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# Toxicity Effect of a Potential Nanomaterial Using Wistar Rats

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**Akhtar Rasool[1], Syeda Azeem Unissa[1], M.F Rehman[2], Zamin Ahmad[3]**

*1- Department of Environmental Sciences, Osmania University, Hyderabad (India).*

*2- Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad (India).*

*3- Department of Zoology, Osmania University, Hyderabad (india).*

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**ABSTRACT**

In the present investigation, an attempt was made to study the *in-vitro* toxicity effect of a potential nanomaterial (Cobalt) with special references to biochemical enzymes, Aspartate aminotransferase (ASAT), and Alanine aminotransferase (ALAT) in different tissues of Wistar rats. Aspartate aminotransferase (ASAT) and Alanine aminotransferase (ALAT) are the enzymes widely used for the rapid detection to predict early warning of xenobiotics toxicity studies. The biochemical changes induced by a test chemical have significance in its toxicological studies because alterations in biochemical parameters before clinical sign symptoms indicate either the safety of the toxicant or its detrimental effect. The biochemical or enzymatic variations are powerful tools in assessment of toxicity. Present study has demonstrated that cobalt (<50 NM) has significantly activated in serum, liver and kidney ASAT and ALAT activities in exposed female rats at dose dependent order.

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## INTRODUCTION

AST and ALT formerly called as SGOT and SGPT respectively are the enzymes found mainly in the liver, also found in RBC's, Heart cells, kidneys and Pancreas. Amount of AST and ALT in the blood is directly related to the tissue damage i.e. more or less amount leads to the increased or decreased tissue damage respectively.

Due to the clinical importance of AST and ALT in monitoring patients with liver diseases, AST and ALT detection have been researched by a number of scientists all over the world as well as International Federation of Clinical Chemistry (IFCC) and Scandinavian Committee on enzymes [1-11.] These liver enzymes act as biological catalysts; they catalyze the transfer of amino groups to generate products in gluconeogenesis and amino acid metabolism[12,13]. Serum levels of ALP are commonly used in clinical practice as a marker of liver or bone diseases[14]. Elevated AST levels may be caused by disorders that affect organs or tissues other than the liver, with the most common being striated muscles. Elevated values up to 300 u/l of AST and ALT are considered nonspecific. Normal elevated levels occur during 2nd trimester in asymptomatic normal pregnancy[15] whereas elevated levels of AST predominate in patients with cirrhosis[16].

## MATERIALS AND METHODS

### Treatment of rats:

Wister rats (*Rattus norvegicus*) weighing 100±150 g were obtained from **National Institute of Nutrition (NIN) Hyderabad,** and maintained under controlled conditions in the Indian Institute of Chemical Technology (IICT) animal house, for a week prior to the experiment. Prior to studying the ethical clearance was obtained from animal ethical committee. Standard rats were maintained at 22°C and relative humidity of 30-70 percent. Lightning arrangements were 12 hrs light and 12 hrs dark.

### Biochemical Studies:

The rats were killed by decapitation. Before the decapitation, blood was collected without any anticoagulant. Serum was obtained by centrifuging the blood at 1500 rpm for 10 min. Liver and kidney of both control rats were dissected out and quickly homogenized separately ice-cold sucrose solutions using Yarco speed homogenizer to make 10 percent homogenate

(w/v). The pellet was discarded and the supernatant was used as enzyme source. Serum, kidney and liver homogenates were used to determine *in vitro* Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in controlled and nanomaterials exposed rats.

**Protein Estimation:**

The protein was estimated in liver and kidney of control and treated rats with Folin-Phenol reagent using the standard procedure as described by Lowry *et al*., (1951).

**Calibration Curve for Protein:**

To draw the calibration curve for protein different aliquots of BSA solution were taken in the range of 10 to 60 µg and made up to 0.2 ml with Millipore water. Blank was prepared by taking 0.2 ml of Millipore water. Then 3 ml of Reagent 'C'{100 Parts of Reagent A+1 Part of Reagent B, Where Reagent A is 2% $Na_2CO_3$+0.4% NaOH+0.16% Na/K tartarate+1% SDS and Reagent B is(Copper Reagent) 4.0% $CuSO_4$} was added in each test tube, mixed thoroughly and allowed to stand for 30 minutes at room temperature. Then 0.3 ml of Folin-Phenol reagent added in each test tube. The samples were read in High-Performance Double beam spectrophotometer (XP 2001 Explorer) at 660 nm. The concentration of protein in micrograms was plotted on X- axis against their optical densities on Y –axis as shown in figure 1.

