group-1:*** received 1ml distilled water; served as control. (n=5)
- ***Group-2:*** received lead nitrate (20mg kg^{-1} body weight per day) dissolved in distilled water
- ***Group-3 and 4:*** received dead biomass of *Aspergillusfoetidus* MTCC 8876 at a dose of 150 & 250 mg kg^{-1} body weight per day, with normal dietrespectively.
- ***Group-5 and 6:*** received lead nitrate at a dose of 20mg kg^{-1} body weight per day along with a dose of dead biomass of *Aspergillusfoetidus* MTCC 8876 at a dose of 150 &250 mg kg^{-1} body weight per day with normal diet, respectively.

The dose for lead was decided and selected as described by Mishra *et al*. (2000). The concentration of lead nitrate used in the experiment was 1/ 45 of LD_{50} (Plastunov and Zub, 2008). The fungal doses were decided on the basis of standardization experiments conducted in our own laboratory.

Processing of the tissues for experiment

After 30 days of treatment the mice were fasted overnight and then sacrificed under light ether anesthesia. The soft tissues, like brain, kidney, liver and testis were dissected out, washed immediately with ice-cold normal saline to remove blood, and then wet weight was noted and then stored at -80°C for various biochemicals, histochemical, histological studies.

Biochemical analysis

Organs such as liver, kidney, testis and brain were cut into pieces and homogenized with a homogenizer in ice-cold 0.1 M sodium phosphate buffer (pH-7.4) at 4^0C to give 10% homogenate (w/v). The homogenate was centrifuged at 10,000 rpm for 15-20 min at 4^0C twice to get cell free enzyme extraction. The resulting supernatant was separated and used for various biochemical and histochemical estimations.

The content of malondialdehyde (MDA), a final product of lipid peroxidation, was determined using the modified method as described by Dhindsa *et al*. (1981).

Catalase activity was measured using the method of Chance and Maehly (1955) with few modifications.

The activity of Glutathione S-transferase was estimated according to the method of Habig *et al*. (1974) with slight modifications.

Reduced glutathione content was assayed according to the modified method of Ellman (1959).

The method of Lowry *et al*. (1951) was used to measure protein content in the above experiments

Histochemical analysis

For the determination of histochemical changes in the organs, activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) were determined using the diagnostic or pathological kit of Span Diagnostic limited India.

Histological examination

Histological staining of test organs was done according to the method of Mc Manus Mowry (1965). Tissues were removed, washed in saline to make it blood free, and fixed in Buin's fluid at room temperature for 72 h. After fixing the tissue, it was thoroughly washed under running water and dehydrated in ascending grades of ethyl alcohol, cleared, and then embedded in soft paraffin. Tissue sections of about 6µm were obtained through microtome technique. Now the tissue sections were stained by hematoxylin and eosin, and examined under light microscope.

Lead quantity estimation through flame AAS

For lead quantity analysis, accurately measured wet tissue samples were digested in concentrated HNO3 and HCl mixture (1:3) using Microwave Digestion System. Lead was analyzed using PerkinElmer AAnalyst 200 atomic absorption spectrometer equipped with mercury/hydride lamp and a quartz tube atomizer. Air/acetylene (ultrahigh purity 99.995%) was used to sheath the atomizer and to purge internally and a wave length of 293.3 nm for lead analysis was used against suitable standards processed identically.

Serological Analysis

For serological analysis whole blood was collected from test animals in an eppendorf tube. After collection of the whole blood, the blood was allowed to clot by leaving it undisturbed at room temperature for 15- 30 minutes. After that the clot was removed by centrifugation at 1,000-2,000 Xg for 10 minutes in a refrigerated centrifuge. The resulting supernatant was designated serum. Now the supernatant was immediately transferred into a sterilized eppendorf tube. The samples were maintained at 2-8°C while handling. This resultant supernatant was used for the analysis of Iron –TIBC and calcium activity using the diagnostic or pathological kit of Span Diagnostic limited, India.

Statistical Analysis

All determinations were carried out in triplicates in each case. Statistical analysis was done by one-way ANOVA followed by post-hoc multiple comparisons by Duncan's method. The difference was considered as significant when $p<0.05$.

RESULTS

Changes in Biochemical parameters in test organs
Liver

Figure 1. illustrates the effect of lead nitrate alone, and effect of treatment with dead biomass of *A.foetidus* MTCC 8876 individually during lead nitrate exposure on lipid peroxidation, activity of antioxidant enzymes (GST and CAT), and level of non-antioxidant biomolecules (GSH) and protein content in the hepatic tissues of control and Pb-treated animals.

Protein content in hepatic tissue was significantly decreased in lead treated mice compared withcontrol group. The decrease was almost 1.81-fold lower than that of control. In Group III and Group IV there were no changes in protein content observed compared with control. In case of Group IV and V, the protein content showed significant changes. In group V the protein content was increased almost 1.25 fold and in group VI the protein content was found to be increased almost about 1.5 fold greater than that of lead treated group

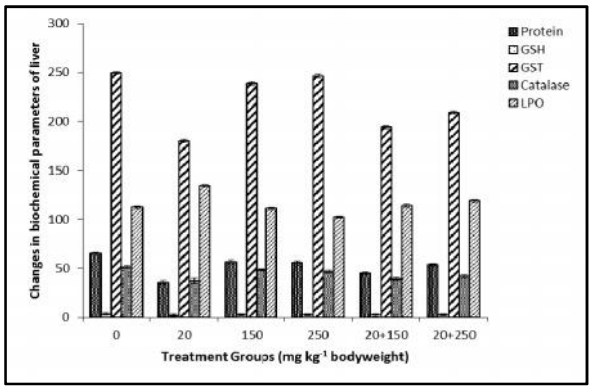

Figure 1 Prophylactic effects of dead biomass of A. foetidus on some oxidative stress parameters in hepatic tissue of lead nitrate exposed mice.Protein activity (mg g-1 wet fresh tissue); GSH activity (mg GSH g-1 tissue); GST activity (nmole CDNB formed min-1 mg-1 protein); LPO (nmole MDA g-1 fresh wet tissue); CAT (µmoles of H2O2 degraded minute-1 mg-1 protein). Data were found to be significant at $P < 0.05$

In group II the reduced glutathione content was significantly decreased compared with the group I. GSH content was decreased almost about 1.62 fold than that of the group I. As expected the GSH content in group III and IV were more or less as same as group I. But in group V and VI the GSH contents were increased about 1.13 fold, and 1.25 fold greater than that of group II respectively.

