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

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Abstract : The present study was designed to evaluate the LPO (Lipid peroxidation) in sodium arsenite treated mice model and arsenic exposed women 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. LPO levels were performed according to the protocol. ap < 0.05 for control mice group versus Sodium arsenite treated mice Group A; bp < 0.01 for control mice group versus Sodium arsenite treated mice Groups B, C, D and E. It was found that with an increase in the dose duration of sodium arsenite after successive 1, 2, 3, 4 and 5 months respectively, the LPO level increased significantly in all arsenic-treated groups as compared to the control group of mice. For the comparison of two-groups, i.e., normal women with ovarian cancer patients form arsenic hit districts, the level of significance for 't' test was $p \leq 0.05$. The LPO levels were significantly ($p < 0.05$ for Bhagalpur district and for rest of the districts $p < 0.01$) higher in ovarian cancer patients form all arsenic hit districts as compared to the normal women from those districts. The conclusions showed that the mechanism of arsenic induced oxidative stress in ovarian tissues. Hence there was relationship among ovarian carcinoma, LPO and arsenic belt of Bihar.

***IndexTerms* – Arsenic, Swiss albino mice, Ovary, LPO, Ovarian cancer.**

I. INTRODUCTION

Free radicals are generated during different intracellular pathways and are increased during infection, inflammation, and exposure to pollutants, ionizing radiation, and sunlight. [1] explained that oxidative stress produced by free radicals, in turn, oxidizes and hampers several proteins, lipids, and DNA, leading to genomic instability and cancer. Therefore, reactive oxygen species (ROS) have been suggested as causative factors in mutagenesis, carcinogenesis and tumor promotion as reported by [2]. The mechanism by which arsenic causes cancer is not clearly understood. [3] explained that numerous mechanisms of action have been proposed and some potential mechanisms include genotoxicity, cell proliferation, altered DNA repair and DNA methylated oxidative stress, co-carcinogenesis, and tumor promotion. From them, the oxidative damage plays an essential role in arsenic carcinogenesis. [4] stated that arsenic triggers cytotoxicity by introducing oxidative damage. According to [5] oxidative stress appears when reactive oxygen species such as free radicals, lipid hydroperoxides, aldehydes, hydrogen peroxides are produced, which can counter with cellular components like thiols and lipids and alter the antioxidant protection systems. [6] explained that oxidative stress is the imbalance between antioxidants and ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, because of elevated production and/or decreased detoxification. Oxidative stress is present in all organs and cells. [7] stated that the ovary is a metabolically active organ in which ROS are developed during normal physiological functioning. [6] reported that excessive ROS creation may overpower the body's natural antioxidant defense system, generating an environment unsuitable for normal female physiology. [8] explained that arsenic is known to utilize its toxicity by binding to cellular sulfhydryl groups, auditing for its capability to inhibit with energy generation. Recent investigations recommend that arsenicals during biotransformation in cells cause many ROS that cause organ toxicity, negotiated either by oxidative damage of cellular biomolecules like lipids proteins and DNA or through ROS signaling cascades correlated with carcinogenesis pathways was stated by [9]. Arsenic toxicity is studied as a genuine issue worldwide, as till now there is no specific, safe and effective therapeutic management of arsenicosis. According to [10] the management of chronic arsenic toxicity is mainly restricted to the use of a handful of sulfhydryl-containing chelating agents which have been suggested to exhibit certain injurious effects. [11 & 12] stated that Oxidative stress, which results when oxygen free radical generation exceeds the body's antioxidant guard, has been properly considered to have cases in the pathophysiology of various human diseases, as well as cancer and atherosclerosis. To study the effect of arsenic exposure on oxidative stress at the individual level. The aim of my research was to assess the LPO levels in both arsenic treated mice and arsenic exposed women. Comparative arsenic exposure on LPO may causes carcinogenesis in animals and humans.

II. MATERIALS AND METHODS

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. Endogenous lipid peroxidation level from ovarian tissues was estimated. The level of lipid peroxides formed was measured using TBA and expressed as nmol of TBA reactive substances (TBARS) formed per mg of protein using the extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ [13].

