

## **Bovine serum albumin rescued SH-SY5Y cells from neurotoxic effects of lead**

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**Abstract:** Lead has long been identified as an environmental pollutant all over the world. It is a metal that has the ability to affect every organ and system in the body. Lead in low levels can adversely affect the central nervous system, resulting in memory loss and other neurological abnormalities. Bovine Serum Albumin (BSA) is a well known globular protein that has the tendency to aggregate in the macromolecular assemblies. Albumin has a high affinity for fatty acids, hematin, bilirubin and a broad affinity for small negatively charged aromatic compounds. It forms covalent adducts with pyridoxyl phosphate, cysteine, glutathione, and various metals, such as Cu (II), Ni (II), Hg (II), Ag (II), and Au (I). To investigate the possible effect of lead on the structure and function of BSA, it is treated with lead in different concentrations and for different time incubations. The results of the present study reveal that the BSA after treatment with lead rescued the cells from the cytotoxic effects of lead at higher lead concentration (higher than 200µM). The fluorescence study also shows a significant decrease in intrinsic fluorescence at 335nm and absorbance at 280nm has been significantly increased. SDS-PAGE results also shows aggregations in lead treated BSA samples. Phase contrast microscopy reveals a significant rescue of cells in lead treated BSA group as compared to lead control group. However further studies are required to investigate the possible role of lead in affecting the structure and function of proteins.

**Keywords:** BSA, SH-SY5Y cells, neurotoxicity, lead.

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### **INTRODUCTION**

It is a well known fact that the native structure of protein plays a key role in its biological and chemical properties in the complex environment of cell. Under some conditions, proteins fail to fold correctly in their native form which results in a wide range of diseases like amyloidoses, which involves the deposition of aggregated proteins in various tissues<sup>1-3</sup>. Neurodegenerative pathologies like Alzheimer's and Parkinson's diseases belong to this category. Protein aggregation process is going in competition with the normal folding pathways [4-5] and it takes place from misfolded and partially unfolded state<sup>6-7</sup>.

Bovine Serum Albumin (BSA) is a well known globular protein that has the tendency to aggregate in the macromolecular assemblies<sup>8</sup>. Its three dimensional structure is composed of three domains, each one is formed by six helices, and its secondary structure is essentially alpha helix [9-10]. The structure of BSA shows stability at room temperature but as temperature increases it results in the formation of soluble aggregates through disulphide and non-covalent bonds<sup>11-12</sup>.

It has been reported that BSA can undergo glycation particularly with ribose which lead to high cytotoxicity of SH-SY5Y cells<sup>13</sup>. Albumin has a high affinity for fatty acids, hematin, bilirubin and a broad affinity for small negatively charged aromatic

compounds. It forms covalent adducts with pyridoxyl phosphate, cysteine, glutathione, and various metals, such as Cu (II), Ni (II), Hg (II), Ag (II), and Au (I).

Heavy metals compromise normal brain development and neurotransmitter function, leading to long term deficits in learning and social behavior. Lead (Pb) has long been recognized as a harmful environmental pollutant all over the world<sup>14-15</sup>. It is a toxin that has the ability to impede the development and function of every organ and system in the body. The time has come to acknowledge the real dangers of lead contamination. Lead toxicity could be the reason for the increasing number of subjects suffering from various psychological problems, like depression and tension<sup>14-15</sup>. Rate of suicides have also increased during the last few years.

Lead is the most well known toxic heavy metals. Severe and acute lead intoxication can cause encephalopathy, convulsion, coma and even death<sup>16</sup>. During the last few decades, environmental investigations, education services, and laws prohibiting the use of lead in many fields of industry have contributed to the lowering of the lead level in the environment, but the exposure to low-level lead, which causes mild and subclinical symptoms, is nonetheless still a significant public health problem<sup>17-19</sup>. In particular, the neurotoxic effect of low-level lead on higher brain functions, such as

cognition, learning and memory are attracting increasing interest.

In the present study, based on the information regarding the misfolding and aggregation of BSA leading to many neurodegenerative diseases, the possible role of lead on BSA has been investigated to figure out the effect of environmental exposure of lead on the structure and physiological properties of BSA.