The activity of GST was significantly decreased in Group II in comparison with group I. The decrease was 1.38 fold lower than that of control group. Like previously mentioned other biochemical parameters Group III and IV exhibited similar GST activity as observed in group I. In group V and VI the GST activity was increased about 1.07 and 1.16 fold than that of group II.

The most common toxicity biomarker, catalase exhibited significant decrease in activity in group II with respect to group I, and it was more than 1.36 fold higher than that of group I. In group III and IV the catalase activity was found to be same as group I. But in Group V and VI the catalase activity was increased almost about 1.04 fold and 1.11fold higher than that of group II respectively.

Due to metal stress the LPO content was significantly increased in group II and this increase was more than 1.19 fold higher than that of group I. In groups III and IV the LPO content was more or less as similar as Group I. In group V and VI the LPO content was decreased and the decrease was almost about 1.18 fold and 1.12 fold lower than that of group II, respectively.

Kidney

Figure2. exhibited the effect of lead nitrate with and without dead biomass of *A. foetidus* MTCC 8876 on lipid peroxidation, activity of antioxidant enzymes (GST and CAT), and level of non-antioxidant enzyme (GSH) and protein content in the renal tissues of animals.

It was found that protein content in renal tissue was significantly decreased in group II mice in comparison to control group.

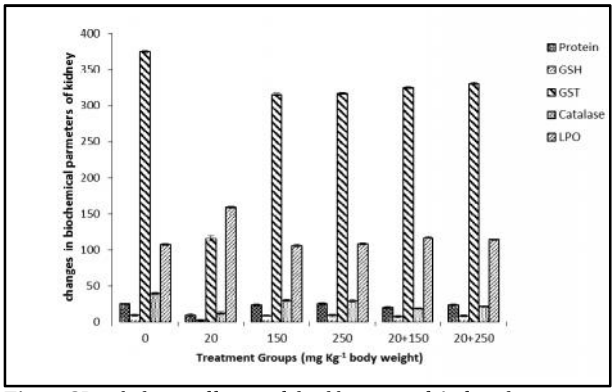

Figure 2 Prophylactic efficacy of dead biomass of A. foetidus on some oxidative stress parameters in renal tissue of lead nitrate exposed mice.Protein activity (mg g-1 wet fresh tissue); GSH activity (mg GSH g-1 tissue); GST activity (nmole CDNB formed min-1 mg-1 protein); LPO (nmole MDA g-1 fresh wet tissue); CAT (µmoles of H2O2 degraded minute-1 mg-1 protein). Data were found to be significant at $P < 0.05$

The decrease was almost 2.64 fold lower than that of control. In Group III and Group IV, there was almost same protein content as in control. In case of Group V and VI the protein content showed significant changes. In group V the protein content was increased almost 2.16 fold and in group VI the protein content was found to be increased almost about 2.46 fold higher than that of lead treated group and in both group the protein content was similar as in control group after the treatment with dead biomass of *A. foetidus.*

The reduced glutathione content was found to be drastically decreased in group II compared with group I. It was observed that the GSH content was decreased almost about 3.47 fold lower than that of group I. As expected the GSH content in group III and IV were more or less same as in group I. But in group V and VI the GSH contents were increased almost about 2.90 fold and 3.15 fold higher than that of group II, respectively.

In group II the activity of GST was found to be significantly decreased in comparison to group I. The decrease was 3.24 fold lower than that of control group. Like previously mentioned other biochemical parameters Group III and IV exhibited more or less similar GST activity as same as group I. The GST activity was found to be increased in group V and VI which was more than about 2.81 and 2.85 fold higher than that of group II.

Catalase enzyme showed a significant decreased activity in group II compared with group I and it was more than 3.29 fold higher than that of group I. In group III and IV the catalase activity was same as in group I. But in Group V and VI the catalase activity was increased almost about 1.55 fold and 1.79 fold higher than that of group II, respectively.

The extent of lipid peroxidation was found to be significantly increased in group II and this increase was more than 1.48 fold higher than that of group I. In group III and IV the LPO content was similar as in Group I. In group V and VI the LPO content was decreased almost about 1.36 fold and 1.39 fold respectively, than that of group II.

Testis

Figure3.exhibited the toxic effect of lead nitrate and effect of treatment with dead biomass of *A. foetidus* MTCC 8876 individually during lead nitrate exposure on lipid peroxidation, GST, CAT, GSH activity and protein content in the testicular tissues of control and experimental groups of animals

In testicular tissue it was found that protein content was significantly decreased in lead treated mice in comparison to control group. The decrease was almost 2.80 fold lower than that of control. In Group III and Group IV there was almost same protein content was observed as control. In case of Group IV and V the protein content showed significant changes. In group V the protein content was increased almost 1.60 fold and in group VI the protein content was found to be increased almost about 1.79 fold higher than that of lead treated group.

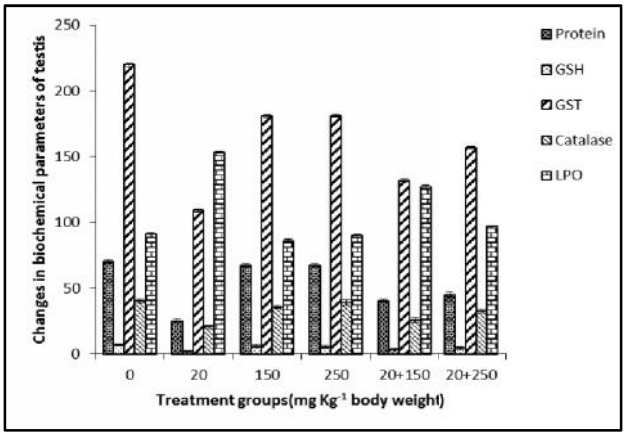

Figure 3 Prophylactic effects of dead biomass of A. foetidus on some oxidative stress parameters in testicular tissue of lead nitrate exposed mice.Protein activity (mg g-1 wet fresh tissue); GSH activity (mg GSH g-1 tissue); GST activity (nmole CDNB formed min-1 mg-1 protein); LPO (nmole MDA g-1 fresh wet tissue); CAT (µmoles of H2O2 degraded minute-1 mg-1 protein). Data were found to be significant at $P < 0.05$

In group II the reduced glutathione content was found to be significantly decreased with respect to group I. It was observed that the GSH content was decreased almost about 3.58 fold lower than that of group I. As expected the GSH activity in group III and IV were more or less as same as group I. But in group V and VI the GSH content were found to be increased almost about 1.64fold and 2.27 fold higher than that of group II respectively.

