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Effect of BPA on Protein, Lipid Profile in Serum of Albino Rats and Impact of Vitamin E and Thyronorm in BPA Induced Albino Rats

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Abstract: ***BPA is an estrogenic chemical produced in large quantities for use primarily in the production of polycarbonate and epoxy resins. The present study was conducted to estimate protein, lipid and TSH levels in serum of BPA induced and vitamin E supplemented rats. The physiological and biochemical activities in the albino rats were completely disturbed after the oral administration of BPA. The high dose of BPA induces serum damage affecting protein, total cholesterol and thyroid level as a result of reactive oxygen species in rat serum and cause damage and the condition is reversed by Vitamin E supplementation.***

Keywords: BPA, Albino pregnant rat, Aminotranferases and GDH activity, Serum cholesterol, Immunohisto chemistry

1. Introduction

Bisphenol- A is an organic compound with two phenol functional groups. It is produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins 1 (Kang JH, *et al* 2006) Polycarbonate plastics are used in certain food and drink packaging, e.g., water and infant bottles, compact discs, impact-resistant safety equipment, and medical devices, and can be blended with other materials to create molded parts for use in mobile phone housings, household items, and automobiles. Epoxy resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some polymers used in dental sealants or composites contain BPA-derived materials. BPA induced the molecular and morphological alterations in the uterus of adult rats (Steinmetz R, *et al* 1998). Previous studies through analyses of BPA in the serum of pregnant women and in cord blood collected at birth have indicated that BPA accumulates early in fetuses (Takahashi O and Oishi S 2016). Therefore, attention has been drawn towards the possibility that even low doses of BPA could possibly affect human development and reproduction (Welshons WV.*et al* 2006*)* Proteins are the ubiquitous macro molecules in the biological system and are derivatives of high molecular weight polypeptides (Murray IV *et al*2007) . They constitute about one fifth of an animal body on wet weight basis (Swaminathan, M.1983). The concentration of proteins on serum is a balance between the rate of their synthesis and degradation. The overall protein turnover in animal is the dynamic equilibrium between synthesis and degradation rates (Tavill AS, et al 1983;. Chitra KC *et al* 2003; Alonso-Magdalena P, *et al* 2012). BPA induced oxidative stress in cells are known to damage proteins and showed decreased protein content in BPA administered rats. Aminotransferases are widely acknowledged for their significant in protein metabolism by virtue of their ability to regulate both the synthesis and degradation of amino acids. Changes in their activities, whether induced by endogenous or exogenous factors, are often associated with changes in many other metabolic functions and may thus represent wide spread alteration in the organism's physiological state. They couple the protein, fat metabolism under altered physiological, pathological and induced environmental stress conditions (Zeinab K, et al 2012) Mai A Dose of BPA (50 mg/kg) significantly increased the serum cholesterol of rat. Serum cholesterol is a term that includes the total level of cholesterol that is found in the bloodstream. The endocrine disruptors are widespread in the environment and food chains and include some common environmental contaminants such as pesticides, plastic ingredients, dioxins,and biocides. Although several authors have speculated that specific environmental chemicals might bind to thyroid receptors(TRs) and alter thyroid hormone signaling .In vitro studies have demonstrated that BPA binds weakly to the thyroid hormone receptor and suppresses transcriptional activity that is stimulated by T3. BPA can be hydrolyzed under high temperature and acidic or basic conditions leading to leaching into food and drink containers.The present study aimed to evaluate the effect of high doses of BPA on thyroid gland function.

2. Literature Review

A 2007 review concluded that bisphenol-A has been shown to bind to thyroid hormone receptor and perhaps has selective effects on its functions. 2009 review summarized BPA adverse effects on thyroid hormone actions.BPA act as an agonist for the estrogen receptors. Although an epidemiologically based investigation has suggested that BPA disrupt thyroid function in animals. BPA inhibits TR-mediated transcription by acting as an antagonist.

Proteins are the ubiquitous macromolecules in the biological system and are derivatives of high molecular weight polypeptides. They constitute about one fifth of an animal body on wet weight basis. The concentration of proteins on serum is a balance between the rate of their synthesis and degradation. The overall protein turnover in animal is the dynamic equilibrium between synthesis and degradation rates. BPA induced oxidative stress in cells are known to

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damage protein and showed decreased protein content in BPA administrated animal. Aminotransferase are widely acknowledged for their significant in protein metabolism by virtue of their ability to regulate both the synthesis and degradation of amino acids. Changes in their activities whether induced by endogenous or exogenous factors are often associated with changes in many other metabolic functions and may thus represent wide spread alteration in the organism's physiological state. Aminotransferase such as ALT and AST catalyze the reaction of transamination of alanine, glutamic and aspartic acids. They couple the protein, fat metabolism under altered physiological, pathological and induced environmental stress conditions.

