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Research Article

Effect of BPA on Protein, Lipid Profile and Immuno-Histo Chemical Changes in Placenta and Uterine Tissues of Albino Rat

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ABSTRACT

Bisphenol-A (BPA) is an estrogenic chemical produced in large quantities for use primarily in the production of polycarbonate and epoxy resins. The potential teratogenic effects and fetal toxicity of environmental estrogenic endocrine disruptors have become a great concern in recent years, and they have yet to be fully characterized. The present study was conducted to estimate protein, lipid contents and immunohisto chemical changes in placental and uterine tissues of BPA exposure during the period of pregnancy in Rattus norvegicus. Pregnant rats were administered 50 and 500mg/kg.b.w/day of BPA orally using sesame oil as a vehicle from days 8th - 15th of gestation. The control group received sesame oil only. On completion of the treatment period, the experimental animals were sacrificed under light anesthesia. BPA also induced changes in placental and uterine tissues, dose-dependent decrease in protein and lipid contents in serum of rat. The present study suggested that, in immunohisto chemical studies the presence of caspase-3 protein was observed in the placental and uterine tissues, dose -dependent decrease in protein and lipid contents adversely affected the embryo fetal, placental and uterine development of the pregnant female rats.

Key words: BPA, Albino pregnant rat, Aminotranferases and GDH activity, Serum cholesterol, Immunohisto chemistry.

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]. 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[2]. 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[3]. Therefore, attention has been drawn towards the possibility that even low doses of BPA could possibly affect human development and reproduction[4]. Proteins are the ubiquitous macro molecules in the biological system and are derivatives of high molecular weight polypeptides[5]. They constitute about one fifth of an animal body on wet weight basis[6]. 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[7-9]. 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. Aminotransferases such as ALT and AST catalyse the reactions of transamination of alanine, glutamic and aspartic acids. They couple the protein, fat metabolism under altered physiological, pathological and induced environmental stress conditions10. Low dose of BPA (50 mg/kg) significantly increased the serum AST, ALT indices of liver function of rat and normal in the control. Glutamate dehydrogenases (GDH) play a crucial role in the cells affected by a variety of effectors of protein metabolism in the cells[11]. This enzyme has several metabolic functions with great physiological significance and closely associated with the detoxification mechanisms of serum. Hence, the activities of AST, ALT and GDH are considered as sensitive indicators of stress[12]. Increase in GDH activity, AST and ALT favors trans-deamination of amino acids to incorporate them into TCA cycle for energy releasing purposes to meet the imposed toxic stress as keto acids and the elevation in GDH activity under toxic stress[13]. Serum cholesterol is a term that includes the total level of cholesterol that is found in the bloodstream. Measuring the level of total cholesterol includes

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Table 1: Changes in protein levels (μ moles / ml of serum / hr) of BPA treated rats and controls. Values in parentheses indicate percent change over control.

Name of the proteins	Control	Low dose (50 mg/kg/ body wt))	High dose (500 mg/kg/ body wt))
Total proteins Mean SD PC	1.546± 0.072	1.239± 0.011 (19.80)	0.679± 0.350 (56.00)
Free amino acids Mean SD PC	2.520± 0.553	3.012± 0.493 (-19.50)	4.921± 0.228 (-95.20)
Aspartate Aminotransferase Mean SD PC	11.886± 1.797	14.298± 0.778 (-20.20)	18.726± 0.770 (-57.50)
Alanine Aminotransferase Mean SD PC	12.876± 1.314	14.783± 0.995 (-14.80)	18.720± 1.115 (-45.30)
Glutamate de hydrogenase vMean SD PC	4.886± 0.428	6.264± 0.256 (-28.20)	8.472± 0.125 (-73.30)