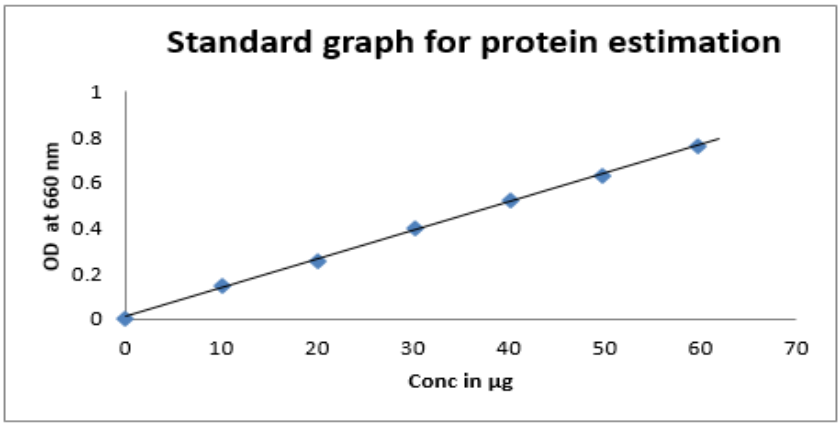


**Fig. 1. Graph representing standard protein.**

**ASTand ALT estimation**

AST and ALT activity in serum, kidney & liver of rat was estimated by spectrophotometer method of Yatzidis (1960).

**Calibration curve of Aminotransferase:**

To draw a calibration curve of Aminotransferase, different aliquots of pyruvic acid in separate glass tubes ranging from 50 to 350 micrograms were taken and volume adjusted to 1.5 ml with saline (0.9% NaCl). The tubes were placed in refrigerated shaking incubator for 60 minutes at 37°C. The 0.5 ml of diluted citrated aniline pipetted into the tubes, to stop the enzymatic reaction. Immediately, 0.5 ml of the 2, 4-DNPH was added and the contents vigorously mixed. Exactly 5 minutes after the addition of the later reagent (the time required to form pyruvate-dinitrophenyl hydrazine but excluding dinitrophenyl hydrazine but excluding the formation of the α-ketoglutarate dinitrophenyl hydrazine) 3 ml of sodium hydroxide was added, the contents mixed by the inversion of the tubes and the color produced was measured after 30 minutes in a High Performance Double beam spectrophotometer (XP 2001 Explorer) at 500 nm against the blank set at zero optical density. The blank was a mixture of the reagents in the same way as that of the standard, except that the pyruvic acid is replaced with saline. A graph was plotted taking the concentration of pyruvic acid in µg/ml on the X-axis and their optical densities on Y axis (Figure 2).

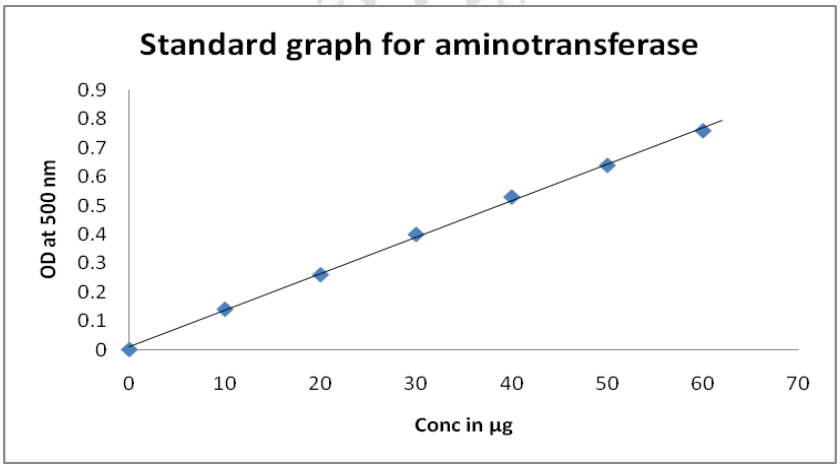


**Fig 2. Graph representing aminotransferase**

**Calculations:**

$$\textbf{Serum} = X/(0.025) \times 1/88$$

= (µmole/hr/ml)

$$\textbf{Tissue} = X/mg\ protein \times 1/88$$

= (µmole/hr/mg protein)

- Where X stands for pyruvic acid formed in µg
- 0.025 stands for volume of serum in ml and
- 88 stands for molecular weight of pyruvic acid (for converting µg into µmoles).

**Statistical Analysis:**

Data represents mean and standard deviation (S.D.) of three biological replicates from each group of experimental animals. The data were analyzed by Dunnett test to illustrate the significant difference between the exposed groups and the control. Statistical significance was considered at $P<0.05$.