Serum cholesterol is a term that includes the total level of cholesterol found in the bloodstream. Measuring the level of total cholesterol includes identifying all types or classes of cholesterol that found in the system. Intestinal cholesterol absorption plays a major role in maintaining total body cholesterol homeostasis, the present study is investigate whether BPA affects cholesterol level.

The aim of the study was to estimate the intensity of oxidative damage and alterations in antioxidant enzymes activities in blood sample of bisphenol -A treated albino rats. The study investigates the level of thyroid stimulating hormone and low density lipids. The protein estimation was also carried out in chemical induced animals**.**

3. Materials and Methods

Animals
Male albino rats of Wistar strain weighing around 160 to 180gms were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai. The Animals were acclimatized to the laboratory conditions, fed with commercial pelleted rats chow (Hindustan Lever Ltd, Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the guidelines of institutional animal ethics committee.

Experimental Protocol
The rats were divided into 3 groups comprising of 6 animals in each group.
Group I Control-sham operated animals (C)
Group II BPA induced (by oral) 20 days animals-Control (EC)
Group III BPA induced animals after 20 days given Vitamin E (ET vitE_{15})
The study was carried out to assess the oxidative stress, antioxidant enzymes, TSH, LDL and Protein in the BPA induced animal blood and under the influence of BPA induced and vitamin-E and tablet Thyronorm supplementation.

BPA induction:
BPA was given to the albino rats by oral 20mg/ kg body weight for 20 days.

Vitamin E supplementation
Rats were fed with vitamin E (-tocopherol) of 200mg/Kg body weight, as reported earlier (*Tang, 1989; Bauersachs***et al.** 1993) for 15 days (*Manju**et al.**2005*).

Thyronorm Supplementation
Rats were fed with thyroid tablet named thyronorm of 200mg/kg body weight.

Sample Preparation
The experimental animals were sacrificed by cervical dislocation at the end of the appropriate experimental period. The various experimental groups viz. C, EC, ET vitE, ETvitE_{15}&THY_{15} were dissected and heart punctured and blood sample were collected for analysis.

Protein estimation
The protein content was determined by the method of *Lowry **et al.*** (1951**SDS** (8.1%): 8.1 gm SDS was dissolved in 100 ml of distilled water.

Low Density Cholestrol
The activity level of LDL was measured in all the blood samples viz. C, EC, ET vitE, ET vitE_{15} &THY_{15}animals.

TSH
TSH was measured in all the blood samples viz. C, EC, ET vitE, ET vitE_{15} &THY_{15}animals.

Statistical Analysis
The statistical analysis of the various parameters of the present study in all animal groups was carried out.

Student't' test was conducted to test the difference between two sample means by using given formula as suggested by Gupta (1978)

4. Result

After biochemical analysis, the activity levels of Cholesterol was significantly elevated to16.08% when compared to that of control (Table-1; Figure-1,2) The activity levels of Cholesterol was significantly reduced to 35.54% in the BPA Vitamin E induced albino rats when compared to that of control (Table -1; Figure -1,2). The activity level of cholesterol was significantly reduced to 49.39% in Vitamin E and thyronorm induced albino rats when compared to that of control and Experimental control.

After biochemical analysis, the activity levels of protein was significantly decreased 52.27% in BPA induced to albino rats when compared to that of control (Table-1; Figure-1,2) The activity levels of Protein was significantly increased to 104.76% in BPA Vitamin E induced albino rats when compared to that of control and Experimental control (Table -1; Figure -1,2). The activity levels of Protein was significantly increased to 92.85% in BPA Vitamin E induced albino rats when compared to that of control and Experimental control (Table 1- Figure -1,2).
The results of the present study (Table, 2 fig 3) revealed that no significant differences were was observed in serum TSH and T3concentration between BPA-treated groups and control. On other hand a significant ($P \leq 0.05$) decrease in serum concentrations of T4 in 100 mg/kg bodyweight BPA-treated groups compared with control . Finally in the same table a significant ($P \leq 0.05$) increase in serum AST and ALT levels were found in all BPA-treated groups compared with control.