All the values are mean ± SD of six individual observations
SD: Standard deviation
PC: Percent change over control

identifying all types or classes of cholesterol that are found in the system. This helpful measurement makes it possible to determine if the balance between the HDL or good cholesterol and LDL or bad cholesterol is within acceptable limits. It also involves identifying the current level of very low density lipoprotein content, as well as the Intermediate Density lipoprotein levels. These readings, along with the LDL and HDL cholesterol levels, help to provide a complete picture of the lipids and proteins currently present in the body. Triglycerides, as major components of very-low-density lipoprotein (VLDL) and chylomicrons, play an important role in metabolism as energy sources and transporters of dietary fat. They contain more than twice as much energy as carbohydrates[14,15]. The confluent cultures of 3T3-L1 fibroblasts treated with BPA presented an increase in triglyceride content, lipoprotein lipase activity, and glycerol phosphate dehydrogenase activity, suggesting that BPA by itself can promote 3T3-L1 fibroblasts to differentiate into adiposities. In this study aimed was examined immunohistochemical changes (immune-histochemcal staining of active caspase-3 expressions) in BPA treated placenta and uterine tissues, it indicated that apoptotic changes occurred both in uteroplacental tissues. In vitro studies show that BPA in other endometrial cells seems to induce different effects. Indeed, decreased proliferation and increased of apoptosis was observed in human uterine endometrial endothelial cells[16]. Immunohistochemical changes were observed in placenta and uterine tissues of ADM $_{22\text{-}52}$ treated pregnant rats[17]. High expression of active caspase-3 was localized at the apoptotic germ cells in mice exposed to oral BPA at puberty[18].

MATERIALS AND METHODS

Maintenance of Experimental Animals

Healthy rats of Wistar strain were purchased from authorized vendor (M/S Raghavendra Enterprises, Bangalore, India). All rats were housed in polypropylene cages (18" 10"x 8") lined with sterilized paddy husk, and provided filtered tap water and rat food ad libitum in an air-conditioned environment (25±2°C) with a 12-h light and 12-in dark cycle. The experiments were carried out in accordance with the guidelines of the committee for the purpose of control and supervision on experiments on animals.

Experimental Protocol

Female Wistar rats three months old, weighing 250g to 300 g were used for the experiment. The status of estrous cycle stages were determined every morning between 7:00 am by collecting of vaginal secretion with a plastic pipette filled with 10 μL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina. One drop of vaginal fluid was placed on glass slides the unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40 x objective lense. Two females of pro-estrous stage were paired with a male overnight and the next morning, males were removed and females were assessed for the presence of sperm in the vaginal flush. Animals with positive sperm in the flushes are designated as day 1 of gestation. Six pregnant rats were used in each experimental group.

Treatment

BPA was given orally on 8th to 15th day of gestation period (total 8 days) of female albino rats. After that animals were sacrificed and took the serum and selected tissues were isolated for further investigations.

Estimation of proteins contents in rat serum after administration of BPA

Serum samples was used for the assay of protein contents namely total proteins according to Lowery[19], free amino acids according to Colowick and kalpan, aspertate aminotransferase and alanine aminotransferase according to Reitman and Frankel[20], glutamate dehydroginase according to Lee and Lardy[21].

Estimation of lipid contents in rat serum after administration of BPA

Serum samples was used for the assay of lipid contents namely total cholesterol and triglycerides (Cell counter (PocH 100i) Sysmex – Transasia, 2004).

Immunohistochemistry of placenta and uterine tissues

Immunohistochemical assay was estimated by the method of Stemrmberger[22]. After deparaffinization of tissue sections, antigen retrieval was performed by heating in 10