**RESULTS**

Present study has demonstrated that cobalt (< 50 NM) has significantly activated in serum, liver and kidney AST and ALT activities in exposed female rats at dose dependent order. However, in case of Serum AST significant activation was observed at all the three doses i.e. 1.0, 2.5 and 5.0 mM. The observed $IC_{50}$ observed was < 1.00 mM and the maximum activation of 149 percent was observed at 5.0 mM dose (Table 1). Similarly, in case of serum ALT, significant activation was observed at 1.0, 5.0, and 10.0 mM doses and $IC_{50}$ observed was 3.186 ± 1.20 mM. Further maximum activation of 149 percent was observed at 10 mM dose (Table 1). In case of kidney ASAT, significant changes were observed at 0.5, 1.0, 2.5, 5.0 and 10.0 mM doses and the $IC_{50}$ observed was 1.138 ± 0.270 mM. Maximum increase of 242 percent was observed at 10.0 mM dose (Table 2). Similarly, in case of kidney ALAT significant changes were observed at 1.0, 2.5, 5.0 and 10.0 mM doses and the $IC_{50}$ observed was 3.00 ± 1.640 mM. A maximum activation of 175 percent was observed at 10.0 mM dose (Table 2). Liver AST was significantly activated at 0.5, 1.0, 5.0 and 10.0 mM doses and the $IC_{50}$ observed was 0.273 ± 0.405 mM. Further, 212 percent activation was observed at 10.0 mM dose (Table 3). Similarly, liver ALAT was significant at all the exposed doses i.e. 0.5, 1.0, 2.5, 5.0 and 10.0 mM doses and the $IC_{50}$ observed was 3.217 ± 1.10. The maximum increase of 106 percent was observed at 10.0 mM dose (Table 3).

**Table 1: *In-vitro* toxicity of serum AST and ALT in rat exposed to Nanomaterial**

| Conc. mM | Rat No. | Activity of AST | Activity of ALT | Mean ±S.D of AST | Mean ±S.D of ALT | Percent activation of AST | Percent activation of ALT | $Ac_{50}$ ± S.E. mM of AST | $Ac_{50}$ ± S.E. mM of ALT |
|---|---|---|---|---|---|---|---|---|---|
| Control | 1 | 4.13 | **5.45** | | | | | | |
| Control | 2 | 4.34 | 5.5 | 4.33±0.0173 | **5.46±0.028** | | | | |
| Control | 3 | 4.34 | 5.45 | | | | | | |
| 1 | 1 | 9.09 | 6.59 | | | 110 | 16.39 | | |
| 1 | 2 | 9.18 | 6.13 | **9.13±0.045**** | **6.39±0.235*** | 110 | 16.39 | | |
| 1 | 3 | 9.13 | 6.45 | | | 110 | 16.39 | | **3.186 ± 1.20** |
| 5 | 1 | 9.43 | 8.81 | | | **120** | 64.8 | | |
| 5 | 2 | 9.59 | 9.08 | **9.52±0.081**** | **6.39±0.235*** | **120** | 64.8 | **< 1.00** | |
| 5 | 3 | 9.54 | 9.13 | | | **120** | 64.8 | | |
| 10 | 1 | 10.68 | 14.2 | | | **149** | 161 | | |
| 10 | 2 | 10.9 | 14.36 | **10.82±0.127**** | **13.95±0.565**** | **149** | 161 | | |
| 10 | 3 | 10.9 | 14.31 | | | **149** | 161 | | |

Data represents Mean ± S.D. of three biological replicates from each group. **P<0.01 significantly different from control.

**Table 2: *In vitro* toxicity of Kidney AST and ALT in rat exposed to Nanomaterial**

| Conc. mM | Rat No. | Activity of AST | Activity of ALT | Mean ±S.D of AST | Mean ±S.D of ALT | Percent activation of AST | Percent activation of ALT | $Ac_{50}$ ± S.E. Mm of AST | $Ac_{50}$ ± S.E. Mm of ALT |
|---|---|---|---|---|---|---|---|---|---|
| Control | 1 | 15.76 | 20.12 | | | | | | |
| Control | 2 | 18.31 | 19.61 | 16.81±1.33 | 19.86±0.255 | | | | |
| Control | 3 | 16.36 | 19.87 | | | | | | |
| 0.5 | 1 | 19.4 | 21.42 | | | 18 | 7 | | |
| 0.5 | 2 | 20 | 21.29 | 19.88±0.44** | 21.33±0.075 | 18 | 7 | | |
| 0.5 | 3 | 20.25 | 21.29 | | | 18 | 7 | | |
| 1 | 1 | 24.28 | 23.89 | | | 47 | 18 | | |
| 1 | 2 | 25.19 | 23.5 | 24.75±0.46** | 23.58±0.27** | 47 | 18 | | |
| 1 | 3 | 24.8 | 23.37 | | | 47 | 18 | | |
| 2.5 | 1 | 30.64 | 28.18 | | | 79 | 44 | 1.138±0.270 | 3.00 ± 1.640 |
| 2.5 | 2 | 30.51 | 28.18 | 30.12±0.78** | 28.57±0.47** | 79 | 44 | | |
| 2.5 | 3 | 29.22 | 28.44 | | | 79 | 44 | | |
| 5 | 1 | 45.19 | 44.67 | | | 171 | 128 | | |
| 5 | 2 | 46.1 | 44.8 | 45.58±0.47** | 45.45±1.24** | 171 | 128 | | |
| 5 | 3 | 45.45 | 46.88 | | | 171 | 128 | | |
| 10 | 1 | 57.4 | 52.2 | | | 242 | 175 | | |
| 10 | 2 | 57.14 | 57.14 | 57.61±0.61** | 54.67±2.47** | 242 | 175 | | |
| 10 | 3 | 58.31 | 54.67 | | | 242 | 175 | | |