The activity of GST was found to be significantly decreased in Group II in comparison with group I. The decrease was 2.02 fold lower than that of control group (group I). Like previously mentioned other biochemical parameters Group III and IV exhibited more or less similar GST activity as same as group I. In group V and VI the GST activity was found to be increased about 1.20 and 1.44 fold higher than that of lead treated group (group II).

Catalase enzyme exhibited significant decrease in activity ingroup II with respect to group I and it was more than 1.95 fold higher than that of group I. In group III and IV the catalase activity was found to be as same as group I. But in Group V and VI the catalase activity was found to be increased almost about 1.24 fold and 1.59 fold higher than that of group II respectively.

The extent LPO was found to be significantly increased in group II and this increase was more than 1.69 fold higher than that of group I. In group III and IV the LPO content was found to be more or less as normal as Group I. In group V and VI the LPO content was found to be decreased and the decrease was almost about 1.21 fold and 1.58 fold lower than that of group II respectively.

Brain

The effect of lead nitrate and dead biomass of *A. foetidus* MTCC 8876 either alone or in combination on neurological biochemical variables has been exhibited in Figure 4.

In neurological tissue it was found that protein content was significantly decreased in group II in comparison to group I. The decrease was almost 3.76 fold lower than that of control. In Group III and Group IV there was almost same protein content was observed as control. In group V the protein content was increased almost 2.44 fold and in group VI the protein content was found to be increased almost about 2.19 fold higher than that of lead treated group.

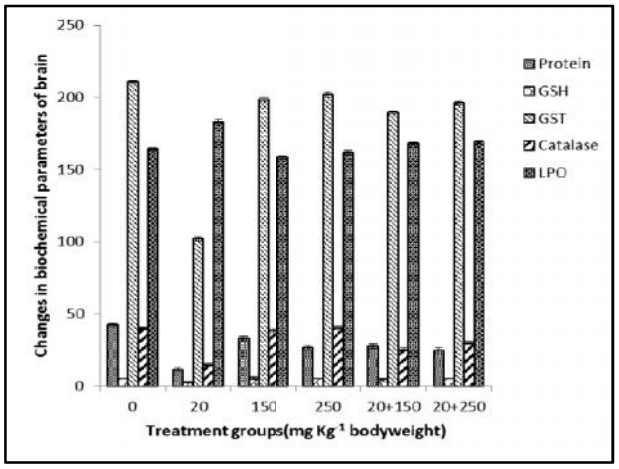

Figure 4 Prophylactic effects of dead biomass of A. foetidus on some oxidative stress parameters in nervous tissue of lead nitrate exposed mice.Protein activity (mg g-1 wet fresh tissue); GSH activity (mg GSH g-1 tissue); GST activity (nmole CDNB formed min-1 mg-1 protein); LPO (nmole MDA g-1 fresh wet tissue); CAT (µmoles of H2O2 degraded minute-1 mg-1 protein). Data were found to be significant at $P < 0.05$

In group II the reduced glutathione content was found to be significantly decreased with respect to group I. It was observed that the GSH content was decreased almost about 1.93 fold lower than that of group I. As expected the GSH content in group III and IV were more or less as same as group I. But in group V and VI the GSH content were found to be increased almost about 1.66 fold and 1.84 fold higher than that of group II respectively.

The activity of GST was found to be significantly decreased in Group II in comparison with group I. The decrease was 2.06 fold lower than that of control group. Like previously mentioned other biochemical parameters Group III and IV exhibited more or less similar GST activity as same as group I. In group V and VI the GST activity was found to be increased about 1.85 and 1.91 fold higher than that of lead treated group (group II).

Catalase exhibited significant decrease in activity in group II with respect to group I and it was more than 2.70 fold lower than that of group I. In group III and IV the catalase activity was found to be as same as group I. But in Group V and VI the catalase activity was found to be increased almost about 1.57 fold and 2.02 fold higher than that of group II respectively.

The extent LPO was found to be significantly increased in group II and this increase was more than 1.11 fold higher than that of group I. In group III and IV the LPO content was found to be more or less as normal as Group I. In group V and VI the LPO content was found to be decreased and the decrease was almost about 1.09fold and 1.08 fold lower than that of group II respectively.

Histochemical assay

Liver

The histochemical study of liver (Fig 5) revealed that the lead treatment altered some of the important histochemical parameters in a significant level. It was found that a significant elevation in the concentration of AST, ALT, ACP, and ALP took place.

It was found that AST and ALT activity in hepatic tissue was significantly increased in group II mice in comparison to control group. The increase was almost 1.60 and 1.92 fold higher than that of control group respectively. In Group III and Group IV, there was almost same AST and ALT content was observed as control. In case of Group V and VI the AST and ALT content showed significant changes. In group V the AST and ALT content was decreased almost 1.48 and 1.47 fold lower and in group VI the AST and ALT content was found to be decreased almost about 1.53 and 1.29 fold lower than that of lead treated group respectively. However in both groups the AST and ALT activity was found to be more or less normal as control group after the treatment with dead biomass of *A. foetidus.*

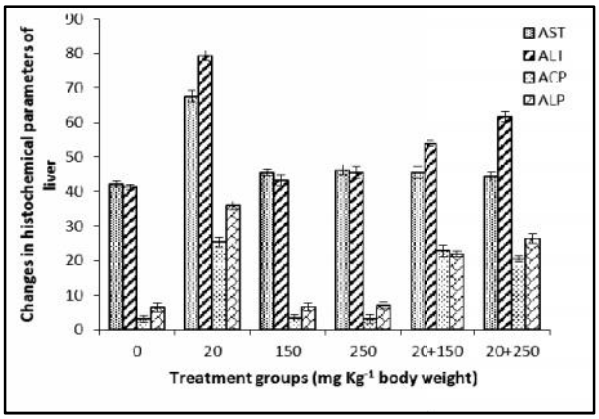

Figure 5 Effects of dead biomass of A. foetidus on some histochemical parameters of the hepatic tissue of lead nitrate exposed mice.AST: Aspartate transaminase (IU L-1); ALT: Alanine transaminase (IU L-1); ACP: Acid phosphatase (µM of PNP formed min -1 g-1 tissue); ALP: Alkaline phosphatase (µM of PNP formed min -1 g-1 tissue. Data were found to be significant at $P < 0.05$

Very same results have been observed in the case of ALP and ACP. It was observed that the ACP and ALP content increased in a significant manner in lead treated mice. In the hepatic tissue of group II mice ACP and ALP content was found to be increased almost about 8.21 and 5.69 fold higher than that of group I mice. In Group III and Group IV there was almost same ACP and ALP content was observed as control. In group V the ACP and ALP content was decreased almost 1.11 and 1.64 fold lower and in group VI the ACP and ALP content was found to be decreased almost about 1.23 and 1.36 fold lower than that of lead treated group respectively.