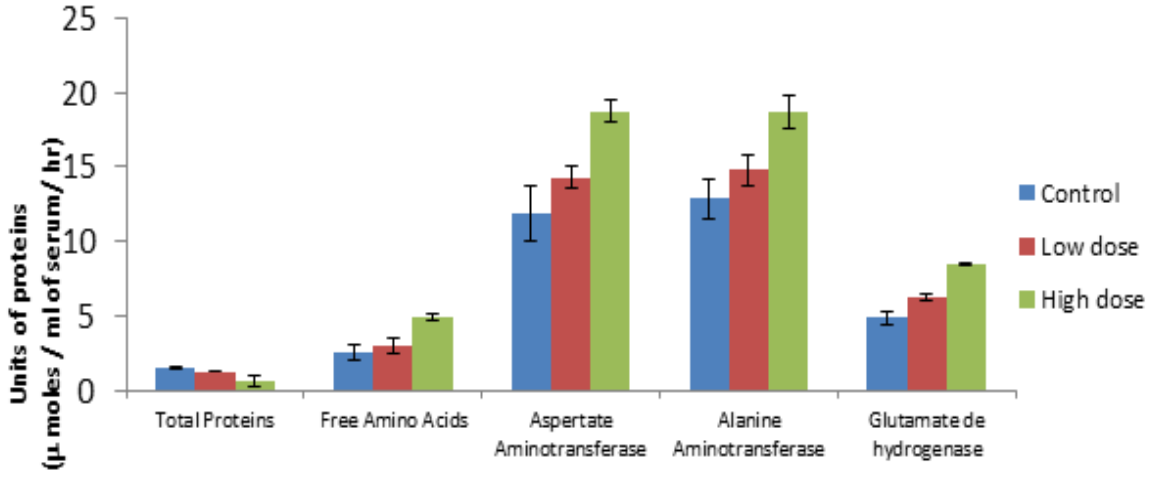

Figure 1: Total Protein content, Free amino acid content, Aspartate aminotransferase, Alanine aminotransferase, Glutamate dehydrogenase activity levels in different serum samples of albino rats exposed to BPA

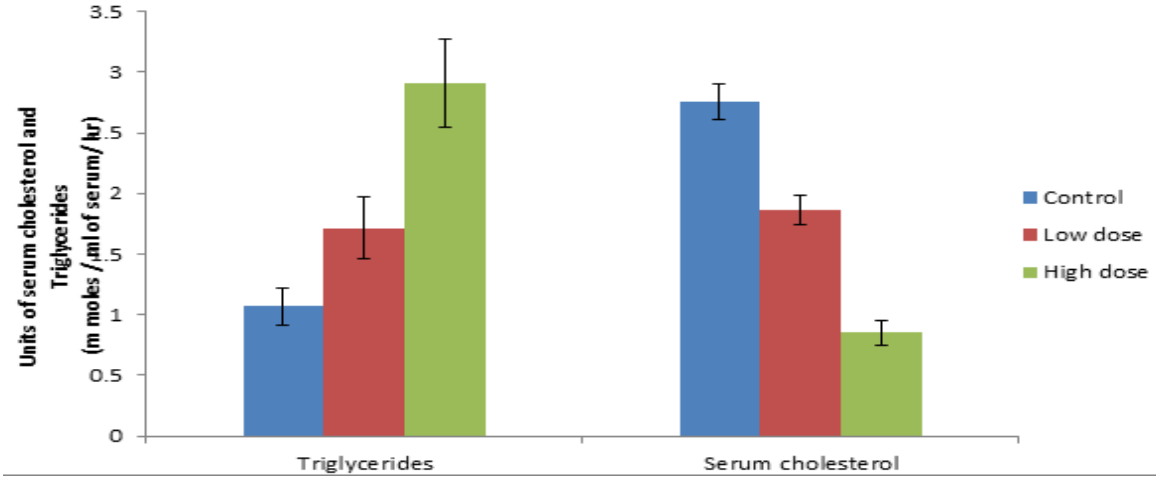

Figure 2: Triglycerides and serum cholesterol activity levels in different serum samples of albino rats exposed to BPA

mM citrate buffer (pH 6.0) for 10 min. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min. After blocking with 5% normal rabbit serum, sections were incubated with the primary antibody for caspase-3 at 48° C overnight. They were rinsed in PBS and incubated with biotinylated goat anti-rabbit immunoglobulin (Ig) G, followed by avidin-biotinperoxidase solution. Next, DAB with 0.003% H_2O_2 in PBS was added to each slide. The tissues were lightly counterstained with hematoxylin and examined by light microscopy. Negative controls were obtained using normal rabbit serum in place of primary antibody, with all other steps unchanged.

Statistical analysis

The mean, standard deviation (SD), percent change and one way analysis of variance (ANOVA) (Steel and Torrle) were performed using the Statistical Package for Social Sciences (SPSS) Package programming techniques on "Intel Core 2 Duo Processor" personnel computer. Probability values less than 0.05 were considered significant[23].

