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# LIPID PEROXIDATION INDUCED NUCLEIC ACID DAMAGE IN LIVER AND BRAIN OF BISPHENOL-A TREATED CHICK EMBRYOS

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**Abstract**

***A study was conducted on effect of Bisphenol-A (BPA) toxicity on nucleic acid damage during embryonic development in chicks. Bisphenol-A dissolved in distilled water in three concentrations of 100µM, 250µM and 500µM injected into the aircell separately to 11th and 14th day old chick embryos. Embryos were sacrificed after 24 and 48 hours of BPA exposure to collect liver and brain tissues for estimation of Thiobarbituric acid reacting substances (TBARS) and nucleic acids (DNA & RNA). A dose dependent decrease in DNA levels was observed in all treatment groups after 24 hours in both 11th and 14th day BPA treated chick embryos compared to control. A significant dose dependent decrease in RNA levels was observed after BPA exposure after 24 hours in both 11th and 14thday BPA treated chick embryos compared to control.***

***Key words: Brain, Liver, Lipid peroxidation, Nucleic acids, TBARS***

## I. INTRODUCTION

There has been increasing scientific concern since last two decades regarding, the adverse effects of chemical pollutants in the environment which interferes with normal functioning of different organs in animals and humans. Among endocrine disrupting chemicals, BPA is one of the most studied because of its extensive use (Gioiosa et al., 2013). The chemical bonds between BPA molecules are unstable and the chemical leaches into materials with time of storage and use. Studies in animals have shown that BPA exposure is associated with early puberty in females, low sperm counts, increased susceptibility to reproductive tract cancers and altered brain development in males and females (Newbold et al., 2009).

Bisphenol-A induced mullerian duct malformation in female quail embryos and feminization of the left testis in male chicken embryos (Berg et al., 2001). Highest concentrations of BPA was found in adipose tissue and liver (100%) followed by brain (70%) (Geens et al., 2011). There is evidence that several teratogens affect the developing embryo by increasing its oxidative stress, because of its relatively weak anti-oxidant defense especially at early stages of organogenesis, resulting in severe embryonic damage (Ornoy, 2007). It was reported that BPA exposure during embryonic/fetal life and infancy induces tissue oxidative stress and peroxidation, ultimately leading to underdevelopment of the brain, kidney and testis (Kabuto et al., 2004).

Oxidative stress influenced by excess reactive oxygen species (ROS) produced with BPA in mitochondria and microsome damage nucleic acids, lipids and proteins (Chitra et al., 2003). It binds covalently to DNA and induces modifications which leads to hepatotoxicity (Atkinson and Roy, 1995). Exposure to high concentration of BPA results in the induction of oxidative damage and also causes damage to the biological membranes and cellular structures in brain tissues of chicken embryos.

## II. MATERIAL AND METHODS

### A. Source of Fertilized Eggs and Incubation Conditions:

The present study was conducted at the Department of Veterinary Biochemistry, College of Veterinary Science, Tirupati. Freshly laid wild Bobcock strain zero day old fertilized eggs were procured from Department of Poultry Science, College of Veterinary Science, Tirupati. They were incubated at 37.5±0.5˚C with a relative humidity of 65% in an egg incubator.

### B. Experimental groups:

Group I : Control group (distilled water)
Group II : Bisphenol- A (100µM)
Group III : Bisphenol -A (250µM)
Group IV : Bisphenol -A (500µM)

The embryos were sacrificed after 24 and 48 hours for the collection of liver and brain tissue samples.

### C. Biochemical Analysis:

Thiobarbituric acid reacting substances in tissues were estimated by the method of Ohkawa et al. (1979).

### D. Estimation of Nucleic acids:

Maximum induction in TBARS observed in both liver and brain tissues after 24 hours in both $11^{th}$ and $14^{th}$ day of BPA treated chick embryos. Hence RNA and DNA estimations were carried out after 24 hours in both liver and brain tissues in $11^{th}$ and $14^{th}$ day BPA injected chick embryos. DNA and RNA contents were estimated by standard kits supplied by Himedia laboratories Pvt. Ltd., Mumbai using the following protocols.

