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**Original Article**

# Variations in proteins and transaminases following experimental induction of Bisphenol A in mice

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**Abstract**
The current work was aimed to find the effect of Bisphenol A (BPA) which is widely used in the manufacturing of epoxy resins and variety of plastics to which humans are exposed routinely. BPA (12.5%) was injected in mice for consecutive 15 days while control animals were not exposed to any treatment (n=3). Serum samples were processed for the estimation of total protein and albumin and aminotransaminases activity. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was also run to analyze protein bands. Statistically significant increase in total protein contents, albumin and aminotransaminases were noted in sample in comparison with control. Comparative analysis between experimental and control samples revealed protein fractions (KDa) of 149, 132, 101, 72, 75 and (163, 114, 91, 75, 72, respectively. Conclusively, it can be stated that extensive use of BPA in various application cause serious health hazards, as noted by changes in serum proteins and transaminases. Therefore, further investigation of its toxicity is necessary in order to consider it safe.
**Key words:** Albumin, BPA, PAGE, transaminases, total protein

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## INTRODUCTION

Bisphenol A (BPA) is a colorless organic solvent, belongs to a class of diphenylmethane derivatives, routinely employed to make consumer goods including plastics (water bottles/ baby bottles, sports equipment, dental prostheses & sealants, CDs, & DVDs etc.) and epoxy resins which are being used as inside coatings of edible cans (Brotons *et al.,* 1995; Brede *et al.,* 2003). It also acts as antioxidant in some plasticizers (Erickson, 2008). Despite short half-life of BPA; effluent discharge from wastewater treatment plants, combustion of domestic waste and the natural breakdown of plastics are the most common modes of its emission in the environment (Sidhu *et al.,* 2005; Kinney *et al.,* 2006; Crain *et al.,* 2007; Kang *et al.,* 2007; Oehlmann *et al.,* 2009). Humans are highly exposed to BPA through diet which causes its ingestion through contaminated water and food (Vom and Hughes, 2005). It leached out from plastics when cleaned with harsh detergents or even under normal washings and sterilizing conditions (Wilson *et al.,* 2007). A considerable number of *in vivo and in vitro* studies have been conducted to examine the effects of BPA using different solvents, doses and routes of administration. Structural and functional changes in multiple organs have also been cited (Quesada *et al.,* 2002; Zoeller *et al.,* 2012). BPA, even in minimal dosage (10−9 mol) induced significant effects not only on structure & function but on behavior and memory processes in mice (Richter *et al.,* 2007; Jones and Miller, 2008). Ogiue-Ikeda *et al.* (2008) reported BPA causes hyperactivity as result of enhancement of dopamine action. Yang *et al*. (2009) reported oxidative damage in hepatic and extra hepatic organs in rodents. Furthermore, it was also found that reactive oxygen species (ROS) were elevated in some cell line, indicating its oxidative stress-related cytotoxicity. Diamanti and coworkers (2009) noted effect of BPA on Cardiovascular system

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(CV). Acute BPA exposure results in the development of arrhythmias while chronic exposure results in cardiac remodeling, atherosclerosis and hypertension. Soto *et al.* (2008) and Zhu *et al.* (2010) reported malfunctions of thyroid gland in neonates and children. Rezg *et al.* (2014) recently reported correlation of high levels of BPA with multiple disorders *viz.* obesity, diabetes, cardiovascular diseases, polycystic ovarian disease as well as low sperm count. In view of the foregoing literature cited, a considerable number of studies have been published on the effects of BPA exposure in experimental animals and in human subjects. To the best of our knowledge relatively very few is known regarding changes in transaminases and proteins of BPA induced animal models. The current work was aimed for this notion.

## MATERIALS AND METHODS

All chemicals, materials and detection systems (kits) used were of analytical grade and obtained from commercial sources as indicated: BPA (Lot# BCBJ1020V) from SIGMA® Life Sience, kits for the estimation of amino-transferases and albumin activities from Randox Laboratories, Ltd (U.K) and Protein ladder (Cat. # 10747012) from Life technologies.

### *Colonies of mice Animals*

Approximately 30 g of mice were used for the study and taken care according to the institutional guidelines. All the animals were acclimatized under standard laboratory condition for a period of 2 weeks before the commencement of the experiment.

### *Preparation of dose*

BPA was dissolved in alcohol which was then mixed with distilled water. Dose was so prepared that each 0.1ml of solution contained the 12.5% of BPA.