RESULTS

Protein contents

In the present study, protein level in serum of BPA treated rats (lower dose and higher dose) showed significant variations (Fig-1), compared to control group. On treatment with BPA, serum samples showed significantly decreased protein level compared to control group. The maximum decrease was observed in higher dose administration followed by lower dose when compared to control rats. The highest decreased protein level was found in the serum of higher dose BPA treated rats. Free amino acids, Asparatate aminotransferase, Alanine aminotransferase, Glutamate dehydrogenase (GDH) activities were enhanced in all the experimental groups of animal (Fig-1), compared to control group. The elevated free amino acids, Asparatate aminotransferase, Alanine aminotransferase, Glutamate dehydrogenase (GDH) activities were dose dependent in BPA treated groups.

Lipid profile

Triglycerides

Triglycerides activity was found to show statistically significant variations ($P<0.05$) in BPA treated groups. The results obtained are shown in Fig-2. When compared to the control group, there is a significant elevation of triglycerides activity observed in the serum of BPA treated rats with low dose and high dose groups. The elevated activity of triglycerides was dependent on dose concentration. There for in the present study, high

Table 2: Changes in lipid levels (μ moles / ml of serum/ hr) of BPA treated rats and controls. Values in parentheses indicate percent change over control.

Name of the Lipids	Control	Low dose (50 mg/kg/ body wt))	High dose (500 mg/kg/ body wt))
Triglycerides Mean SD PC	1.072 ± 0.152	1.712 ± 0.254 (-59.70)	2.913 ± 0.364 (-171.70)
Serum cholesterol Mean SD PC	2.754 ± 0.142	1.864 ± 0.124 (32.30)	0.854 ± 0.103 (68.90)

All the values are mean ± SD of six individual observations
SD: Standard deviation
PC: Percent change over control

triglycerides activity was observed in high dose than in low dose when compared to control group.

Serum cholesterol

The present data revealed that, the serum cholesterol activity was statistically decreased ($P<0.05$) in serum of BPA treated groups when compared to control group (Fig-2). A significant decrease in cholesterol activity ($P<0.05$) was observed in serum of BPA treated groups compared to control group. The decreased serum cholesterol activity was dose dependent in BPA treated groups. There for in this study, serum cholesterol activity was drastically decreased in serum of high dose BPA treated group.

Immunohistochemical changes in placenta and uterus:

In this study of results, figures- 3.A.2, 3.A.3 & 3.B.2, 3.B.3, demonstrate representative active caspase-3 protein immunostainings within the sections of the placenta and uterus of rat's treatment with 50 mg/kg and 500 mg/kg BPA compared with the negative controls on day 15th pregnancy. Brown-red color, which indicates active caspase-3 protein immunoreaction, was recognized in labyrinth layer of placenta and deciduas of the uterus in the BPA treated group (Fig-3. A.2, 3.A.3, & 3.B.2, 3.B.3). The active caspase-3 protein appeared to be abundant in the uterine decidual layer and placental labyrinth layer of the BPA treated rats compared with untreated controls. Brown staining indicates a positive reaction, and specificity of the staining was confirmed by the absence of staining when primary antibody was omitted in the reaction. 3. A.1. Immunohistochemical staining for active caspase-3 protein in section of placenta from control rats on day 15 of gestation. No staining occurred in the Labryinth layer (L.Z) of control placenta (original magnification, X40). 3. A.2. Immunohistochemical staining for active caspase-3 protein in section of the placenta from lower dose BPA (50 mg/kg) treated group. Arrows indicates active caspase-3. Positive immune reactivity was seen in Labyrinth layer (L.Z) of placenta compared with the untreated control rats (Fig-3. A.1). Brown staining indicates a positive reaction (original magnification, X40). 3. A.3. Immunohistochemical staining for active caspase-3 protein in section of the placenta from higher dose BPA (500 mg/kg) treated group. Arrows indicates active caspase-3. Positive immune reactivity was abundantly seen in Labyrinth layer (L.Z) of placenta compared with the untreated control rats (Fig-3. A.1). Brown staining indicates a positive reaction (original magnification, X40). 3. B.1. Immunohistochemical staining for active caspase-3 protein in section of the uterus from control rats on day 15 of gestation. No staining occurred in the decidual layer (D.L) of control uterus (original magnification, X40). 3. B.2. Immunohistochemical staining for active caspase-3 protein in section of the uterus from lower dose BPA (50 mg/kg) treated group. Arrows indicates active caspase-3. Positive immune reactivity was seen in decidual layer (D.L) of uterus compared with the untreated control rats (Fig-3. B.1). Brown staining indicates a positive reaction (original magnification, X40). 3. B.3. Immunohistochemical staining for active caspase-3 protein in section of the uterus from higher dose BPA (500 mg/kg) treated group. Arrows indicates active caspase-3. Positive immune reactivity was abundantly seen in decidual layer (D.L) of uterus compared with the untreated control rats (Fig-3. B.1). Brown staining indicates a positive reaction (original magnification, X40).