### E. Statistical Analysis:

Statistical significance between the groups was analysed by one way ANOVA followed by Tukey's post–hoc test using statistical package for social sciences (SPSS 15.0 version).

## III. RESULTS

The results showed a significant dose dependent increase in TBARS in liver of both $11^{th}$ and $14^{th}$ day BPA treated chick embryos compared to control. Maximum induction was observed after 24 hours which was found to be nearly 2 and 2.5 fold in Group III & IV respectively in $11^{th}$ day treated embryos. In $14^{th}$ day BPA treated embryos, it was found to be 8%, 25%, 62% increase after 24 hours in Group II, III & IV respectively. The percentage of increase was decreased after 48 hours of treatment in liver of both $11^{th}$ and $14^{th}$ day treated embryos (Figs. 1&2).

Significant increase in TBARS was observed after 24 hours in $11^{th}$ day compared to $14^{th}$ day in brain of BPA treated embryos. It was found to be 45, 53 & 100% increase in brain of $11^{th}$ day compared to 20, 37 & 64% in $14^{th}$ day in Group II, III & IV respectively. Decreased production of TBARS observed with increase in growth of the embryo (Figs. 3&4).

Maximum reduction in DNA was noticed in $11^{th}$ day liver compared to $14^{th}$ day treated embryos (Table 1, Fig.5). A significant dose dependent reduction was noticed in brain of both $11^{th}$ and $14^{th}$ day BPA treated chick embryos compared to control (Table 2, Fig.6). Maximum reduction in DNA levels was observed in liver compared to brain tissue with BPA exposure.

A significant dose dependent decrease in RNA levels was observed in liver (Table 3, Fig.7) and brain (Table 4, Fig. 8) of both $11^{th}$ and $14^{th}$ day BPA treated chick embryos compared to control.