Kidney

From this study it was found that (Fig. 6) the trends of the present results were almost same as hepatic tissue. AST and ALT content in renal tissue was significantly increased which was almost 4.97 and 2.85 fold higher than that of control group respectively. In Group III and IV there was almost same AST and ALT content was observed as control. In case of Group V and VI the AST and ALT content showed significant changes. In group V the AST and ALT content was decreased almost 2.16 and 1.90 fold lower and in group VI the AST and ALT content was found to be decreased almost about 1.87 and 2.25 fold lower than that of lead treated group respectively.

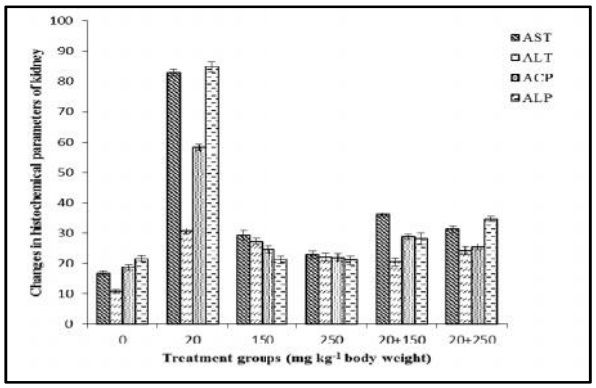

Figure 6 Effects of dead biomass of A. foetidus on some histochemical parameters of the renal tissue of lead nitrate exposed mice.AST: Aspartate transaminase (IU L-1); ALT: Alanine transaminase (IU L-1); ACP: Acid phosphatase (µM of PNP formed min -1 g-1 tissue); ALP: Alkaline phosphatase (µM of PNP formed min -1 g-1 tissue. Data were found to be significant at $P < 0.05$

It was observed that the ACP and ALP content increased in a significant manner in group II mice. In the renal tissue of group II (lead treated) mice ACP and ALP content was found to be increased almost about 3.12 and 3.93 fold higher than that of group I (control) mice. In Group III and Group IV, there was almost same ACP and ALP content was observed as control. In group V the ACP and ALP content was decreased almost 2.02 and 3.02 fold lower and in group VI the ACP and ALP content was found to be decreased almost about 2.28 and 2.45 fold lower than that of lead treated group respectively.

Testis

It was evident from the histochemical studies of testicular tissue that lead has a certain toxic effects on the histochemical parameters of testis (Fig 7).

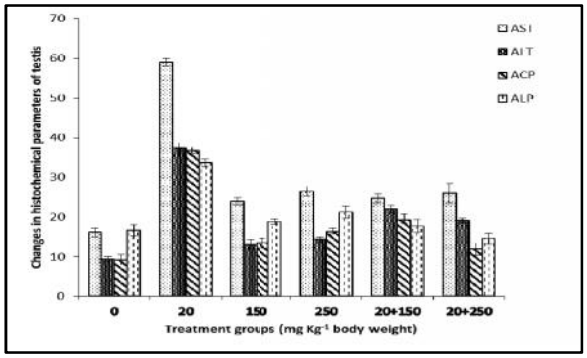

Figure 7 Effects of dead biomass of A. foetidus on some histochemical parameters of the testicular tissue of lead nitrate exposed mice.AST: Aspartate transaminase (IU L-1); ALT: Alanine transaminase (IU L-1); ACP: Acid phosphatase (µM of PNP formed min -1 g-1 tissue); ALP: Alkaline phosphatase (µM of PNP formed min -1 g-1 tissue. Data were found to be significant at $P < 0.05$

It has been observed that treatment with lead nitrate showed a significant increase in parameters which include AST and ALT. The increase was almost 3.65 and 4.01 fold higher than that of control group respectively. In Group III and Group IV there was almost same AST and ALT content was observed as control. In group V the AST and ALT content was decreased almost 2.38 and 1.70 fold lower and in group VI the AST and ALT content was found to be decreased almost about 2.27 and 1.96 fold lower than that of lead treated group respectively.

It was observed that the ACP and ALP content increased in a significant manner in group II mice. In the testis of group II (lead treated) mice ACP and ALP content was found to be increased almost about 3.99 and 2.02 fold higher than that of group I (control) mice. In Group III and Group IV there was almost same ACP and ALP content was observed as control. In group V the ACP and ALP content was decreased almost 1.90 and 1.90 fold lower and in group VI the ACP and ALP content was found to be decreased almost about 3.08 and 2.31 fold lower than that of lead treated group respectively.

Brain

Lead toxicity produced a significant elevation of different histochemical marker enzymes like AST, ALT, ACP, and ALP of brain (Fig 8).

It was found that AST and ALT content of brain was significantly increased in group II mice in comparison to control (group I). The increase was almost 4.13 and 4.73 fold higher than that of control group respectively. In Group III and Group IV there was almost same AST and ALT content was observed as control. In case of Group V and VI the AST and ALT content showed significant changes. In group V the AST and ALT content was decreased almost 2.18 and 2.11 fold lower and in group VI the AST and ALT content was found to be decreased almost about 2.38 and 2.44 fold lower than that of lead treated group respectively.

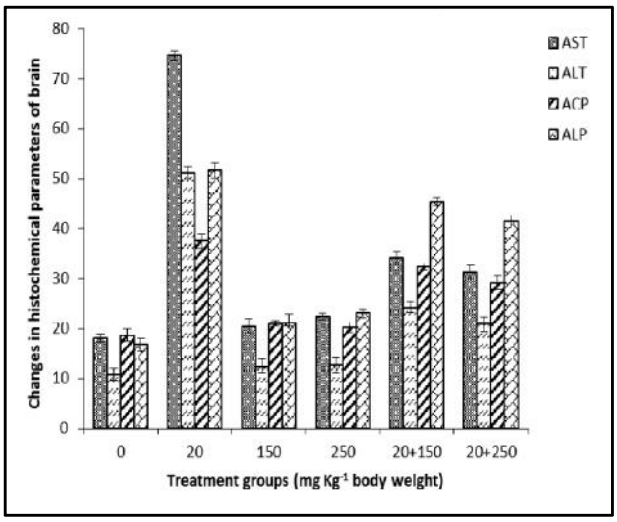

Figure 8 Effects of dead biomass of A. foetidus on some histochemical parameters of the nervous tissue of lead nitrate exposed mice.AST: Aspartate transaminase (IU L-1); ALT: Alanine transaminase (IU L-1); ACP: Acid phosphatase (µM of PNP formed min -1 g-1 tissue); ALP: Alkaline phosphatase (µM of PNP formed min -1 g-1 tissue. Data were found to be significant at P <0.05

It was observed that the ACP and ALP content increased in a significant manner in group II mice. In the brain of group II (lead treated) mice ACP and ALP content was found to be increased almost about 2.00 and 3.07 fold higher than that of group I (control) mice. In Group III and Group IV there was almost same ACP and ALP content was observed as control. In group V the ACP and ALP content was decreased almost 1.16 and 1.14 fold lower and in group VI the ACP and ALP content was found to be decreased almost about 1.29 and 1.25 fold lower than that of lead treated group respectively.