DISCUSSION

The present work results showed that, changes in protein metabolism 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 total serum protein values were significantly ($P<0.05$) decreased in all BPA treated groups[24]. Decreased levels of total 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[25]. 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 mitochondria and microsomes in cells are known to damage proteins. The depletion of total protein content was observed in this investigation (Figure-1) can be correlated to this fact. Bisphenol-A is converted to bisphenol O-quinone. The quinone intermediates of BPA may be the ultimate DNA binding metabolites[26]. This interaction might prevent RNA polymerase transcribing the DNA and can inhibit the formation of mRNA. A failure in mRNA formation can result in an inhibition of protein synthesis, which may be considered to be the cause of cell necrosis. Significant and dose-dependent reduction in

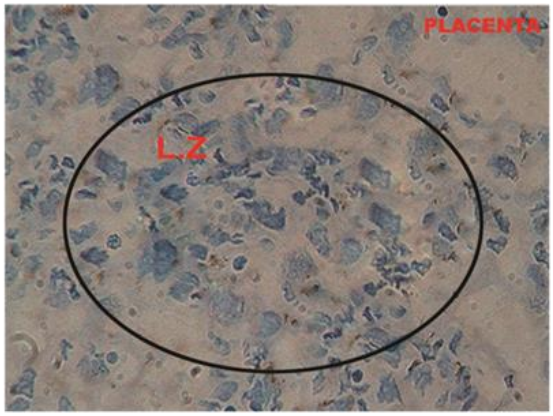

Fig.3.A.1

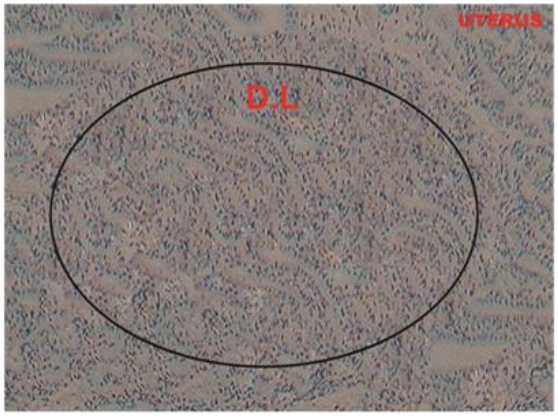