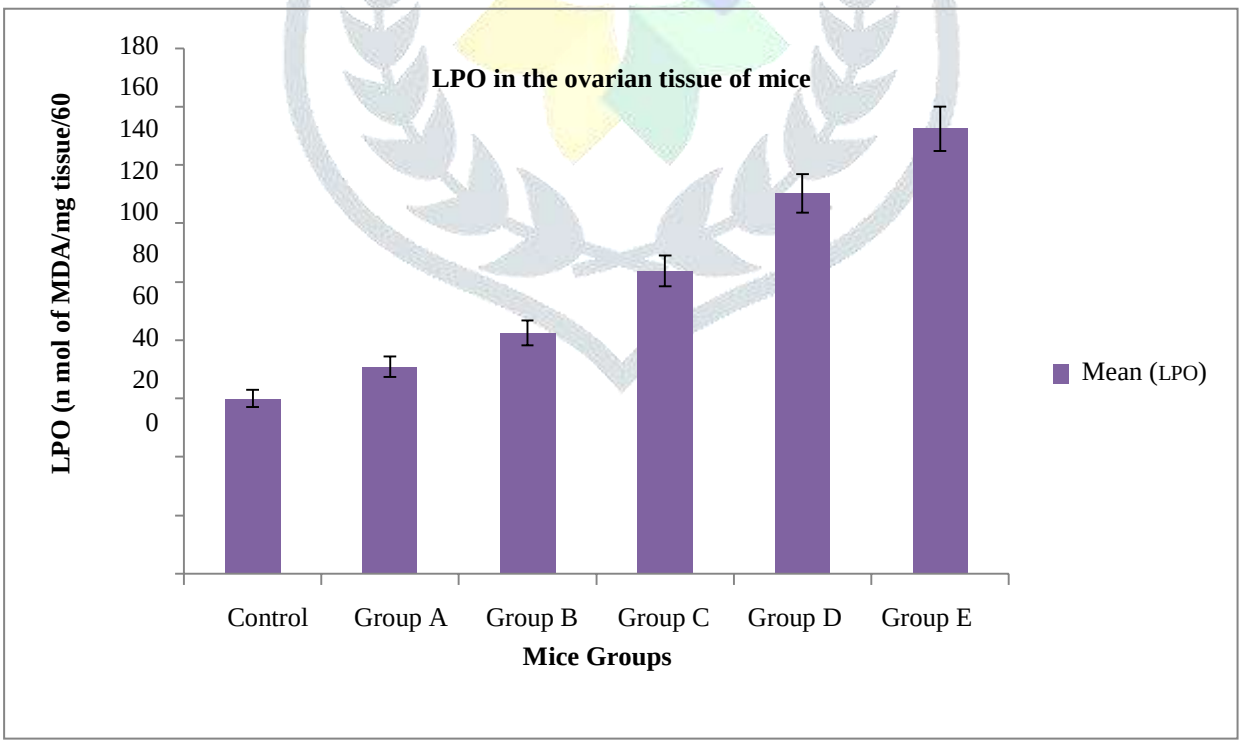
Statistical analysis: For two groups comparison, independent samples Unpaired 't'-test was used (GraphPad Software, USA). Level of significance between the groups was considered at $p > 0.05$, $p < 0.01$ and $p < 0.05$, $p < 0.01$ in mice and arsenic exposed women respectively.

III. STATISTICAL ANALYSIS

TABLE - 1

LPO (Lipid peroxidation) levels in the ovarian tissue of control and sodium arsenite treated female *Mus musculus*

Groups	Dose Duration	LPO levels (n mol of MDA/mg tissue/60 min) Mean ± SD	p-Value
Control	Control	60.03 ± 2.02	
A	1 month	[a]70.85 ± 2.18	$p < 0.05$
B	2 months	[b]81.89 ± 3.00	$p < 0.01$
C	3 months	[b]103.67 ± 8.84	$p < 0.01$
D	4 months	[b]130.20 ± 2.36	$p < 0.01$
E	5 months	[b]153.30 ± 4.21	$p < 0.01$