Histological Analysis

Liver

The histological examination showed normal architecture of the hepatic tissues of the group I animals (Fig. 9a). The microscopic analysis revealed the presence of normal hexagonal or pentagonal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes were found to be arranged in trabecules running radiantly from the central vein and were separated by sinusoids containing Kupffer cells. The nuclei were found to be regular large spherical shape. Mice of group I showed radially arranged hepatic cords around the Central vein and Sinusoids were evident.

Assessment of the hepatic tissue section of group II mice which showed that the normal structural organization of the hepatic lobules ware damaged and the regular cordlike arrangement of the normal liver cells was lost. It was observed that the central and portal veins were congested. Hepatic sinusoids were found to be dilated and apparently contained more kupffer cells as compared to control liver of mice Considerable number of hepatic cells were damaged and lost their characteristic appearance while others showed marked cytoplasmic vacuolization which was so extensive in some cells. The nuclei of thesecells were pyknotic. Some leukocyte infiltration and fatty deposition was also clearly found (Fig. 9b).

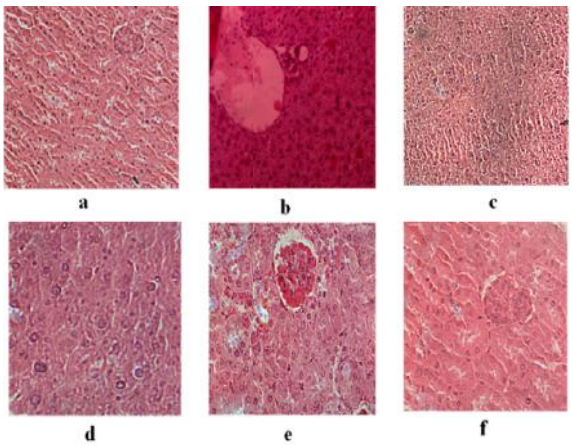

Figure 9 Transverse section of hepatic tissue.(a) group I; (b) group II;(c) group III; (d) group IV; (e) group V; (f) group VI

In group III and IV showed almost normal ultrastructures which were more or less as same as control group (Fig. 9c and d).

In group V and VI it was found that most of those deleterious histopathological changes were reduced but some hepatocytes appeared with vacuolized cytoplasm and Kupffer cells were activated in low doses groups (Fig.9 e). In high doses groups, the liver tissue restored most of its normal structure and was able to lessen the fibrosis, congestion, incidence of inflammatory cells infiltration, centrilobular hepatocytes swelling, hepatocytes vacuolization, fatty changes and hemorrhagic clots (Fig 9f).

Kidney

A section of the renal tissue of the control group of mice showed normal structure of both the renal corpuscles and tubules. Renal sections of group I showed normal rounded glomeruli and did not show any signs of damage. Renal tubules are lined with typical thick cubic epithelium. The tubules had a relatively regular distinct lumen. It was found that the renal tubules were well arranged and uniformly stained (Fig 10a).

The microscopic studies of the renal tissue sections in the group II mice are that of dilation of tubules; marshing of epithelium indicated advanced degeneration of renal tubules. At remnants of dead renal tubules were found to scattered everywhere. Shrinked glomeruli, widened urinary space of the Bowman's capsule has been observed. A few proximal convoluted tubule cells were vacuolated and swollen. Inflammatory cells were observed in the intertubular spaces. Most of the cells of the convoluted tubules were highly swollen and their lumens were nearly obliterated. Some blood sinusoids appeared to be filled with erythrocytes (Fig. 10b).

The renal tissue sections of group III and IV showed almost normal appearances which were more or less as same as control group (Fig.10c and d).

In these tissue sections (Fig 10e and f) it was found that the renal tissue more or less retain its normal appearance. Glomeruli were found to be quite normal. Cell debriswas absent. Renal tubules were observed to be compact, rounded and at places thin-walled but neither dilated nor damaged. There was no evident for the presence of inclusion of blood cells. These findings suggested that the dead fungal biomass might have some positive intrinsic effect in bringing about functional improvement on the renal tissues of lead treated animal.

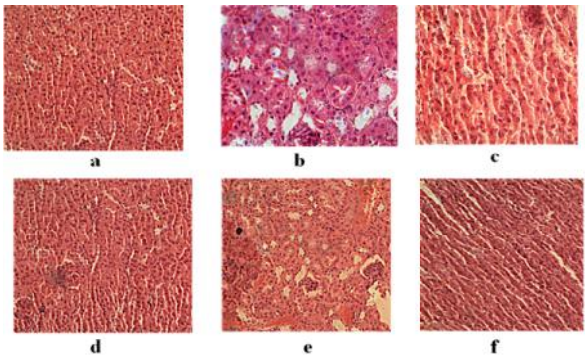

Figure 10 Transverse section of renal tissue.(a) group I; (b) group II;(c) group III; (d) group IV; (e) group V; (f) group VI

Testis

In group I, the testicular tissues exhibited that the tunica albugenia and blood vessels were found to have normal appearance. Seminiferous tubules were observed to be richly populated and in good physical shape (Fig. 11a). All the testicular cells of the spermatogenic process such as spermatogonia, spermatocyte, spermatids, and spermatozoa, even Sertoli cells could be identified in the tubules. Lumen could easily be delineated in almost all the tubules, and majority of them were occupied by mature spermatozoa. Leydig cells were well observed in between the tubules.

