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RESEARCH ARTICLE

THE EFFECT OF BISPHENOL-A IN KIDNEY'S TISSUE OF PREGNANT RAT

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ABSTRACT

Bisphenol-A (BPA) is an artificial compound that has been widely used in milk and water bottles, medical equipments, food containers. It is known to be toxic to mammalian cells. Extensive studies have suggested potential links between BPA exposure and diseases including cancer, obesity, diabetes and disorders of the reproductive, neuroendocrine and immune systems. Recent evidence has also identified the renal system as a potential target of BPA. These findings suggest that BPA exposure may be a risk factor for a range of renal abnormalities such as renal injuries & renal dysfunction. The present study investigated the ability of BPA to cause renal toxicity via aberration in the expression of oxidative stress, histopathological, apoptotic changes in pregnant rats. 2nd and 3rd groups of pregnant rats were orally administered BPA at 50mg/kg/b.wt/day (2ndgroup) & 500mg/kg/b.wt/day (3rd group) for gestational day 8th to 15th. The first group was given sesame oil with vehicle. The aim of this study was to determine the effects of BPA in kidney of pregnant rats. The activities of antioxidant enzymes like- SOD, catalase and glutathione peroxidase were seen to be decreased significantly in both doses; while, the level of MDA, a biomarker of lipid peroxidation, was increased in BPA treated rats. Histo pathological alterations were observed in the sections of treated kidney tissues. Caspase-3 activity levels were increased & Bcl2 activity levels were decreased in treated group's kidney tissues as compared to control group. The study showed that, BPA induced nephro toxicity through oxidative stress and by altering the apoptotic path way involved. In this review, we discuss these recent findings that point to the potential nephro toxicity of BPA, and highlight the knowledge gaps in this growing research area.

INTRODUCTION

Bisphenol-A is one of the environmental contaminants, it is currently one of the highest volume chemicals produced worldwide, with a global production capacity of 11.5 billion pounds in 2008 (Burridge, 2008). It is widely used in the manufacture of water bottles, baby bottles, inside coating in metallic food cans and as a non–polymer additive to other plastics (Hernandez- Rodriguez *et al*., 2007). Hence, it becomes an integrated part of the food chain (Huang *et al*., 2011). BPA has an abnormal effect of low dose exposure depending on non-monotonic dose responses and acts like a hormone, altering cellular function at very low concentrations with maximum safe levels of 5mg/ kg/ day (Vandenberg *et al*., 2010). BPA absorbed in gastrointestinal tract, may affect reproduction and development and has also seen found in human blood. It is conjugated by glucuronic acid in intestine and liver excreted in urine as BPAglucorinide (Dekant and volkel, 2008) can also disrupt immune system and maybe carcinogenic (Prins *et al*., 2008). The potential risk that BPA posses to the human health have attracted much attention from regulatory agencies and the general public, and have been extensively studied. An emerging and rapidly growing area in the study of BPA's toxicity is its impact on the kidney system. Recent epidemiological studies have shown that higher urinary BPA concentration in humans is associated with various types of kidney diseases.

Experimental studies have demonstrated that acute BPA exposure shows short term & subchronic toxicity in pregnant rat kidney. The underlying mechanisms may involve oxidative stress, histopathological changes and apoptotic protein activity. The role of BPA as an oxidative stressor in heart, kidney, brain and other tissues has been reported in rat and mice models (Chitra KC, 1995; Gong Y and Han XD, 2006). They suggested that BPA induces the formation of reactive oxygen species (ROS) in the cells (Bindhumol V, 2003; Kabuto H, 2004). Moreover, the study of Bindhumol *et al*, revealed that low doses of BPA generate ROS by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation thereby

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causing oxidative stress in kidney of rats. An increase in oxidative stress is considered an important pathogenic mechanism in the development of various complications like kidney diseases. Apoptosis is cell death to remove aberrant cells and involves two main families of proteins cystein proteases called caspase enzymes and Bcl-2 family (Pio *et al*., 2013). There are 2 major apoptosis signaling pathways, mitochondrial and death receptor pathways. The mitochondrial apoptotic pathway is initiated within the cell in which pro-apoptotic proteins are released from mitochondria to activate caspase proteases triggering apoptosis (Lessene *et al*., 2008). Apoptosis can result from multiple stimuli, including free radicals (Pio *et al*., 2013). Reactive oxygen speices are formed by exposure to several agents, causing oxidative damage in tissues may lead to mitochondrial injury consequently playing an important role in the apoptotic mechanisms (Al-Shobaili *et al*., 2013). The aberrant production of reactive oxygen species due to contact with chemicals may result in a number of clinical disorders (Roy *et al*., 2012). While several studies have reported the toxic effect of BPA on reproductive, neurological and endocrine tissues in animals, accordingly the aim the present study was to investigate the toxic effect of BPA in the kidney of pregnant rats.