Fig.3.B.1

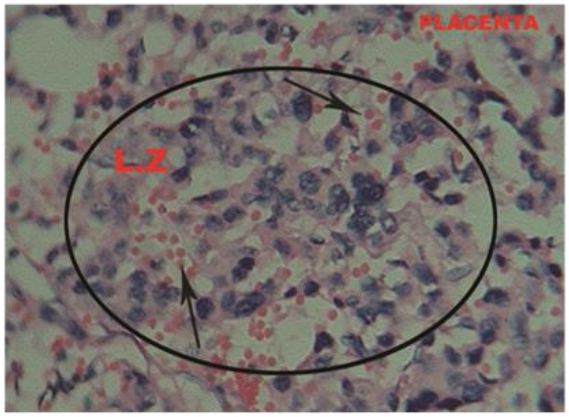

Fig.3.A.2

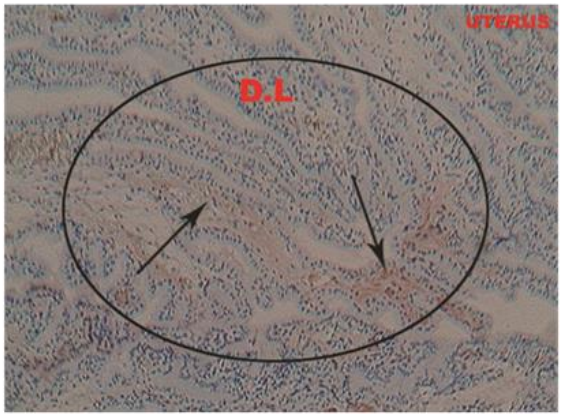

Fig.3.B.2

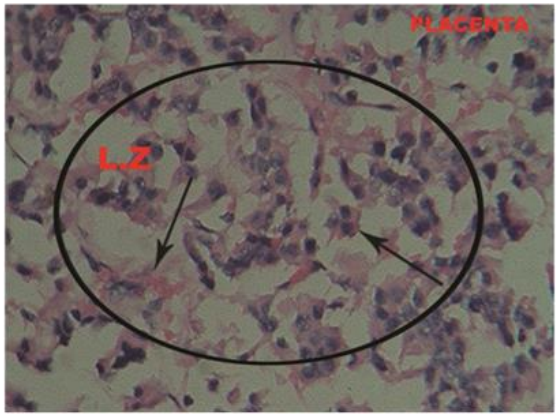

Fig.3.A.3

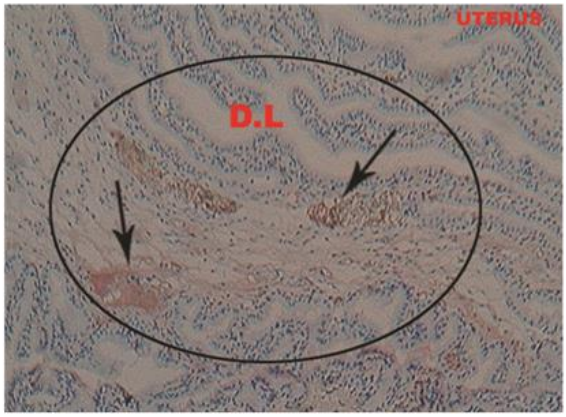

Fig.3.B.3

Figure 3: Immunohistochemical changes in placenta and uterus

mRNA expression and protein content was observed in AvPv (anteroventral periventricular nucleus) region of hypothalamus of female pups treated with BPA[27]. Thus, alterations in DNA, RNA and protein contents affect the overall process of protein synthesis[28].The ability of BPA to form DNA adducts both in vitro and in vivo in rodent liver. Decreased total protein content and increased alkaline phosphatase activity were observed in male rats exposed to BPA[29]. BPA induced oxidative stress in mitochondria and microsomes of cells are known to damage proteins. This can lead to various diseases, including cancer, infertility and neuro degenerative diseases[30]. In conclusion, the present work indicates that a significant decrease in total protein content of serum