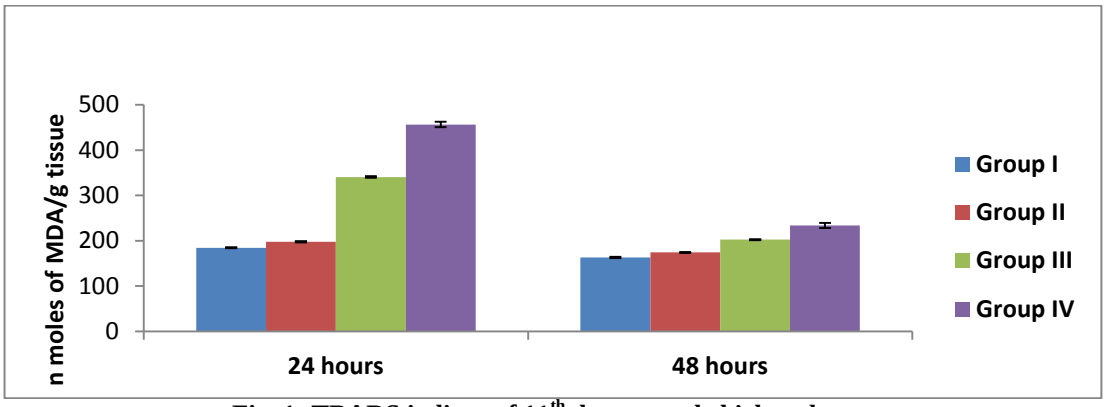


Fig. 1: TBARS in liver of 11th day treated chick embryos

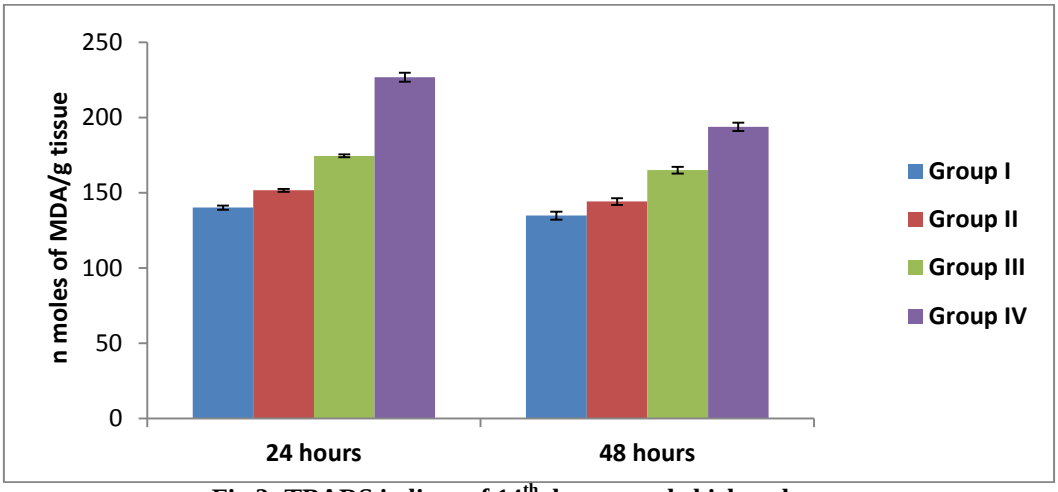


Fig.2: TBARS in liver of 14th day treated chick embryos

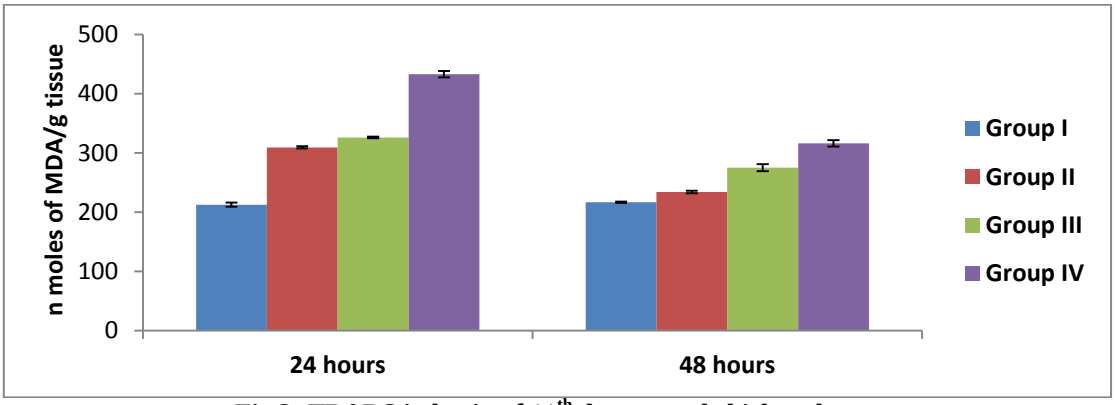


Fig.3: TBARS in brain of 11th day treated chick embryos

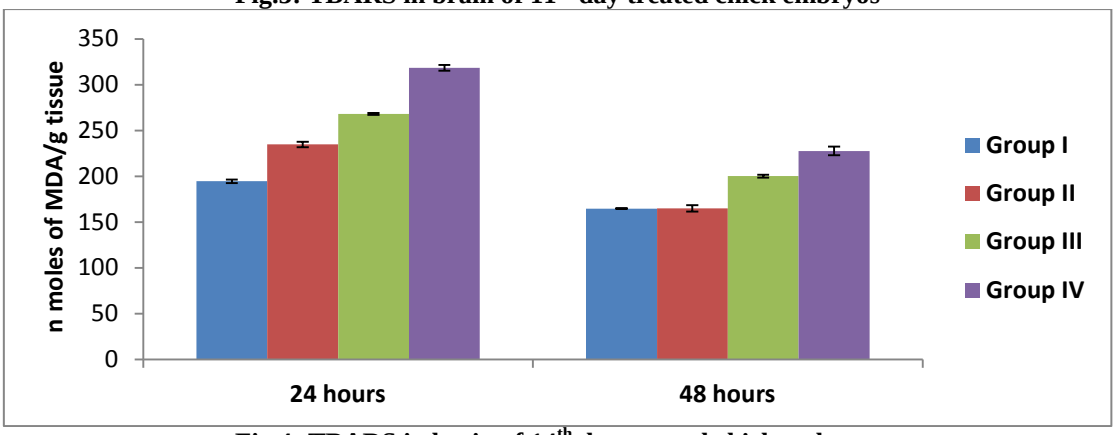


Fig.4: TBARS in brain of 14th day treated chick embryos

**Table 1: Mean values of DNA (µg/100 mg tissue) in liver tissue**

| Treatment | 11th day | 14th day |
|---|---|---|
| Group-I | 110.23 ± 0.50$^{d}$ | 112.89 ± 0.30$^{a}$ |
| Group-II | 102.41 ± 0.57$^{c}$ | 104.19 ± 0.42$^{b}$ |
| Group-III | 49.85 ± 3.44$^{b}$ | 69.95 ± 0.52$^{c}$ |
| Group-IV | 36.15 ± 0.32$^{a}$ | 56.08 ± 0.47$^{d}$ |
| df | (3, 20) | (3, 20) |
| F | 439.75 | 3881.25 |

**Table 2: Mean values of DNA (µg/100 mg tissue) in brain tissue**

| Treatment | 11th day | 14th day |
|---|---|---|
| Group-I | 95.75 ± 1.49$^{c}$ | 109.36 ± 0.31$^{a}$ |