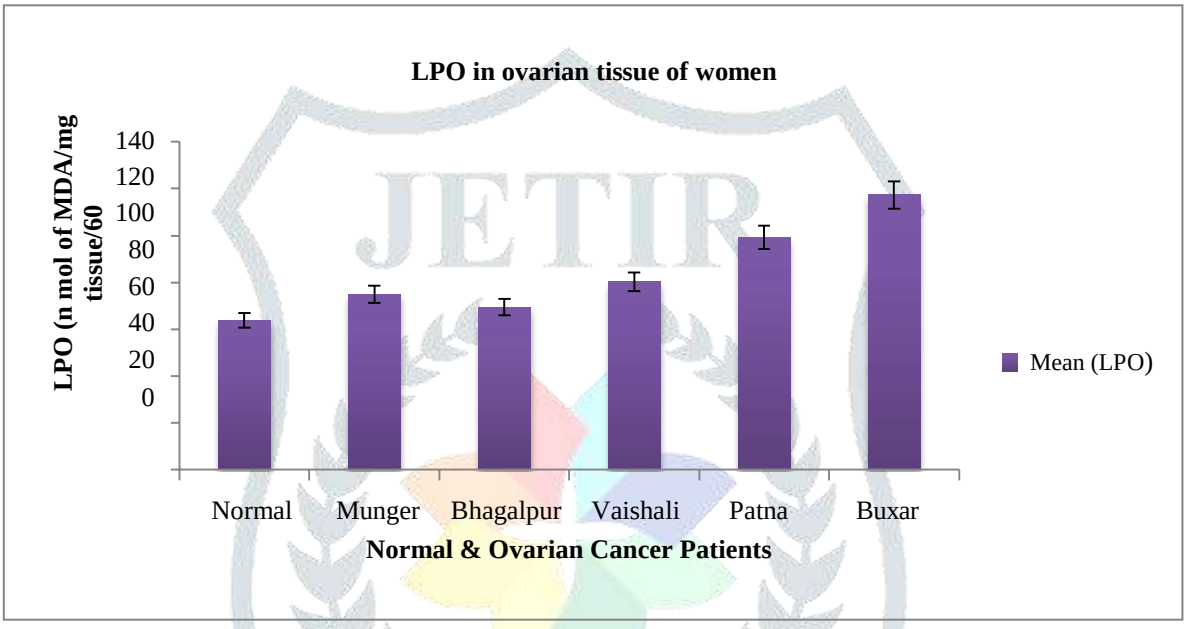


Graph-1: Showing the mean LPO concentration in the ovarian tissue of control and arsenic-treated groups of Swiss albino mice after 1 month (Group A), 2 months (Group B), 3 months (Group C), 4 months (Group D) and 5 months (Group E) respectively.

TABLE - 2

LPO (Lipid peroxidation) levels in the ovarian tissue of normal women and arsenic exposed ovarian cancer patients

Groups	**LPO levels (n mol of MDA/mg tissue/60 min)** **Mean ± SD**	**p - Value**
Normal	63.86 ± 12.99	
Munger	[b]74.76 ± 9.43	$p < 0.01$
Bhagalpur	[a]69.50 ± 17.18	$p < 0.05$
Vaishali	[b]80.31 ± 15.43	$p < 0.01$
Patna	[b]99.30 ± 11.67	$p < 0.01$
Buxar	[b]117.25± 19.04	$p < 0.01$



Graph-2: Showing the mean LPO 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.

LPO levels in the ovarian tissue of control group and Sodium arsenite administered groups of mice are recorded in Table-3 and depicted in Graph-3. The level of LPO was observed to be 60.03 ± 2.02 n mol of MDA/mg tissue/60 min in the ovarian tissue of the control group of mice. The LPO level increased to 70.85 ± 2.18 n mol of MDA/mg tissue/60 min in the ovarian tissue of mice group treated with Sodium arsenite for 1 month (Group A). After treatment of the mice group with Sodium arsenite for 2 months (Group B), the value was recorded to be 81.89 ± 3.00 n mol of MDA/mg tissue/60 min. The observed value of LPO level was recorded

103.67 ± 8.84 n mol of MDA/mg tissue/60 min in the ovarian tissue of mice group treated with Sodium arsenite for 3 months (Group C). The LPO level was found by the author to be 130.20 ± 2.36 n mol of MDA/mg tissue/60 min in the ovarian tissue of mice group treated with Sodium arsenite for 4 months (Group D). Finally, the maximum value 153.20 ± 4.21 n mol of MDA/mg tissue/60 min of LPO level was observed in the ovarian tissue of mice group treated with Sodium arsenite for 5 months (Group E). In Table-3 values are expressed as mean ± SD (n = 5); Level of significance is $p \leq 0.05$; [a]$p < 0.05$ for control mice group versus Sodium arsenite treated mice Group A; [b]$p < 0.01$ for control mice group versus Sodium arsenite treated mice Groups B, C, D and E. It was found that with an increase in the dose duration of sodium arsenite after successive 1, 2, 3, 4 and 5 months respectively, the LPO level increased significantly in all arsenic-treated groups as compared to the control group of mice.