### *Experimental design*

The mice were randomly divided into two groups, a control (Co) and experimental (Exp) group (n=3). Experimental animals were subjected to i.p. injection for 15 days on daily basis. The animals were sacrificed on the16$^{th}$ day post treatment while control animals did not receive any treatment. All the animals were processed for extraction of serum as described elsewhere (Abbasi *et al*., 2015).

### *Estimation of clinical chemistry parameters*

Quantitative *in vitro* determination of alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total protein and albumin activities were determined according to the manufacturer's instructions.

### *One dimensional electrophoresis*

To resolve low molecular weight proteins 8% polyacramide gel was prepared and proceded as described elsewhere (Abbasi *et al*., 2015).

### *Statistical analysis*

Graph pad Prism 5 software (San Diego, USA) was used for the data analysis. Experimental errors are indicated as S.E.M. and statistical significance was calculated by *student's t test*. Significance was accepted at P<0.05.

## RESULTS

### *Total protein and albumin*

A statistically significant increase in total protein contents and albumin was found in the experimental group. Regarding total protein 84.72% rise in the value was noted compared to the control (P<0.01) and for albumin about 41.07% positive change was noted in BPA treated animals compared to control (P<0.01; Fig. 1).

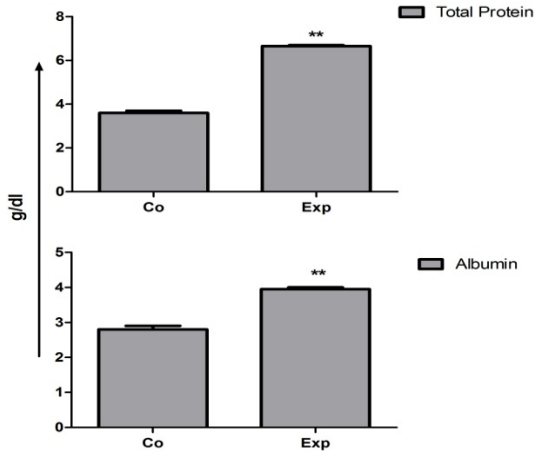


**Figure 1: Changes in Total protein and albumin concentration (g/dl). columns show averages with error bars showing the respective Standard Error of Mean (SEM). Statistically significant changes are marked with asterisks (*P<0.05, **P<0.01, ***P<0.001).**

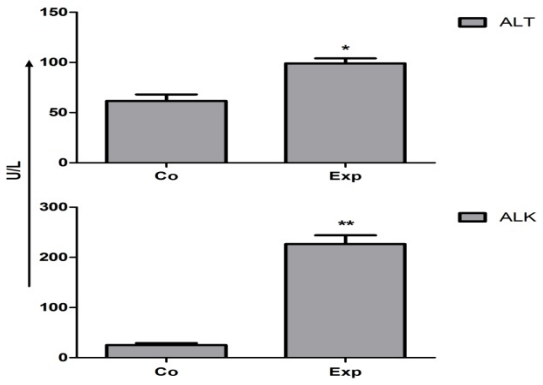


**Figure 2: Changes in serum tranaminsease (U/L) in ALT and ALP. Columns show averages with error bars showing the respective Standard Error of Mean (S.E.M.).Statistically significant changes are marked with asterisks (*P<0.05, **P<0.01, ***P<0.001).**

***Transaminases***

A profound elevation in the serum level of hepatic enzymes ALT (60.97%) and ALP (800%) were noted in the BPA induced experimental group compared to control (P<0.05, <0.01; Fig. 2).

***SDS-PAGE analysis***

Figure 3 showed analysis of protein variation and their densities. Dense bands were observed in experimental group as compared to respective bands in control sample. Protein fractions ranged from 72 KDa to 149 KDa was seen against protein ladder (10-220 KDa). Comparative analysis revealed protein fractions of 149 KDa, 132 KDa, 101 KDa, 72KDa, 75kDa in BPA treated animals sera compared to control (163 KDa, 114KDa, 91KDa, 75KDa, 72KDa).

