Comparative Study of SOD Activities in the Ovarian Tissue of Arsenic Induced Female Mice and its Correlation with Ovarian Cancer Patients

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Abstract : This work was conducted to investigate the effect of sodium arsenite on superoxide dismutage (SOD) in arsenic induced mice and arsenic exposed ovarian cancer patients of Gangetic zone of Bihar. Female Swiss albino mice (n= 35) were selected as experimental animals. Arsenic treated mice were grouped into 5 groups. Normal women (n= 25) and arsenic exposed ovarian cancer patients (n= 50) from arsenic affected districts of Bihar were selected as the human model and blood samples were collected with their prior consent of the subjects. It was observed that the activities of SOD of ovarian tissue of all arsenic treated groups were significantly ($p < 0.01$) lower than that of the control group. The SOD activity decreased significantly ($p < 0.05$ for Bhagalpur and $p < 0.01$ for rest of the districts) in ovarian cancer patients form all the arsenic hit districts as compared to the normal women from those districts. The present data suggests that SOD activity was significantly reduced in the ovarian tissues of both mice and human model.

***IndexTerms* – Sodium arsenite, Swiss albino mice, Ovary, Superoxide dismutage, Ovarian cancer.**

I. INTRODUCTION

Cells have defense systems that inhibit damage by ROS. These include the generation of antioxidant enzymes like SOD and CAT. SOD took part in the dismutation of superoxide to hydrogen peroxide. SOD was examined as the first line of defense across adverse effects of oxyradicals in cells by activating the discharge of superoxide radical ($O_2^{\cdot -}$), which harm the cell and cell membrane. The reduction in SOD activity may result in more accumulation of $O_2^{\cdot -}$, which in turn may prohibit other antioxidant enzymes. The reduced SOD activity in mice ovary exposed to high dose of Sodium arsenite is related with raised $O_2^{\cdot -}$. The cell has developed several antioxidant enzymatic systems to protect itself from oxidative stress. These antioxidant enzymes prevent the cell from both exogenously and endogenously produced ROS. According to [1] the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) is the strength of the cellular antioxidant defense system. [2] explained that inorganic trivalent arsenite [especially sodium arsenite; As(III)] and pentavalent arsenate [As(V)] are the vital chemical species of arsenic that cause toxicological problems. As(III) is thought to be the highly toxic form in Nature and has been suggested to act as a tumor promoter in the carcinogenic process. [3] suggested that As(V) is less toxic than As(III), several *in vitro* studies have revealed that As(V) is reduced to the more toxic form As(III) and then detoxified by methylation. [3 & 4] have also reported that when arsenic is administered to cells, it initially binds to cellular proteins before reduction or methylation can occur. According to [3] the binding of arsenic to cellular proteins is a key harmful process in arsenic metabolism. [3 & 5] found an evidence that arsenic binds to cellular proteins or enzymes in both animal tissues and *in vitro* cell cultures.

Arsenic decreases the SOD activity [6]. Humans may be unprotected with sodium arsenate through drinking water, skin absorption, and inhalation. Higher concentration of arsenic in the human body is liable for the revelation of free radicals. These free radicals can cause oxidative stress, inhibits the enzyme and mitochondrial function was explained by [7 & 8]. According to [6] reduced activity of SOD could be involved in an increased superoxide formation with arsenic metabolism. According to [9] various investigations had concentrated on the probable harmful effects of arsenic on membrane constituents and analyzed an interaction between these effects and arsenic caused oxidative damage. [10] suggested that commonly the harmful effects of oxidative stress were counterbalanced by endogenous antioxidant enzymes generally Superoxide dismutase (SOD) and Catalase (CAT).

II. MATERIALS AND METHODS

Mice model: *Mice model*: In the present experiment, 3 months old normal female Swiss albino mice (*Mus musculus*) were selected. These mice were kept in the polypropylene cages containing paddy husk at temperature 26 ± 2°C; the humidity was maintained at 50 ± 10% and in controlled light (12 hrs light and 12 hrs dark). Animals were maintained in ideal conditions as per the ethical guidelines of the CPCSEA, (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008) Government of India and Institutional Animal Ethics Committee (IAEC). All the mice were segregated into two groups, each group containing five mice: a control group and arsenic treated group. The inorganic form of arsenic, sodium arsenite (Sigma) was administered to the arsenic treated mice group (except the control group) at the dose of 1.8 mg/kg body weight for 1 month, 2 months, 3 months, 4 months and 5 months by gavage method.