| Group-II | 68.13 ± 12.59$^{b}$ | 86.84 ± 6.35$^{b}$ |
| Group-III | 75.09 ± 1.53$^{b}$ | 73.32 ± 3.45$^{c}$ |
| Group-IV | 33.15 ± 0.36$^{a}$ | 58.20 ± 0.43$^{d}$ |
| df | (3, 20) | (3, 20) |
| F | 16.62 | 35.85 |

**Table 3: Mean values of RNA (µg/100 mg tissue) in liver tissue**

| Treatment | 11th day | 14th day |
|---|---|---|
| Group-I | 333.67 ± 2.39$^{d}$ | 368.77 ± 4.63$^{d}$ |
| Group-II | 222.17 ± 1.38$^{c}$ | 285.63 ± 1.03$^{c}$ |
| Group-III | 210.90 ± 1.30$^{b}$ | 271.77 ± 0.86$^{b}$ |
| Group-IV | 199.07 ± 1.87$^{a}$ | 255.10 ± 1.40$^{a}$ |
| df | (3, 20) | (3, 20) |
| F | 1206.76 | 404.73 |

**Table 4: Mean values of RNA (µg/100 mg tissue) in brain tissue**

| Treatment | 11th day | 14th day |
|---|---|---|
| Group-I | 346.77 ± 2.69$^{d}$ | 369.40 ± 1.14$^{d}$ |
| Group-II | 280.37 ± 14.12$^{c}$ | 335.60 ± 1.57$^{c}$ |
| Group-III | 252.47 ± 1.16$^{b}$ | 281.53 ± 1.29$^{b}$ |
| Group-IV | 210.93 ± 1.86$^{a}$ | 207.23 ± 2.50$^{a}$ |
| df | (3, 20) | (3, 20) |
| F | 61.58 | 1711.14 |