Data represents Mean ± S.D. of three biological replicates from each group. **P<0.01 significantly different from control.

**Table 3: *In vitro* toxicity of Liver AST and ALT in rat exposed to Nanomaterial**

| Conc. mM | Rat No. | Activity of AST | Activity of ALT | Mean±S.D of AST | Mean±S.D of ALT | Percent activation of AST | Percent activation of ALT | $Ac_{50}$ ± S.E. mM of AST | $Ac_{50}$ ± S.E. mM of ALT |
|---|---|---|---|---|---|---|---|---|---|
| Control | 1 | 13.54 | 20.45 | | | | | | |
| Control | 2 | 13.45 | 20.11 | 13.54±0.09 | 20.45±0.35 | | | | |
| Control | 3 | 13.63 | 20.81 | | | | | | |
| 0.5 | 1 | 20.45 | 20.36 | | | 57 | 12 | | |
| 0.5 | 2 | 21.59 | 20.1 | 21.30±0.75** | 20.91±1.18** | 57 | 12 | | |
| 0.5 | 3 | 21.87 | 22.27 | | | 57 | 12 | | |
| 1 | 1 | 23.72 | 23.45 | | | 78 | 17 | 0.273 ± 0.405 | 3.217 ±1.10 |
| 1 | 2 | 24 | 25.36 | 23.63±0.42** | 23.91±1.19** | 78 | 17 | | |
| 1 | 3 | 23.18 | 23.18 | | | 78 | 17 | | |
| 5 | 1 | 37.09 | 35.63 | | | 180 | 73 | | |
| 5 | 2 | 38.45 | 35.54 | 37.95±0.75** | 35.57±0.52** | 180 | 73 | | |
| 5 | 3 | 38.27 | 35.54 | | | 180 | 73 | | |
| 10 | 1 | 40.9 | 42.09 | | | 212 | 106 | | |
| 10 | 2 | 40.9 | 42.27 | 41.32±32** | 42.21±0.10** | 212 | 106 | | |
| 10 | 3 | 42.18 | 42.27 | | | 212 | 106 | | |

Data represents Mean ± S.D. of three biological replicates from each group. **P<0.01 significantly different from control.

**Table 4: Comparative Effect of cobalt NM in different tissues of Female Rats**

| PARAMETER | MAXIMUM PERCENT ACTIVITY | $IC_{50}$ ± S.E |
|---|---|---|
| SERUM ASAT | 149 | **< 1.00** |
| SERUM ALAT | 161 | **3.186 ± 1.20** |
| KIDNEY ASAT | 242 | **1.138 ± 0.270** |
| KIDNEY ALAT | 175 | **3.00 ± 1.640** |
| LIVER ASAT | 212 | **0.273 ± 0.405** |
| LIVER ALAT | 106 | **3.217 ± 1.10** |

## DISCUSSION AND CONCLUSION

The biochemical enzymes ASAT and ALAT increased significantly in serum, liver and kidney tissues of female rats exposed to cobalt NM. As in the present study, elevated levels

of ASAT and ALAT were reported in animals treated with toxic chemicals and certain toxic plant-derived products[17]. Transaminases are important class of enzymes linking carbohydrates and amino acids metabolism and these enzymes have established a relation between the intermediates of tricarboxylic acid (TCA) cycle. These enzymes increased in serum and simultaneously increased in tissues like liver and kidney, which might be due to increased permeability of plasma membrane. This is also suggestive of an increased synthesis of these enzymes as an adaptive mechanism due to the chemical stress.

The relative toxicity based on IC50 showed that liver ASAT was more potent in causing the toxicity followed by serum ASAT, kidney ASAT, kidney ALAT, serum ALAT and liver ALAT.

These results suggest that due to the *in-vitro* exposure of cobalt NM caused an alteration in the serum and also in cellular activities of vital organs like liver and kidney of exposed female rats, thus inducing changes in the physiology and metabolic activities of the individual.

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