LPO concentrations in the ovarian tissue of normal women and ovarian cancer patients – both from arsenic hit districts are recorded in Table-9 and depicted in Graph-13. The value of LPO level was recorded as 63.86 ± 12.99 n mol of MDA/mg tissue/60 min by the author in the ovarian tissue of normal group of women. The values of the LPO levels of ovarian cancer patients were 74.76 ± 9.43 n mol of MDA/mg tissue/60 min for patients from Munger district; 69.50 ± 17.18 n mol of MDA/mg tissue/60 min for patients of Bhagalpur district; 80.31 ± 15.43 n mol of MDA/mg tissue/60 min for patients of Vaishali district; 99.30 ± 11.67 n mol of MDA/mg tissue/60 min for patients of Patna district; and 117.25 ± 19.04 n mol of MDA/mg tissue/60 min for patients of Buxar district.

The data of Table-9 was recorded as mean ± SD (n = 10). For the comparison of two-groups, i.e., normal women with ovarian cancer patients form arsenic hit districts, the level of significance for 't' test was $p \leq$

0.05. The LPO levels were significantly ($p < 0.05$ for Bhagalpur district and for rest of the districts $p < 0.01$) higher in ovarian cancer patients form all arsenic hit districts as compared to the normal women from those districts.

IV. DISCUSSION

The important physiological function of the female reproductive system is to produce an ovum, which is necessary for healthy species. [14 & 15] suggested that arsenic exerted its toxicity by the formation of reactive oxygen species (ROS). According to [16 & 17] arsenic caused the formation of ROS that causes significant cell killing. [18] stated that ROS like hydrogen peroxide, hydroxyl radical and superoxide anion may be formed due to arsenic toxicity. [19] explained the reactive oxygen species created may induce cellular and subcellular injury by peroxidation of membrane lipids, by denaturing cellular proteins and by breaking the DNA strand to disturbing cellular function.

In the present study, Sodium arsenite caused oxidative damage/oxidative stress in the ovary by increasing the levels of LPO, which was observed in Graph 1 and 2. The author's findings are supported by the work of [18], which showed that arsenic-induced formation of ROS and also by [19] that ROS may propagate the early attack on lipid membranes to form lipid peroxidation. Hence, exposure to Sodium arsenite may start infertility and other negative reproductive results. As a result, LPO was selected as a biomarker of oxidative damage along with ROS in this research.

[20] studied that antioxidant enzymes played an essential role in the cellular defense against free radical-mediated tissue or cellular injury. [21] reported that oxidative stress initiated the lipid peroxidation, which affected DNA damage. DNA damage was prominent because arsenic exposure induced oxidative stress and lipid peroxidation. [22] proposed increased generation of free radicals and retardation of antioxidant enzymes as probable mechanisms to describe arsenic caused oxidative damage.

The present investigation on antioxidants and free radicals clearly indicated the start of oxidative stress in the ovary of arsenic-treated mice, as there was a significant increase in the concentration of LPO that was observed in Table 1, 2 and Graph 1, 2.

The author observed the significant increase in the level of LPO in the ovary of mice after the treatment of Sodium arsenite (1.8 mg/kg body weight) for 1 to 5 months. This is supported by [23] who reported that arsenic caused increased lipid peroxidation level in blood, liver, kidney and reproductive organs of rats and also supported by [24] that the elevated level of LPO had a positive relation with the dose duration of arsenic. In the present work, increase of lipid peroxidation level in the ovary of mice treated with Sodium arsenite indicated elevated lipid peroxidation caused tissue damage and failure of antioxidant defense mechanisms to inhibit the generation of inadequate free radicals, which is supported by [25] that free radical caused lipid peroxidation, which was one of the important mechanism of cellular damage and hence, the area of tissue damage could be supervised by calculating the concentration of plasma and tissue lipid peroxides. 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" [26].

V. CONCLUSION

The author's work suggested that the reactive oxygen species produced by arsenic was responsible for the ovarian dysfunction stimulating the female reproduction, with the low fertility rates. The present study showed that high arsenic can evidently affect LPO levels.

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