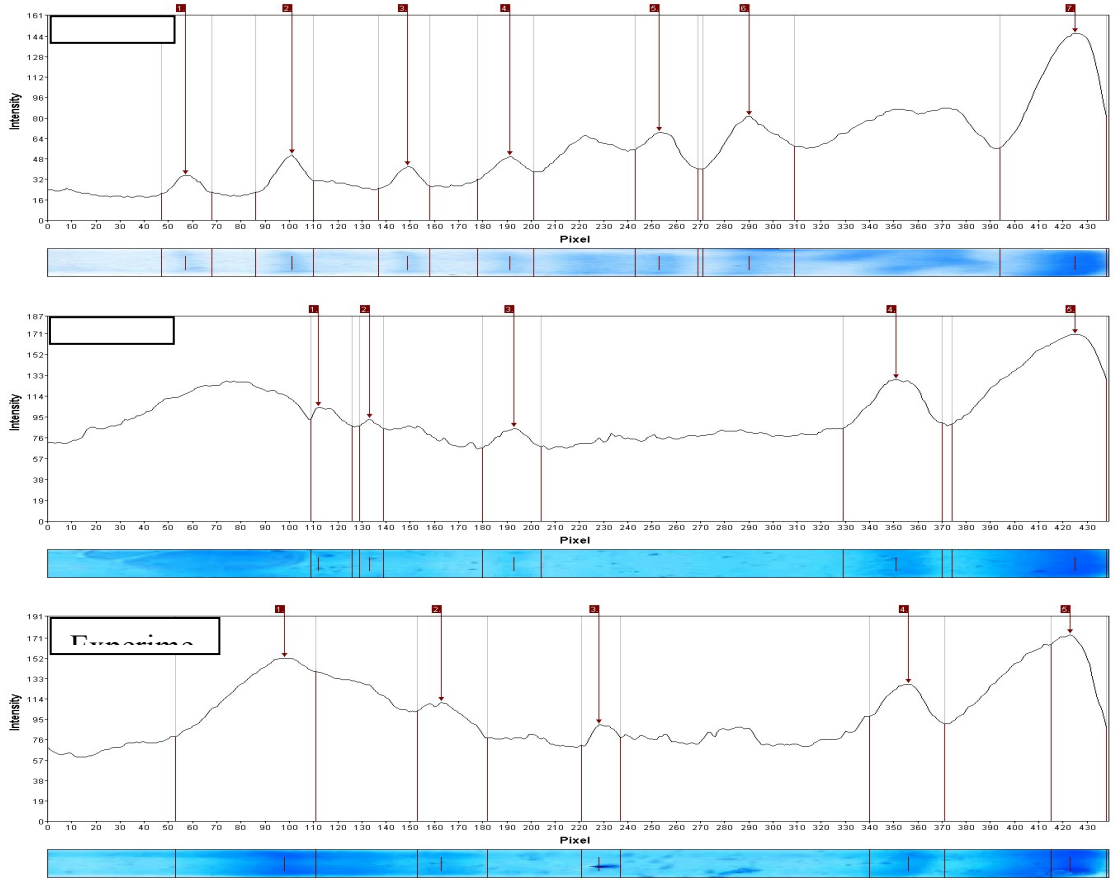


**Figure 3: One dimensional SDS-PAGE densitometric analysis of electrophoretically resolved serum proteins of consecutive 15 days BPA treated mice as compared with control and ladder.**

## DISCUSSION

The current work is done with an attempt to compare changes in serum proteins and transaminases following induction of BPA in mice. In the present study hyper-proteinemia and albuminemia were observed. This might occur due to intoxication of BPA resulted in liver damage hence perturbed protein biosynthesis. Raised level of the proteins is also a suggestive of inflammatory conditions as clinically, albumin solely acts as indictor of several diseases (Farrugia, 2010) A similar condition of hyper-proteinemia and hyperalbuminemia was observed in BPA treated rats (Helal *et al.,* 2015). Statistically significant rise amino transferases (ALT&ALP) were noted in the current study which is clear suggestive of the cellular damage. This rise might reflect that hepatotoxic effects of BPA. Moon *et al.,* (2015) reported liver changes with low dose BPA (1.2 mg/kg) treated rats for 5 days and noted an increase in oxidative stress due to damage and mitochondrial dysfunction. Shakoori *et al*.,(1992) reported 76% increment in SGOT in insecticide induced rabbits.

In BPA treated mice fractions of 149 KDa, 132 KDa, 101 KDa, 72KDa, 75KDa were resolved. The protein fraction of 72KDa and 75KDa might be related to matrix metalloproteinase-2 (MMP-2) or related endopeptidases. Cheung (2000) reported MMP-2 as contributor of cardiac mechanical dysfunction. The presences of such protein fractions in BPA treated animals suggest its correlation with heart related problems.

Conclusively, it can be stated that extensive use of BPA in various application may cause serious health hazards, as revealed by changes in serum proteins and transaminases. Furthermore, this little finding might tend to provide a strong justification that continuous exposure of chemicals like BPA may pose serious damage to humans.

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