## MATERIALS AND METHODS

BSA used was free from fatty acids and purchased from Sigma (USA). Lead in the form of Lead acetate was also purchased from Sigma. All the other chemicals used were of analytical grade.

### Sample preparation

BSA was dissolved in 20mM Tris-HCl pH 7.4 to yield a stock of 0.1mM. BSA solution was then resuspended with lead solution prepared in 20mM Tris-HCl to prepare four different ratios which were 1: 20, 1:40, 1:50 & 1:100. The final concentration of BSA was 10uM and that of lead were 200uM, 400uM, 500uM & 1000uM. BSA alone and four controls of lead were also used. The samples were then incubated at 37°C for 0 to 7 days. All solutions were filtered with 0.22uM membrane (Millipore USA).

### Cell viability test (CCK-8)

SH-SY5Y cells were seeded in 96 well plate at a concentration of  $10^3$  cells per well and either exposed or not exposed to the lead treated BSA (0 and 7 days) for 24 hours. After adding the lead treated BSA, the plates were incubated at 37°C, 5% CO<sub>2</sub> and O.D. was measured at 450nm by Multi Scan MK3.

### Fluorescence measurements

Fluorescence of lead treated BSA was measured on an F-4500 fluorophotometer (Hitachi, Japan) with a circulating water bath at 37°C. The fluorescence spectra were measured at a fixed protein concentration of 10uM. Excitation and emission slits were set at 5nm. The spectra were recorded in 280 to 550nm range and the excitation wave length was set at 288nm.

### Absorption measurements

Absorbance of lead treated BSA was measured on U-2010 spectrophotometer (Hitachi, Japan) with a circulating water bath at 37°C. The absorption spectra were also measured at a fixed protein concentration of 10uM. The range of the spectra was from 200-350nm.

### SDS PAGE

The samples used were of 0 day and 7 days incubations and subjected for SDS PAGE. BSA final concentration was 10uM. One control of BSA alone was used. The samples were subjected to electrophoresis using Bio-Rad (USA), electrophoresis equipment.

### Phase contrast microscopy

SH-SY5Y cells were seeded in 96 well plates at a concentration of  $10^3$  cells per well and either exposed or not exposed to the lead treated BSA (7 days) for 24 hours. After adding the lead treated BSA, the plates were incubated at 37°C, 5% CO<sub>2</sub>. After incubation Hoechst staining was performed according to standard protocol and cell pictures were taken under 10X magnification.

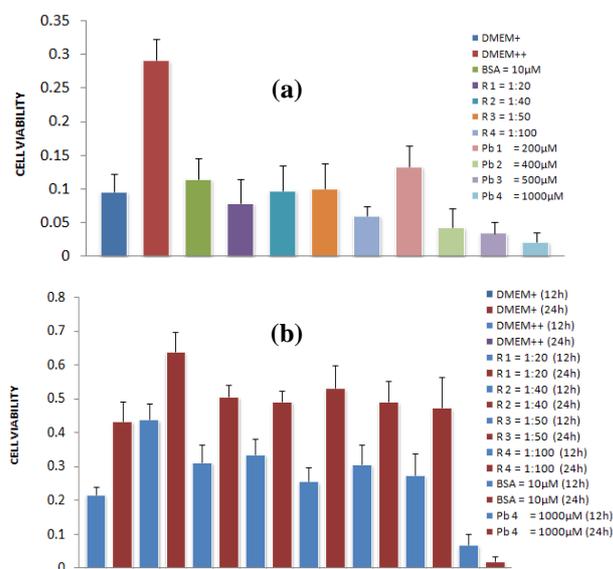
### Statistical analysis

The statistical analyses were performed by Student's t-test. P values <0.05 were considered significant.

## RESULTS

### Cell viability test (CCK-8)

As shown in figure 1(a) and (b) the cell culture study reveals that lead alone at higher concentrations (Pb<400uM) is showing significant cytotoxicity to the SH-SY5Y cell line. Interestingly, the BSA along with lead rescued the SH-SY5Y cells from the cytotoxic effect of lead at higher concentrations.