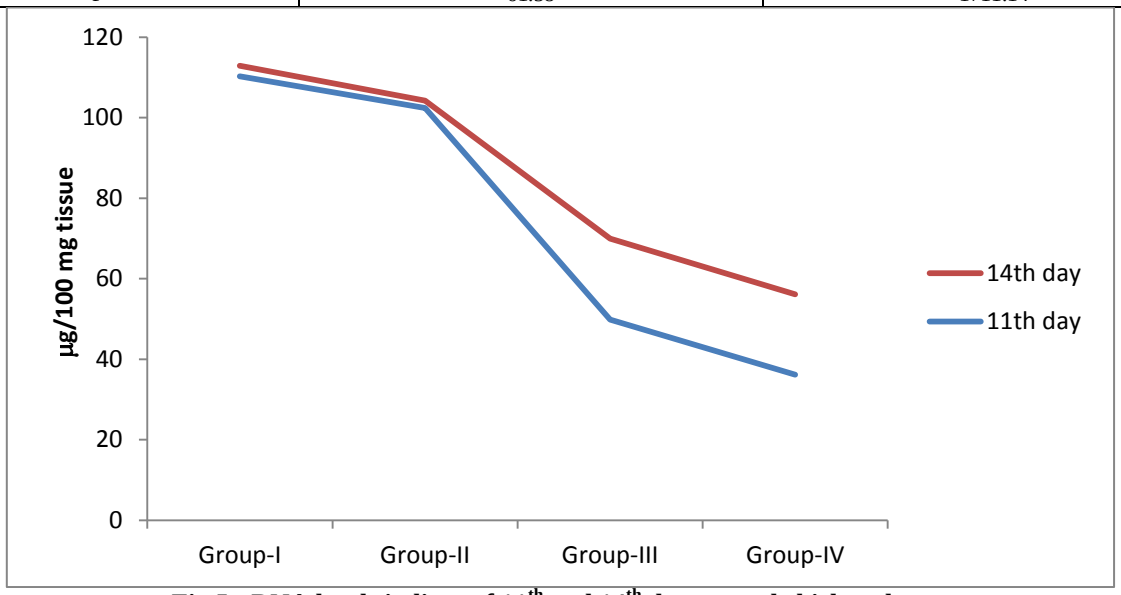


**Fig.5: DNA levels in liver of 11th and 14th day treated chick embryos**

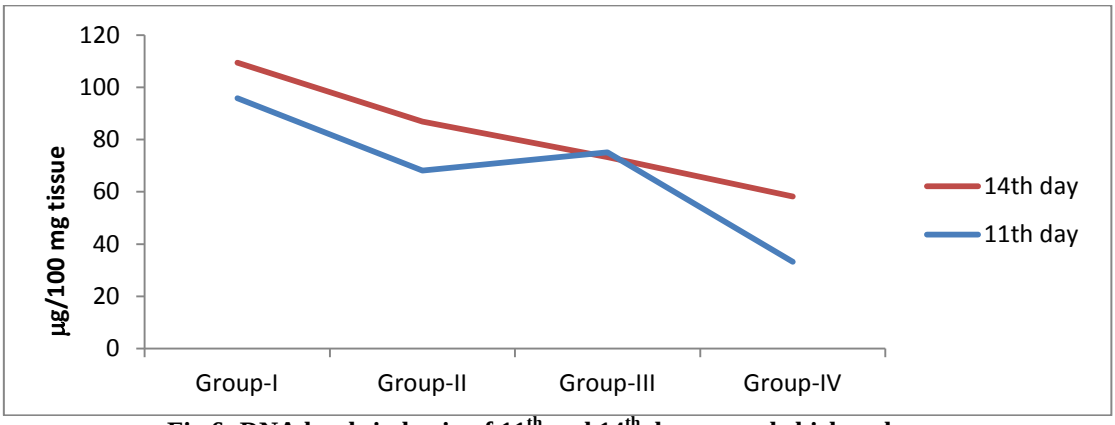


**Fig.6: DNA levels in brain of 11th and 14th day treated chick embryos**

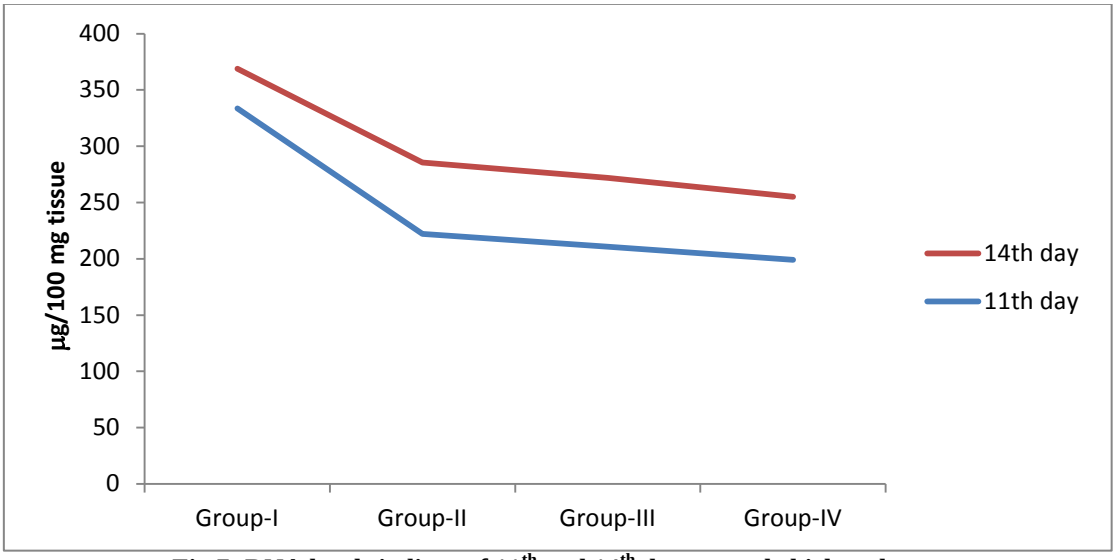


**Fig.7: RNA levels in liver of 11th and 14th day treated chick embryos**

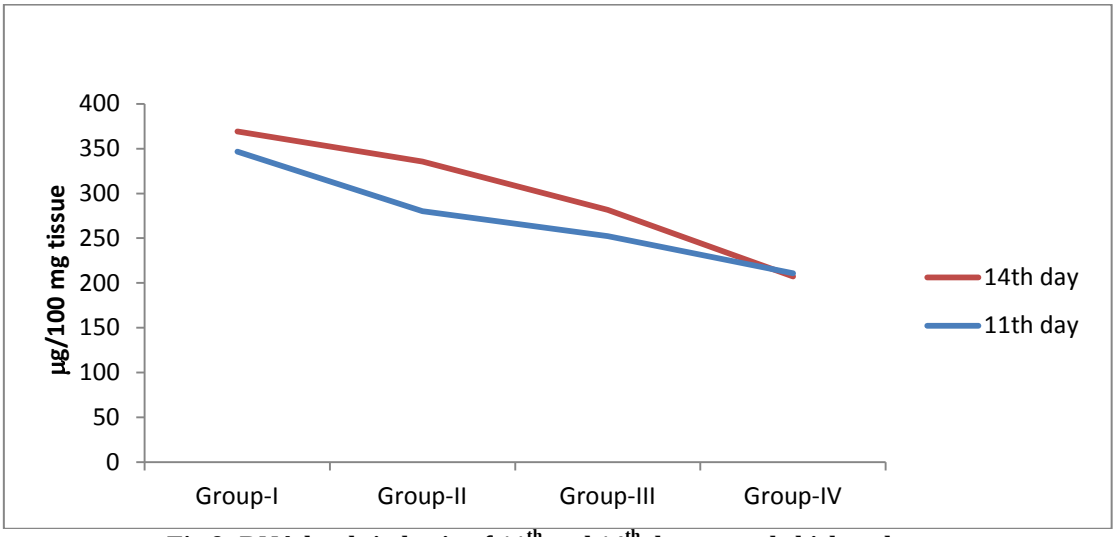


**Fig.8: RNA levels in brain of 11th and 14th day treated chick embryos**

## IV. DISCUSSION

Results of the present study showed significant dose dependent increase in TBARS after 24 hours compared to 48 hours of BPA treatment in both brain and liver tissues and hence further studies on nucleic acids were carried out after 24 hours of BPA exposure. Significant reduction in nucleic acids were observed after 24 hours both in liver and brain tissues of 11th and 14th day BPA

injected chick embryos. Earlier studies also proved that BPA shows potential genetic and embryo toxicity (Fic et al., 2013). Scientific evidence supports that BPA generates ROS during biotransformation which in turn react with DNA and cause DNA damage (Tsutsui et al., 1998).

The elevated TBARS after 24 hours may be responsible for reduction in RNA and DNA levels. In a similar manner a significant increase in DNA strand breakdown observed after 24 hours in BPA exposed cells (Orsolic et al., 2013). Earlier studies on BPA metabolites have shown to bind to DNA in a cellular system (Chen et al., 2002). One of the DNA binding metabolite both in vivo and in vitro may be Bisphenol-o-quinone complex and it was found that modification in DNA by in vivo exposure of BPA may be a factor in the induction of hepatotoxicity (Atkinson and Roy, 1995). These results are in agreement with Sangai and Verma (2012) in which Semiquinone binds with DNA and this interaction might prevent RNA polymerase transcribing the DNA and can inhibit the formation of m-RNA. Decline in nucleic acids may be due to DNA damage caused by the free radicals and inhibition of RNA by direct interaction of ROS (Tabassum et al., 2009).

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