In group II animals, in testicular section tunica albugenia was found to be thickened and blood vessels were thin and collapsed (Fig. 11b). Most of the seminiferous tubules were found to be dried up. The basement membrane of the testicular tissue were thickened and hyalinized. Cellular debri were present in the lumen of the seminiferous tubules. Most of the tubules contained abnormally developed spermatogonia and spermatocytes, which were large in size and contained darkly stained nuclei even in some cells the nuclear membranes had been found to be ruptured. Testicular tubules contained only scanty numbers of bigger sized spermatogonia, with very dark nuclei. The Sertoli cells had been found to be easily identified due to disappearance of other cells. The blood vessels in the interstitium were thin and distorted. The interstitial cells of Leydig were also reduced in number and their characteristic tendency of clumping together to form groups was also reduced.

The transverse sections of testis of group III and IV mice showed almost normal appearances which were more or less as same as control group (11c and d).

In groups Group V and VI recovery of the testicular tissue had been observed which includes accumulation of increased spermatozoa in the luminal areas, normal seminiferous tubules, and thin basement membrane. (Fig. 11e and 7f).

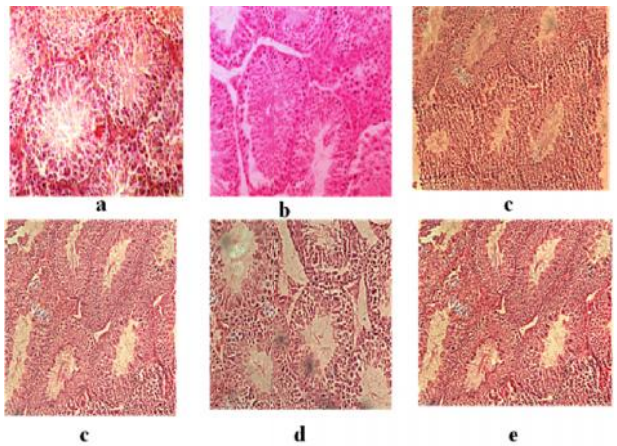

Figure 11 Transverse section of testicular tissue.(a) group I; (b) group II;(c) group III; (d) group IV; (e) group V; (f) group VI

Brain

The histology of neurological tissue of control mice showed well developed neurons. No vascular damage or hemorrhages were observed (Fig.12a).

The brain of lead treated mice divulged that there was (Group II) necrosis of tissue took place. Pyknotic nuclei have been found to have vacuoles. Cells were bigger in size with large vascular spaces around them (Fig.12b).

The transverse sections of brain of group III and IV mice showed almost normal appearances which were more or less as same as control group. No neurotoxic effects were found in those two groups (Fig 12c and d).

The groupV and VI showed very less histological differences when compared with control group I (Figure 12e and f). The combined treatment with dead fungal biomass at a dose of 150 mg kg^{-1}body weight per day along with lead nitrate resulted in some improvement in tissue architecture though vacuolization still persist. However in the high dose better recovery was observed.

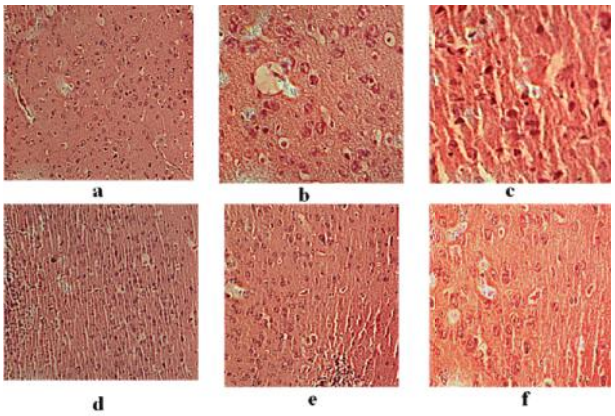

Figure 12 Transverse section of brain tissue. (a) group I; (b) group II;(c) group III; (d) group IV; (e) group V; (f) group VI.

Serological assay

The biochemical analysis of serum of different treatment groups revealed significant changes in serum calcium and iron-TIBC content(fig. 13). It was observed that the calcium activity decreased in a significant manner in group II mice. In the serum of group II (lead treated) mice calcium content was found to be decreased almost about 3.02 fold lower than that of group I (control) mice. In Group III and Group IV where only 150 and 250 mg kg^{-1} body weight per day was introduced respectively there was almost same calcium content was observed as control. In group V the calcium content was increased almost 2.69 higher and in group VI the calcium content was found to be increased almost about 2.98 fold higher than that of lead treated group.

The TIBC level of serum of lead treated group was found to be decreased almost about 2.18 fold lower than that of control group (group I). In Group III and Group IV almost same TIBC content was observed as observed in control group. In group V the TIBC content was increased almost 2.01 higher and in group VI the TIBC content was found to be increased almost about 2.05 fold higher than that of lead treated group.

The serum-iron level of lead treated animal group (group II) was found to be significantly decreased. It was found that the iron content was decreased almost about 2.25 fold lower than that of group I. As usual, Group III and Group IV exhibited almost same serum-iron content as group I. In group V the serum-iron content was increased almost 2.53 higher and in group VI the serum-iron content was found to be increased almost about 2.57 fold higher than that of lead treated group.

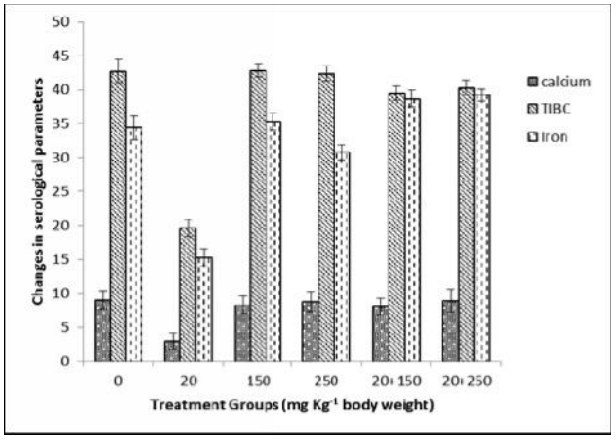

Figure 13 Effects of dead biomass of A. foetidus on some serological parameters of lead nitrate exposed mice. Calcium (mg dL-1); TIBC (µg dL-1); Iron(µg dL-1). Data were found to be significant at $P < 0.05$

Estimation of lead from different tissue

Lead content in different soft tissues like liver, kidney, testis and brain of lead treated animal group (group I) was estimated by flame AAS through wet digestion technique (Table 1). The study revealed that those tissues consisted of significant lead burden due to lead treatment. Maximum lead was absorbed by testis. But in Group V where the animals were treated with 150 mg/kg dead biomass of *A. foetidus* along with lead dose, the lead burden was decreased significantly. 6.05 % lead from liver, 73.25% of lead from kidney, 84.76% of lead from testis and 86.68% of lead from brain was found to be removed. In fact the higher dose of dead biomass of *A.foetidus* along with lead dose was found to be more effective for lead removal. In group VI It was found that 91.31% of lead from liver, 86.68% lead from kidney, 90.80% lead from testis and 87.10% lead from brain was eliminated.