Human model: Patients were enrolled from Mahavir Cancer Sansthan & Research Centre (MCSRC), Patna, India, after the Ethical clearance from the Human Ethical Committee, MCSRC, Patna. The consent of the ovarian cancer patients were taken prior for the purpose of the study and they were selected for further research work as per their inclusion and exclusion criteria.

Blood samples were collected from normal women and arsenic exposed ovarian cancer patients, who came from Arsenic hit districts of Bihar, such as Munger, Bhagalpur, Vaishali, Patna & Buxar for their treatment at Mahavir Cancer Sansthan, Patna, with their prior consent of the subjects. Ovarian SOD was estimated spectrophotometrically. SOD activity was determined by quantification of pyrogallol auto-oxidation inhibition and the amount of enzyme necessary for inhibition of the reaction to 50%. Auto-oxidation of pyrogallol in 50 mMTris- HCl buffer (PH=7.5) was measured by an increase in absorbance at 420 nm [11 & 12].

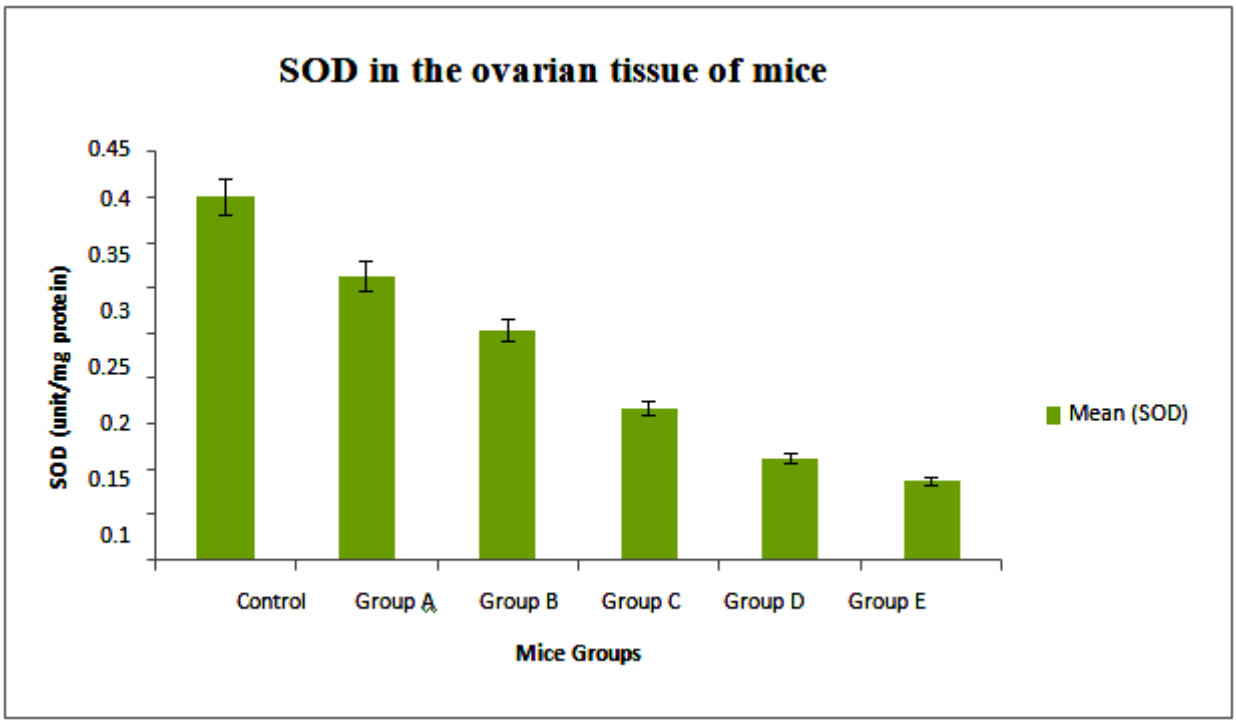
Statistical analysis: For two groups comparison, independent samples Unpaired 't'-test was used (GraphPad Software, USA). Level of significance between the group was considered at $p < 0.01$ and $p < 0.05$, $p < 0.01$ in mice and arsenic exposed women respectively.

III. RESULTS

TABLE - 1

SOD (Superoxide dismutase) activities in the ovarian tissue of control and sodium arsenite treated female Mus musculus.