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After biochemical analysis, the activity levels of TSH was significantly decreased to 18.18% when compared to that of control (Table-2 Figure-3) The activity levels of TSH was significantly increased to 23.07% in the BPA Vitamin E induced albino rats when compared to that of control. The activity levels of TSH was significantly increased to 45.45% in the BPA Vitamin E and Thyronorm induced albino rats when compared to that of control (Table -2; Figure -3) TSH was increased in the vitamin E and Thyronorm given treated rats.

5. Discussion

In this study, lipid parameters in in the BPA induced albino rats was evaluated. A significant increase in the mean LDL-cholesterol level was observed in the Experimental group when compared against the control in the study. This significant increase in lipid profile is an indication that the BPA induced albino rats were affected in lipid metabolism. Lipid metabolism is affected once there is damage since the disturbance of cell membrane integrity is likely to cause some membrane lipids to be released into circulation; while on the other hand, it causes the tissue to compromise its effectiveness in regulating lipid metabolism and also associated with oxidative stress and lipid peroxidation resulting from the imbalance between pro-oxidant and antioxidant chemical species(.Robertson G ***et.al .,*** 2001). These oxidative processes produce free electrons, H_2O_2, and reactive oxygen species (ROS) while depleting the potent antioxidants, glutathione and vitamin E (McCullough AJ. 2002). The increased levels of free fatty acids provide a perpetuating and propagating mechanism for oxidative stress via lipid peroxidation. Lipid peroxidation usually leads to the formation of peroxyl radicals, which are central species in the peroxidation chain reaction. Such reactive oxygen species as hydroxyl and superoxide radicals are known to provoke severe cellular alterations resulting in cell damage or death, due to their high reactivity. These species attack such important cell constituents as proteins, lipids and nucleic acids, and the lipid peroxides that accumulate due to lipid peroxidation are known to be very harmful to cells and tissues (. Linden A ***et al.,*** 2008)

The vitamin E supplemented group animals were characterized by high concentrations of total protein content. In cellular cytoplasm, the mercury bromophenol blue (Hg–BPB) reaction was highly positive and was either in the form of bluish granules of different sizes or in a diffused state, perinuclear or peripheral in position, particularly concentrated adjacent to blood sinusoids and equally distributed. In the nucleus, the chromatic granules and the nucleoplasm were stained to a less extent and gave a diffused faint blue color Total protein was found to exhibit a noticeable decrease in cytoplasms and nuclei of the liver cells in the Cr group because it showed high percentages of protein-devoid hepatocytes with cytoplasmic vacuolation and the protein remnants were primarily located on the periphery of cells.However, oral vitamin E administration concomitant with BPA could ameliorate changes in protein content in rat serum when compared with the BPA induced group.

The above study envisage that the supplementation of vitamin E is an important lipid soluble antioxidant *invivo*, and it is presumed that its principle role is to protect membrane protein and lipids from lipid peroxidation *invivo*, by scavenging lipid alkoxy or peroxy radicals which are capable of abstracting hydrogen from adjacent polyunsaturated lipid molecules to propagate a lipid peroxidation reaction and thus prevent the serum damage due to oxidative stress and maintaining the level of protein and cholesterol in the serum.

The present work results showed that, changes in protein and associated enzyme systems in serum of BPA treated rats. The physiological and biochemical activities in the albino rats were completely disturbed after the oral administration of BPA. In the present study, BPA treated groups showed, decreased protein content when compared to the control. Mean serum protein values were significantly ($P<0.05$) decreased in all BPA treated groups. (Verma RJ and Sangai NP. 2009) . Decreased levels of serum protein might be due to deactivation of protein disulfide isomerase, a multi functional protein critically involved in the folding and shedding of cellular proteins(Hiroi T ***et al.,*** 2006) The decreased protein levels could be related to damage of cells caused by BPA. The decrease in protein content under stress induced by BPA may be attributed to the utilization of aminoacids in various catabolic reactions and the BPA may either act by activating or inhibiting enzyme activities in the cell or destruction of the cell organelles with liberation of particular enzymes is one of the reasons to alter the expression of total proteins and another reason is oxidative stress influenced by excess reactive oxygen species (ROS) produced in serum are known to damage proteins. The depletion of total protein content was observed in this investigation can be correlated to this fact. Decreased total protein content was observed in BPA induced albino rats. BPA induced oxidative stress in serum are known to damage proteins. This can lead to various diseases, including cancer, infertility and neuro degenerative diseases (Zhang X ***et al.,*** 2005). In conclusion, the present work indicates that a significant decrease in total protein content of serum. There is much concern that exposure to BPA causes endocrine toxicity in man and wild life [6]. The dose selected in the present study (100 mg/kg) has been shown to cause dysfunction in rat endocrine system [22]