Table 1 AAS measurement of Pbbiosorption by dead mycelia, total Pb activities in tissues and % Pb removal. The % removal was calculated by measuring the Pb activities of tissue after treatment with dead biomass of A.foetidus. Data were found to be significant at $P<0.05$

Groups Treatments	Organs	Lead absorbed by wet tissue	% of removal
groupI lead 20 mg^{-1} Kg bodyweight	Liver	36.01±1.11	
	Kidney	71. 12±2.31	
	Brain	56.54±1.59	
	Testis	93.50±1.91	
groupV lead +150 mg^{-1} Kg dead biomass	Liver	33.83±1.86	6.05
	Kidney	19.02±0.98	73.25
	Brain	10.35±1.83	86.68
	Testis	10.48±1.69	84.76
groupVI lead + mg^{-1} Kg dead biomass	Liver	3.128±0.9	91.31
	Kidney	9.48±0.91	86.60
	Brain	8.60±0.76	87.10
	Testis	7.29±0.73	90.80

DISCUSSION

In this present study the dead biomass of *Aspergillusfoetidus* MTCC 8876 was found to have an inhibitory activity over lead nitrate induced toxicity. The postulated role of dead biomass of

this fungal strain in prevention of $Pb(NO_3)_2$ toxicity can be explained by its ability in biosorption or chelation of metal ions from the surrounding environment

It was reported that since dead fungal biomass is of little use and is abundant, it may be good source of biomaterial for the removal of metals from industrial wastewaters (Bai and Abraham, 2001). Moreover the use of dead cells offers the following advantages over live cells, the metal removal system is not subject to toxicity limitations, there is no requirement for growth media and nutrients, the biosorbed metal ions can be easily desorbed and biomass can be reused and dead biomass-based treatment systems can be subjected to traditional adsorption models in use. As a result, the use of dead fungal biomass has been preferred in numerous studies on biosorption of toxic metal ions from aqueous solutions (Kapoor and Viraraghavan, 1998).

It has been suggested that the dead fungal cells have the higher potential to bind with the metals than live cells depending on the methods used to kill (pretreated) the live cells (Kogej&Pavko 2001). It has been noticed that the heavy metal ion affinity of the biomass can be alter by pretreating the biomass with acids, alkaline chemical solution and detergents or even by heat. There are various methods that involved in pretreatment of biomass. According to Hima *et al*. (2007) the heat treatment and detergent washing treatment can expose additional heavy metal binding groups to the biomass and thus lead to more binding site that available for the biosorption process.

In this present study it has been found that under lead toxicity the extent of lipid peroxidation was stimulated to each of the soft tissues like liver, brain, kidney and testis. It has been already reported that lead induced oxidative stress could damage the cellular membrane producing oxy-radicals (Halliwell and Gutteridge, 1989). These oxy-radicals attack cell membrane lipid through peroxidation, generating the final product of the peroxidation process, malondialdehyde (MDA) (Marnett, 1999). In this present study it has been found that in lead treated animals the LPO extent was increased, but when the lead intoxicated animals were treated with the dead biomass of *A.foetidus* MTCC 8876 the LPO content was found to be decreased. These results suggested that though *A. foetidus*may not have any medicinal antioxidant property but it could bioabsorb lead through its whole cell wall from the intoxicated animal body so that the metal content was decreased from the animal body and as a result the LPO content was also depleted. The balance between the generation of oxy-radicals and the scavenging of those oxy-radicals was maintained in the cellular environment through a set of antioxidant enzymes (Gibanananda&Hussain, 2002). In the present study, the activities of GST, CAT and GSH antioxidants were reduced by lead nitrate, these results may provide the mechanism responsible for the peroxidative damage to the tissues.

It was suggested that lead induced auto-oxidation procedure may be one of the cause for the production of highly reactive cytotoxic compounds like prooxidant, superoxides or peroxide molecules (Gurer *et al*. 1999) which might be one of the reasons for significant alteration in the activity of antioxidant enzymes. In this present study lead toxicity might result in decreased activities enzyme catalase enzyme. CAT decomposes H_2O_2 to H_2O and O_2.The decrease in CAT activities might suggest that the examined tissue of mice was experiencing oxidative stress, because these enzymes catalyze the decomposition of ROS. In group V and VI the catalse activity was found to be improved as the fungal biomass absorb the lead, lead induced oxidative stress was also diminishing as a result the catalase activity was found to be in its more or less normal level.

Lead directly affect GSH content by binding to the –SH group of GSH of the living cell (Bechara, 2004). GSH is a well-known non enzymatic antioxidant and a major thiol-disulfide metal chealating redox buffer of the cell. In this present study it has been found that in presence of lead, GSH content was decreased. From this result it may be suggested that during lead stress glutathione peroxidase oxidized GSH to GSSG, which in turn decreased the GSH content. (Gibanananda &Hussain, 2002). It was assumed that lead was chelated by GSHand form lead – GSH complex which was subsequently excreted through bile in in-vivo system. In group V and VI it was found that GSH content of the treated animals improved. It may due to the bioadsorption capacity of the fungal strain. During treatment when lead is administered along with the fungal biomass it may be bioadsorped by the strain. For this reason concentration of GSH was found to be improved and increased almost up to normal concentration.

It was observed in this study that the activity of GST enzyme in Group II animals also decreased under lead stress. The decrease may be due to Pb-induced changes in the enzyme structure or the presence of insufficient amount of GSH, as it is the substrate of GST enzyme (Sivaprasad *et al* 2004). The GSH homeostasis in tissues is maintained by these enzymes, such as GPX, GR and GST. GST enzyme and GSH acts mutually in the redox system, hence it may be suggested that the decrease in GSH concentration might trigger the decrease in GST activity. But when dead biomass of the fungi along with lead was administered to the animals, the activity of GST was found to be increased due to the presence of free GSH because the metal was absorbed by the cell wall of fungal biomass, making GSH free for GST induced reaction.