samples under BPA toxicity is dose dependent manner. Decrease in total protein content suggests its metabolic utilization under BPA toxic stress condition. The elevation in free amino acid content in the present investigation (Fig-1) is consistent with the decreased protein level and enhanced transaminase activity during BPA exposure to rats. In the present study the results reported that, the alternations in free amino acids indicate, the condition of the serum sample, and their increase might be considered as the operation of the stress phenomenon in BPA treated groups when compared to control group[31-32]. Free amino acids thus increased may be fed into the TCA cycle, possibly to be utilized for energy production through aminotransferases. Presumably the degradation of proteins has led to FAA accumulation. This higher level of FAA can also be attributed to the decreased utilization of amino acids and is also suggestive of its involvement in the maintenance of osmotic and acid base balance in BPA treated rats. Creatine, a product of amino acid degradation (including glycine), is a major metabolite found mainly in muscle and brain of rats, and it appears to be affected by prenatal exposure to BPA. Conversely, a decrease in essential amino acids, namely valine, leucine, and isoleucine**,** could reflect a disruption in their degradation pathways, which would be consistent with observations in gestational day18 rat fetuses exposed to butylbenzylphthalate. Amino acids may not only act as precursors for the synthesis of essential proteins, but also contribute towards gluconeogenesis, glycogenesis and keto acid synthesis[33,34]. The elevated levels of free amino acids were observed in different albino rats exposed to certain pesticides. In this study, free amino acid levels were elevated in BPA treated groups, the elevation in free amino acids was in consonance with the increased proteolytic activity. The elevated free amino acid levels indicate altered protein homeostasis and nitrogen imbalance due to BPA toxicity in treated groups. In this study of results reveled that, increased levels of AST and ALT activities in BPA treated groups when compared to control group[35-36]. This will give a clear indication of shunting of amino acids into TCA cycle through oxidative deamination and active transamination. Such a phenomenon was necessary to cope up with the energy crisis during stress condition. It has been suggested that stress conditions in general induce elevation in the transmination pathway. AST and ALT are known to play a key role in mobilizing L-aminoacids for gluconeogenesis and functions as link between carbohydrate and protein metabolisms under altered physiological, pathological and induced environmental conditions[37]. Hence they are considered as sensitive indicators of stress. The aspartate and alanine aminotransferases are referred as "markers of cell injury"[38], since they are first to leak out from the cell in the case of injury[39]. BPA can induce hepatic damage and mitochondrial dysfunction by increasing oxidative stress in the liver. The initial effort taken by the animal for raising its energy resources through active transmination and for the synthesis of new proteins required for detoxification of the toxicant and its disposal is an indication in increase in AST and ALT activity in BPA treated groups. Elevated levels of AST and ALT indicate the enhanced transmination of amino acids, which may provide keto acids to serve as precursors in the synthesis of essential organic elements. Activities of superoxide dismutase, catalase, glutathione and malonyl dialdehyde levels in the serum reflect the oxidative status and the serum enzymes like AST and ALAT represent the functional status. The significant dose-dependent change in the activities of ALT, AST shows the toxic effect of BPA[40]. ALT activity is an important index to measure the degree of cell membrane damage, while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme. The activity levels of aminotransferases (AST and ALT) were elevated in the serum samples was observed because increased AST and ALT activity is correlate with increased oxidative stress caused by BPA in treated groups. In the present study, GDH activity was elevated in BPA treated groups when compared to control. During BPA toxicity, the higher expression levels of GDH activity indicates its contribution to enhanced ammonia levels and glutamate oxidation. Increased free amino acid levels and their subsequent transamination results in greater production of glutamate, thus increasing the intracellular availability of substrate, glutamate for consequent oxidative deamination reaction through GDH. Besides the elevation of transaminases and GDH helps in supplying keto acids to the TCA cycle in order to compensate the energy crisis in serum during BPA treated rats. Glutamate dehydrogenase occurs with high activity in the mitochondrial matrix and is commonly used as a marker for matrix space. It has a great importance in neurotransmitter balance in brain. As GDH plays an important role in detoxification of ammonia[41]. In the present study, increase in GDH activity, AST and ALT favors transdeamination of aminoacids to incorporate them into TCA cycle for energy releasing purposes to meet the imposed toxic stress as keto acids in the cells of serum in experimental rats. Exposure of xenobiotic compounds (BPA), capable of modulating or disrupting the enzymatic levels in serum may have harmful consequences for reproductive tissues of pregnant rats. However, serum have shown highly significant changes of GDH activity in BPA treated groups, normal level of expression were seen in control group. Results of the present study revealed a disturbance in lipid profile as reflected by the significant increase in the level of triglycerides with a concomitant decrease in serum cholesterol in BPA treated groups when compared to control[42]. The liver has a complex network of nuclear receptors that coordinately regulates the expression of enzymes involved in lipid metabolism, from fatty acid oxidation and uptake to triacylglycerol synthesis, accumulation and secretion[43]. BPA has the potential to affect lipid and glucose homeostasis by interfering with different nuclear receptors involved in regulation of metabolism[44]. BPA has also found to stimulate lipid accumulation and up-regulate genes involved in lipid metabolism in adipocytes[45]. BPA can induce estrogenic activity in treated groups, where estrogens have a significant effect on serum cholesterol. The effect on cholesterol is probably due to an action of the hormone on