BPA-treatment induction in rats induced a decrease in the specific activities of catalase, SOD and GST and an increase in H_2O_2 generation and lipid peroxidation, indicating an increase in oxidative stress and hypothrodism by decreasing the TSH levels in serum of albino rats, which was effectively reversed by the administration of vitamin E. The BPA -induced decrease in thyroid hormones may be due to multiple mechanisms, such as induction of uridine diphosphate glucuronyl transferases (UDPGTs) and binding to thyroid transport protein transthyretin (TTR) [24]. Vitamin E also restored the BPA-induced decrease in thyroid hormones. Administration of vitamin E and Thyronorm -induced hypothyroid rats has been shown to increase serum levels of TSH by scavenging H_2O_2 in the thyroid gland [25]. This could be the reason for the decreased Serum TSH levels in the BPA-treated rats. Thus its decrease in the BPA-treated rats may suggest TSH deprivation in the serum of albino rats. In conclusion, BPA

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alters the endocrine function by inducing oxidative stress, which could be reversed by vitamin E.

6. Conclusion

Our results indicate that high dose of BPA induces serum damage affecting protein, total cholesterol and thyroid level as a result of reactive oxygen species in rat serum and cause damage and the condition is reversed by Vitamin E supplementation.

Table 1: Parameters of protein and cholesterol in control and BPA induced of male albino rats. Values are mean ±SD (n=10). Values with different letters are significantly different (P < O.OO1).

Parameter	control (C)	% of changes	BPA induced EC*	% of changes	BPA & Vit E Induced ET VitE15**	% of changes	BPA, Vit E & Thyronorm induced ET Vit E& THY 15**
Protein	0.44±.001	-52.27	0.42±.001	+104.76	0.86±.001	+92.85	0.81±0.1
Cholesterol	1.43±0.3	+16.08	1.46±0.7	-35.54	1.07±0.2	-49.39	0.84±0.4

Table 2: Parameters of Thyroid in control and BPA &Vit E induced of male albino rats. Values are mean ±SD (n=10). Values with different letters are significantly different (P < O.OO1)

Parameter	Control C	% of Changes	BPA Induced EC*	% of changes	BPA, Vit E & Thyronorm induced ET Vit E& THY 15**	% of changes
THYROID	0.22±0.6	-18.18	0.26±0.4	-23.07	0..32±0.1	-45.45

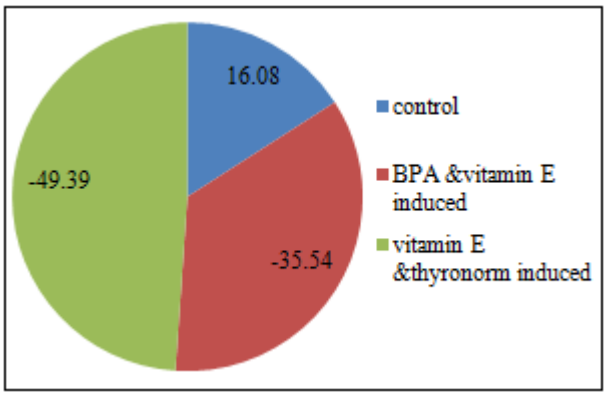

Figure 1: Parameters of protein and cholesterol in control and BPA induced of male albino rats. Values are mean ±SD (n=10). Values with different letters are significantly different (P < O.OO1).

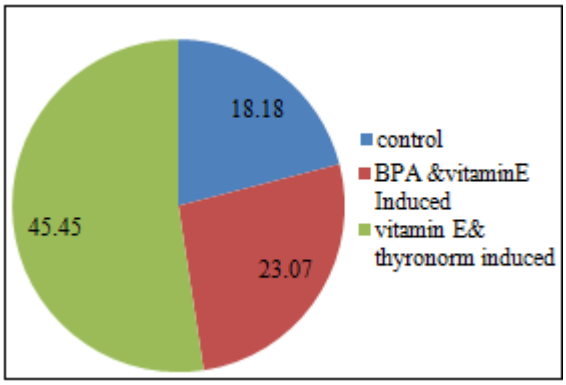

Figure 2: Parameters of Thyroid in control and BPA &Vit E induced of male albino rats. Values are mean ±SD (n=10). Values with different letters are significantly different (P < O.OO1)

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