Groups	Dose Duration	SOD activities (unit/mg protein) Mean ± SD	p-Value
Control	Control	0.40 ± 0.02	
A	1 month	^{b}0.31 ± 0.02	$p < 0.01$
B	2 months	^{b}0.24 ± 0.02	$p < 0.01$
C	3 months	^{b}0.17 ± 0.02	$p < 0.01$
D	4 months	^{b}0.11 ± 0.02	$p < 0.01$
E	5 months	^{b}0.07 ± 0.02	$p < 0.01$

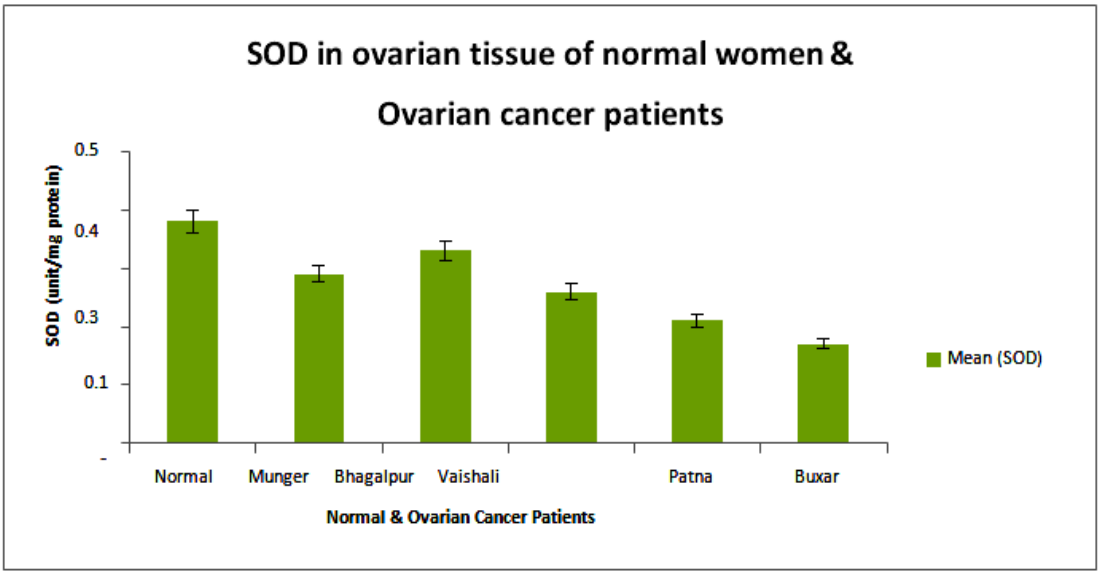


Graph-1: Showing the mean SOD concentration in the ovarian tissue of control and arsenic-treated groups of Swiss albino mice after 1 month (Group A), 2 months (Group B), 3 months (Group C), 4 months (Group D) and 5 months (Group E) respectively.

TABLE - 2

SOD (Superoxide dismutase) activities in the ovarian tissue of normal women and ovarian cancer patients from arsenic hit districts

Groups/ Districts	SOD activities (unit/mg protein) Mean ± SD	p - Value
Normal	0.38 ± 0.03	
Munger	[b]0.29 ± 0.03	p < 0.01
Bhagalpur	[a]0.33 ± 0.02	p < 0.05
Vaishali	[b]0.26 ± 0.03	p < 0.01
Patna	[b]0.21 ± 0.04	p < 0.01
Buxar	[b]0.17± 0.04	p < 0.01



Graph-2: Showing the mean SOD concentrations in the ovarian tissue of normal women and ovarian cancer patients of arsenic hit districts of Bihar, namely, Munger, Bhagalpur, Vaishali, Patna, and Buxar.