the lipoproteins associated with cholesterol in the circulation, so altered the level of serum cholesterol. Mechanistically, environmental BPA is a well-known endocrine-disrupting chemical that binds to estrogen receptors (ER alpha) and (ER beta) and results in competition with estrogen and disrupting the lipid profile. In the present study, BPA might have acted to disrupt estrogen signalling pathways and estrogen plays an important role in decreasing serum cholesterol level. It is manifested through an increase in LDL cholesterol and decrease in HDL cholesterol. Various environmental estrogens can dramatically affect non-reproductive parameters such as cholesterol lowering and bone metabolism[46]. BPA is induced oxidative stress and oxidative stress influenced by excess reactive oxygen species (ROS) are known to altered lipid profile activity (decreased cholesterol and increased triglycerides) in BPA treated rats is another reason in this study[47]. pregnant rats exposed to a low dose of BPA (1mg/L) in drinking water showed increased adipogenesis (because disturbance of lipid profile) in females at weaning period. Decreases in cholesterol and increases in triglyceride were also found in Wistar rats after exposure to BPA during gestation and lactation period[48]. BPA might mimic estrogen properties and alter lipid profile (adipokine release), thereby affecting fetal growth. Similarly, in the present study, 50mg and 500mg/day/kg of bw of BPA treated rats showed decreased serum total cholesterol level when compared to corresponding controls. These results suggest that, early gestational exposure to BPA is sufficient to alter the levels of circulating cholesterol and triglycerides in treated groups when compared to control group. In the present study results revealed that, immuno histo chemical staining of the active caspase-3 protein, indicated abundant apoptotic changes in both the placenta and uterus when compared to control group[49]. Immunohistochemistry for caspase-3, a marker of apoptosis, was performed using a specific antibody capable of detecting the active form of caspase-3. In Gestational day 8^{th} to 15^{th} of BPA treated rats placental &uterine tissues observed a few caspase-3-positive cells in decidual and labyrinth layers, normal cells were observed in the control group. So a few cells exhibited immunoreactivity for caspase-3, but no difference was seen in control groups. Apoptotic changes in early gestation reproductive tissues can leads to precocious puberty. We hypothesized that the reductions of fetal, placental and uterine development in BPA treated rats may involve apoptotic cell death in uteroplacental tissues. During apoptotic cell death, cells undergo a regulated autodigestion, which involves the disruption of cytoskeletal integrity, cell shrinkage, nuclear condensation, and activation of endonucleases[50]. The chief effectors of the apoptotic cell death pathway are the caspase family of cysteine proteases. Caspases are synthesized as inactive precursors that are cleaved at specific aspartate residues to generate the active subunits. Procaspase cleavage can occur by several mechanisms, including proximity- induced autoprocessing or cleavage by other caspases, revealing a caspase cascade with downstream effector caspases such as caspases-3[51]. Apoptotic changes were more apparent in trophoblast cells in the labyrinth zone of placenta and uterine decidua of BPA treated rats when compared with control rats. Immunoreactivity to active caspase-3 protein was abundant in the placenta and uterus of the BPA treated group. The caspase-3 staining was predominant in trophoblast cells in the labyrinth layer of placenta, and was abundant in decidual cells of the uterus. Additionally, these changes in the labyrinth and decidual layer may be related to hypoxia induced by the possible reductions in maternal blood supply in BPA treated rats. Thus, the increased apoptotic effects of labyrinth zone of the placenta, could lead to the reduction in placental weights as well as fetal growth because placenta is an important region for fetomaternal exchange in rats. Early pregnancy in rodents is characterized by a progressive interaction between the embryo and the maternal compartment. Rodent uterine epithelium around the embryo undergoes apoptosis in response to the presence of the blastocyst[52]. The blastocyst signals that induce the apoptotic cascade, which is active caspase-3 protein. In the present study of in vivo examination demonstrating that the BPA action leads to induction of apoptotic changes in the placenta and uterus that result in fetal growth restriction. In particular, we found that the number of caspase-3–positive apoptotic cells were significantly increased in labyrinth cells of the placenta and in decidual cells of the uterus from rats exposed to BPA, suggesting that low & high doses of BPA exposure during early gestation of rats causes augmentation of follicular atresia and luteal regression in the tissues. [53] Immuno histo chemically increased expression of VEGF (vascular endothelial growth factor) in uterus, hepatocytes around central vein, epithelium of oviduct & endometrial glands of BPA treated rats. In the present study, caspase-3 immunoreactivity was more intense in the BPA-exposed groups than in the control group. Although the resulting effects showed in this study are provoked by in early gestation BPA exposure, the mechanism underlying the action of BPA on the fetuses of pregnant rats. Therefore, immunohistochemistry findings suggested that BPA exposure during early pregnancy may cause fetoplacental and uterine growth restriction.

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