In this current study it was evident that the levels of AST, ALT, ACP, ALP enzymes were increased under lead treatment in group II mice tissues. AST and ALT are an important class of enzymes that are linked to carbohydrate and amino acid metabolism. Bersenyi *et al*. (2003) have reported that heavy metals have been found to elevate AST and ALT levels in metal treated tissues. From this histochemical data it might be suggested that administration of lead could cause cell lysis, resulting in the release of cytoplasmic enzymes into the blood circulation, leading to their increase levels in serum or subsequent tissues. But in this present study in group V and VI it has been found that the concentration of AST and ALT was near about normal after the application of the dead fungal biomass. It may be suggested that due to the biosorption capability of this fungal strain the amount of lead from the

intoxicated animal body decreased so that those enzyme activity which was triggered due to the presence of lead in animal body was also come to the normal level.

Lead induced oxidative stress evoked the absorption of fat and lysosomal imbalance which is caused by the destruction of the intact membranes in the tissue leading to the increased ACP activity.ALP has been considered as the marker enzyme for plasma membrane. Increase in the ACP and ALP activities in the lead treated tissues may be suggested that there may be a possibility of increased cellular permeability, damage and or necrosis of cells. In group V and VI it has been found that the ACP and ALP enzymatic activity was decreased at almost normal level due to the ameliorating effects of dead fungal biomass. This biomass may biosorb lead from the intoxicated animal body at a certain level that the enzymes that were evoked due to lead stress were found to be normalized.

Administration of lead nitrate also causes decrease in total protein level in liver, brain .kidney and testis of group II mice. It was reported that lead induced RNA damage, hampers the protein synthesis in the affected tissues (Shalan *et al*., 2005). In group V and VI it has been found that the protein content was increased in each of the test tissue. It may be suggested from this study that due to biosorption of lead through the fungal biomass,the intoxicated cells of the animal body retain its normal protein content as the lead induced toxicity was decreased up to certain level.

In the present analysis, lead exposure produced prominent histopathological damage to liver, kidney, brain and testis which was evidenced by histological studies.

Anomalies in liver which were found in this text were including focal necrosis with hepatocyte vacuolation, swelling, leucocyticinfilteration, pykonotic nuclei, dilation of central vein and sinusoids. Similar results were reported by scientists (Shalan *et al*. 2005; El Sokkary *et al.* 2005).

Lead exposure produced marked histological alternations in kidney in lead treated mice group which include dilation of tubules; congestion of epithelial layer etc. which indicates advanced disintegration of renal tubules. Previous reports (Lin *et al*., 1993) showed that sublethal dose of lead resulted in progressive tubular, glomerular and interstitial alterations.

The histological study of lead induce mice's testicular tissue exerts prominent histopathological damage by the alternations in testis include degeneration of seminiferous tubules, thickening of basement membrane, and condensation of the stroma. There are several reports were present that supported the present result (Thomas and Brogan 1983).

The histological study revealed that lead exerts immense toxicity in brain .It has been found in group II, lead induce vacualisation and blood clot in brain resulting necrosis of the brain tissue.

But in this present study in group V and VI it has been found under treatment with dead fungal biomass all these affected tissues were gradually retained their normal appearance and the sign of toxicity were disappeared from the lead induced tissue to some extent.

The serological study also exhibit deleterious effect on serum calcium and Iron-TIBC activity.

Calcium is the building block element of bone. It was assumed that Lead and calcium compete for the same locations within the body and are stored in the bone. As lead has a greater affinity than calcium for common binding sites. In addition, binding protein calbindin-D, that aids in calcium transport also binds to lead with high affinity and may increase transport of lead in low calcium states (Onalaja and Claudio2000). In this present study, in group II mice the serum calcium was found to be decreased. But the serum calcium level was found to be recovered when the lead treated mice were administered with dead fungal biomass. This result may be due to the absence of lead in the animal body because the biomass adsorb lead from animal body subsequently normalizes the serum calcium level.
It is known that lead interferes with the utilization of iron for the formation of heme. This probably occurs in every cell, although it is best studied in the blood-forming organs. Same results have been found in lead treated group I mice. But in group V and VI may be due to the absorption efficacy of dead fungal biomass lead has been found to be decreased in animal body which in turn increase the serum iron level.

Similar results were observed in serum TIBC activity, TIBC or the total iron binding capacity was found to be decreased in lead treated animal group (group II). It has been found that lead alters the TIBC activity of the serum. For this reason in most lead toxicity cases anemia is the most common symptoms that has been found in not only animals but also in humans (Onalaja and Claudio 2000). In group V and VI the TIBC activity was found to be improved in presence of dead fungal biomass. Because may be the strain can chelate lead from the animal body which helped the intoxicated mice to retain its normal TIBC activity.

Lead burden in liver, kidney, brain and testis was found to be reduced in dead fungal biomass treated groups In different reports it was evident that dead fungal biomass of *Aspergillusspp* have the capability to absorb or biotransform metal ions from metal contaminated site but there are very scanty of reports regarding the administration of fungal biomass to the lead intoxicated animals to detoxify it from metal toxicity. In this study it has been observed that in group II mice lead was administered at a dose of 20 mg kg^{-1} body weight per day. This lead dose was accumulated by different organs in different concentration. Maximum lead was absorbed by testis; it was more or less 93.5 µg/kg bodyweight. Kidney, brain and liver absorb 71.12µg kg^{-1} bodyweight,56-54µg kg^{-1}bodyweight and 36.01µg kg^{-1} bodyweight of lead respectively. In group V the lead burden was found to be decreased significantly. 6.05 % lead from liver, 73.25% of lead from kidney, 84.76% of lead from testis and 86.68% of lead from brain was found to be removed. In group VI It was found that this higher dose was more effective and removed 91.31% of lead from liver, 86.68% lead from kidney, 90.80% lead from testis and 87.10% lead from brain. It may be suggested from this result that the dead fungal biomass might have chelated

lead and enhanced its excretion from the body resulting in reduced lead accumulation in tissues and blood. The results were crosschecked when a certain amount of lead was found to be present in the faeces of the treated mice (group V and group VI).

CONCLUSION

In this present study we demonstrated that dead fungal biomass of *A.foetidus* MTCC8876 as a treatment to detoxify the lead intoxicated animals. The reports regarding the use of dead or inactive fungal biomass to detoxify lead from the environment corroborated the previous studies. But administration of the same to the metal intoxicated animals directly is somewhat a new approach of bioremediation. We administered the dead fungal biomass of *A.foetidus* MTCC8876 with the normal diet of the lead intoxicated mice and got positive results. Further in –depth study with this strain is needed to fully understand the mechanism behind lead removal efficacy of this strain.

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