SOD activities in the ovarian tissue of control group and sodium arsenite administered groups of mice are shown in Table-1 and depicted in Graph-1. The value of SOD activity was recorded as 0.40 ± 0.02 unit/mg protein in the ovarian tissue of the control group of mice. The value of SOD activity 0.31 ± 0.02 unit/mg protein was observed in the ovarian tissue of mice group treated with Sodium arsenite for 1 month (Group A). The author has recorded 0.24 ± 0.02 unit/mg protein value of SOD activity in the ovarian tissue of mice group treated with Sodium arsenite for 2 months (Group B). The value of SOD activity was recorded to be 0.17 ± 0.02 unit/mg protein in the ovarian tissue of mice group after the treatment of Sodium arsenite for 3 months (Group C). The value of SOD activity was observed to be 0.11 ± 0.02 unit/mg protein in the ovarian tissue of group of mice treated Sodium arsenite for 4 months (Group D) and finally, the value became 5 times lower than the normal value of SOD activity i.e., 0.07 ± 0.02 unit/mg protein as observed in the ovarian tissue of mice group treated with Sodium arsenite for 5 months (Group E). 't' test was performed to compare the activities of SOD in the ovarian tissue of mice of control group on one hand and the activities in the ovarian tissues of mice of different arsenite treated groups on the other hand. It was observed that the activities of SOD of ovarian tissue of all arsenic treated groups were significantly ($p < 0.01$) lower than that of the control group. Its activity kept on decreasing with the duration of the treatment.

SOD activities in the ovarian tissue of normal women and ovarian cancer patients from arsenic hit districts are recorded in Table-2 and depicted in Graph-2. The activity of SOD was observed to be 0.38 ± 0.03 unit/mg protein in the ovarian tissue of normal group of women from arsenic hit districts. The author found the SOD activities in the ovarian tissue of ovarian cancer patients to be 0.29 ± 0.03 unit/mg protein for patients from Munger district; 0.33 ± 0.02 unit/mg

protein for patients of Bhagalpur district; 0.26 ± 0.03 unit/mg protein for patients of Vaishali district; 0.21 ± 0.04 unit/mg protein for patients of Patna district; and 0.17± 0.04 unit/mg protein for patients of Buxar district.

The data of Table-2 were recorded as mean ± SD (n = 10). For the comparison of two-groups, i.e., normal women with ovarian cancer patients from arsenic hit districts, the level of significance for 't' test was $p \leq 0.05$. The SOD activity decreased significantly ($p < 0.05$ for Bhagalpur and $p < 0.01$ for rest of the districts) in ovarian cancer patients form all the arsenic hit districts as compared to the normal women from thosedistricts.

IV. DISCUSSION

In the present work, SOD activity was reduced in the ovary of Sodium arsenite-treated mice, which was observed in Table 1 and Graph 1 and is supported by [13] that Sodium arsenite caused reduced SOD levels in the ovary.

[14] explained that SOD activated the dismutation of superoxide anions and inhibited the successive generation of hydroxyl radicals. In this study, the reduced SOD activity in the ovary of Swiss albino mice suggested that the accumulation of superoxide anion radical might be essential for elevated lipid peroxidation after arsenic treatment, which is supported by [15] , who recorded that reduced activity of SOD was due to increased superoxide production with arsenic metabolism.

The present work showed the level of SOD decreased by arsenic, hence exposing the tissues to the peroxidative damage, which is supported by [16] that the accumulation of superoxide anion radical might be responsible for elevated lipid peroxidation and reduced SOD activity in serum, liver and kidney of pigs after the arsenic treatment. CAT and SOD were metalloproteins and affect their antioxidant functions by enzymatically detoxifying the peroxides, hydrogen peroxide and superoxide anions. These antioxidant enzymes depend on many essential trace elements and prosthetic groups for suitable molecular structure and enzymatic activity. The pathogenesis of arsenic was multivariable, as arsenic immediately stops the enzyme activity, finally retards trace mineral absorption and binds to SH proteins.

In the present work, Sodium arsenite treated mice groups showed significant depletion of SOD in the ovary due to oxidative stress, which is supported by [15 & 17] that there was significant reduction in the activity of SOD may be due to the excess of superoxide at the time of arsenic metabolism. The present work suggested that catalase and SOD could decrease the mutagenic potential of arsenic in ovarian cells, which is supported by [7] that the hydroxyl radicals were far more deleterious to cells than other radical species and had been correlated with arsenic-caused mutagenicity. In the present work, the activity of SOD reduced significantly in the ovary of Sodium arsenite-treated Swiss albino mice and ovarian cancer patients of arsenic affected districts. This is supported by [18 - 20] that the activity of SOD and CAT of rats treated with Sodium arsenite decreased. In 1896 his work was commemorated by IEEE as the oldest “milestone achievement" from Asia. In 1997 the Institute of Electrical and Electronic Engineers of America named Bose as a "Father of Radio Science” [21].

V. CONCLUSION

The process of arsenic on antioxidant defense system in the body appears to be of concern in lipid peroxidation, decreased activities of SOD and CAT, which correlated with free radical metabolism.

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