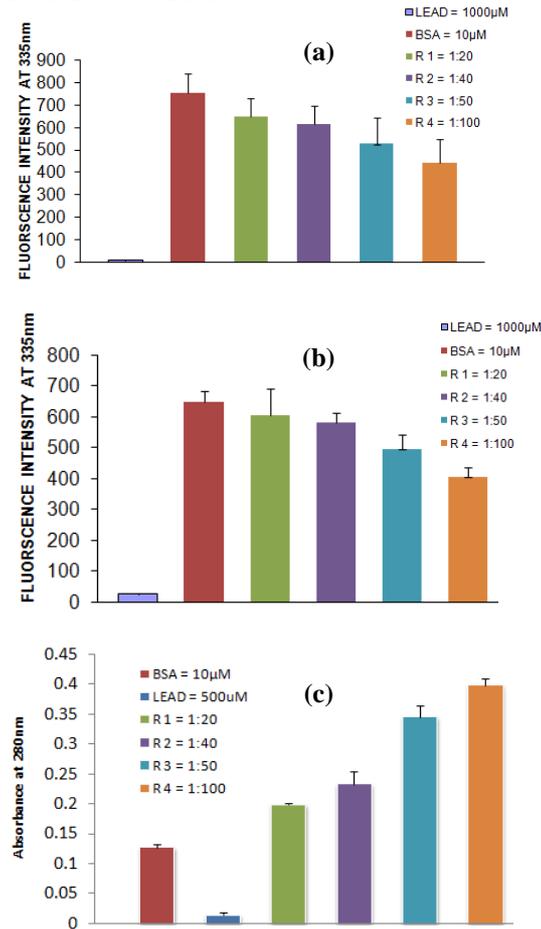
**Figure 1:** (a) Cell viability measured by CCK-8. BSA alone or incubated with different concentrations of lead at 37°C for 7 days, was added to SH-SY5Y cells for 24 hours and cell viability was measured by using the CCK-8 assay. (b) Effect of lead treated BSA (0 day incubation) at 12 hours and 24 hours on SH-SY5Y cells.

**Fluorescence studies**

As shown in figure 2 (a) & (b) fluorescence results show that there is a significant decrease in fluorescence intensity at 335nm at 0 day and 3 days with reference to control BSA at higher concentrations of lead.

**Absorbance studies**

According to figure 2 (c) the absorbance results show that there is a significant decrease in absorbance at 280nm in samples of BSA treated with lead in a concentration dependent manner with reference to control.



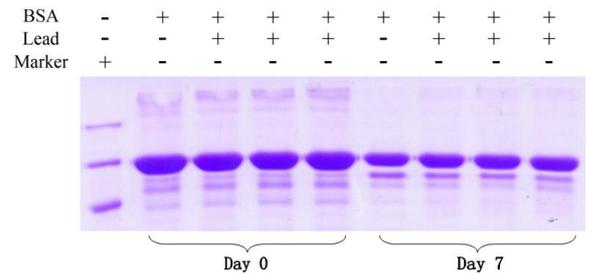
**Figure 2:** (a) Concentration dependent changes in the fluorescence of BSA treated with lead. BSA (final concentration 10µM) in the presence of different concentrations of lead (200, 400, 500 & 1000µM). Aliquots were taken for measurement of fluorescence ( $\lambda_{ex}$  288nm;  $\lambda_{em}$  335nm). (b) Shows that lead significantly decreases the fluorescence intensity of BSA as compared to control at 3 days incubation. (c) Concentration dependent changes in the absorbance of BSA treated with lead. BSA (final concentration 10µM) in the presence of different concentrations of lead (200, 400, 500 and 1000µM). Aliquots were taken for measurement of absorbance at 280nm.

**SDS-PAGE**

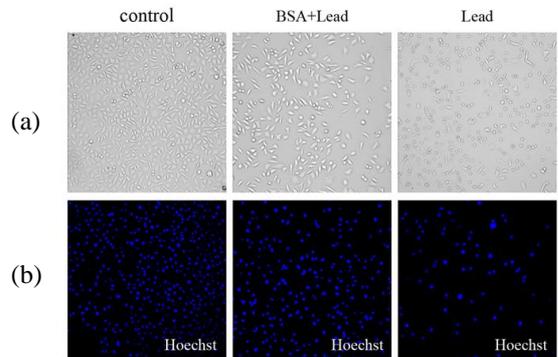
As shown in figure 3, the result from SDS PAGE shows that BSA alone has some aggregation. It is evident from the results that the lead treated samples of BSA show higher aggregations in concentration dependent manner. At day 7, the aggregates started to become soluble.

**Phase contrast microscopy**

It is evident from figure 4a and b, that Bright field microscopy and Hoechst staining pictures show that there is significantly higher cell death in lead alone group as compared to positive control and lead treated BSA group at the same concentration.



**Figure 3:** 10% SDS-PAGE of the products of BSA incubated with different concentrations of lead for 0&7 days at 37°C. BSA (final concentration 10µM) in the presence of different concentrations of lead (200, 400, 500 & 1000µM). Aliquots were taken for SDS-PAGE electrophoresis.



**Figure 4:** (a) Shows bright field pictures of control (DMEM), lead treated BSA and lead alone (0 day samples). (b) Shows Hoechst staining of control (DMEM), lead treated BSA and lead alone (0 day samples).

**DISCUSSION**

Previous research showed that eukaryotic proteomes have a significantly higher occurrence of disordered proteins relative to prokaryotes<sup>20</sup>. The role of many proteins in the neurodegenerative disorders like Alzheimer's and Parkinson's diseases have been investigated in the past research. Previous research showed that partially or fully disordered proteins are prevalent in complex disorders like

neurodegenerative diseases<sup>21</sup>, cancer, cardiovascular disease or diabetes<sup>22</sup>.

It has been reported in our research group that rapid glycation of BSA with ribose could result in the formation of Advance Glycation End Products (AGE's), which would result in the cytotoxicity to the neuroblast cells like SH-SY5Y<sup>13</sup>. It has also been reported in our research group that formaldehyde in low concentrations can react with protein Tau to form globular amyloid like aggregates both in vivo and in vitro, which showed cytotoxicity to SH-SY5Y & HEK 293 cells<sup>23</sup>.

The important finding of the present study is that lead alone at concentrations  $\leq 200\mu\text{M}$  significantly proved to be cytotoxic to SH-SY5Y cells. The interesting result of this study has shown that in the mixture of BSA and lead, the cells have been rescued from cytotoxic effects of lead in the given concentrations. In our previous results (unpublished data), we have shown that BSA alone at a concentration of 0.01mM is also significantly cytotoxic but here in the present study, BSA at a concentration of 10 $\mu\text{M}$  has shown no cytotoxicity as compared to the control. The CCK-8 result for the cells incubated with lead modified BSA for 12 hours and 24 hours also significantly showed the cell rescue by BSA (Figure 1a and b).

To investigate that BSA treated with lead results in any structural change in BSA, we performed the fluorescence studies at the excitation wave length of 288nm. The results showed that there is significant decrease in the intrinsic fluorescence at 335nm for the samples of BSA treated with lead as compared to the control group in both 0 day and 3 days incubation. Figure 2a and b) In order to further elaborate these changes, we have performed absorbance studies. The results of the absorbance studies also showed a significant increase in absorbance at 280nm in a concentration dependent manner (Figure 2c).

The SDS PAGE results also correlate with the above mentioned finding. There is a slight aggregation of BSA alone showing its self aggregating property but this became more significant in the samples of BSA treated with lead in different proportions in a concentration dependant manner.. It is to note down that with long time incubation at day 7, the aggregates started to be solubilized (Figure 3).

Phase contrast microscopy has been done to further elaborate and support the above findings. The results show a significant decrease in the number of viable cells and their morphology in lead alone group

as compared to the positive control and lead treated BSA group (Figure 4).

## CONCLUSION

In conclusion, this study demonstrated that BSA in presence of lead at a concentration above 10 $\mu\text{M}$  can protect cytotoxicity caused by lead in the neuronal cell lines like SH-SY5Y cells. The interesting finding of the present study is that the formation of the aggregates and polymers of BSA in presence of lead at day 0 became soluble at day 7. How ever further studies are required to figure out the possible mechanism of how lead reacted with BSA protected the cells from cytotoxicity and what changes in the structure of BSA resulted in this behavior.

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