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.

Reagents and Chemicals

All the reagents used were of analytical grade. Bisphenol-A (BPA) was purchased from Sigma-Aldrich, oxidized and reduced glutathione, were procured from SRL, India. Eosin and Hematoxylin were procured from Merck, India.

Experimental design

Female Wistar rats (Rattus norvergicus) three months old, weighing 200g to 300 g were used for the experiment. The status of estrous cycle stages were determined every morning between 8:00 and 9:00 a.m 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 8^{th} to 15^{th} day of gestation period (total 8 days) of female albino rats. All pregnant rats were scarified on 15^{th} gestational day using a CO_2 inhalation chamber. Tissues from animals receiving 50 and 500 mg /day of BPA and from vehicle-infused control animals were frozen in liquid nitrogen and stored at -80^0c for enzyme assay, determination of malondialdehyde, histopathology and further analysis of various proteins.

Homogenate preparation

Segments of kidney from all the experimental groups were excised separately and minced in ice-cold saline. A known weight of tissue was homogenized in 10ml buffer (0.1M phosphate buffer, pH 8.0) with 2 mM EDTA and 0.5% Triton-X-100 by a tissue homogenizer on ice. The tissue homogenate was then centrifuged and the supernatant was collected and stored at -20°C for further study.

Biochemical assay

Superoxide dismutase (SOD) activity was measured as per the protocol of Marklund and Marklund, 1974. 1 unit of SOD was defined as the enzyme activity that inhibits the autooxidation of pyrogallol by 50%. Catalase (CAT) activity was measured following the protocol of Sinha *et al*, 1972 with slight modifications. Glutathione peroxidase (GPX) activity was estimated by the method of Rotruck *et al*, 1973. The amount of Malondialdehyde (MDA) was estimated according to the protocol of Devasagayam and Tarachand, 1987.

Histological technique for the morphological study of the kidney tissue

NBF fixed and paraffin impregnated kidney tissue sections were stained with normal hematoxylin-eosin stain according to the method of Bancroft *et al*, 200328 with slight modifications. Briefly, 5µm paraffin section was kept sequentially in xylene and graded ethanol and stained with hematoxylin for 2 minutes. After removing the excess colour the slide was counterstained with eosin and then the stained slides were dehydrated with graded ethanol, cleared with xylene and mounted with DPX and were observed under the microscope (400X magnification). Images were obtained by digital SLR Olympus Camera (E-620) fitted with Olympus light microscope (CH20i).

Western blot analysis

Tissues collected were homogenized with ice-cold homogenizing buffer (50mM Tris-HCl, 150mM NaCl, 1mM EDTA, and 0.5mM Triton X-100, PH 7.4). Kidney protein lysates (50 μg/well) were separated by SDS-PADE (12.5%) under reducing conditions with bovine serum albumin as a standard and transferred to a Nitrocellulose membrane (NCM) membrane. Blocking of membranes was performed with TTBS buffer (20 mM Tris [pH7.4], 150 mM NaCl, and 0.05% Tween

20) containing 5% non-fat dry milk for 1 h and then washed with TTBS buffer. For the detection of proteins on nitrocellulose membrane, antibodies to Bcl-2 (catalog no. SC-492), caspase-3 (catalog no. 9961), were diluted 1:1000 respectively. Polyclonal beta-actin antibody (catalog no. SC-9104) and monoclonal heat shock protein 60 (HSP60) antibodies (catalog no. SC-13115; Santa Cruz Biotechnology) were used at a dilution of 1:1000. Blots were exposed to horseradish peroxidase-conjugated anti-rabbit IgG (caspase-3, Bcl2), secondary antibodies (diluted 2000- to 5000-fold) for 1 h. The anti-rabbit IgG secondary antibody was purchased from Santa Cruz Biotechnology. The blots were rinsed, and the enhanced chemiluminescence reagent (ECL kit) was added and incubated for 1 min and then exposed to Hyper film ECL. The intensity of specific immunoreactive bands was quantified by a Image J software. All replicates from each group were run in one gel, and the proteins are expressed as a ratio of protein signal to the beta-actin signal.

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 (Snedecor and Cochron, 1967).

RESULTS

Effect of BPA on antioxidant enzymes (Super oxide dismutase, Catalase, Glutathione peroxidise, Lipid peroxidise) in rat kidney tissues

In the present study, super oxide dismutase, catalase, glutathione peroxidise activities in tissue of BPA treated rats (lower dose or 50mg/kg.b.w/day and higher dose or 500mg/kg.b.w/day) showed significant variations ($P<0.05$), (Fig-1),

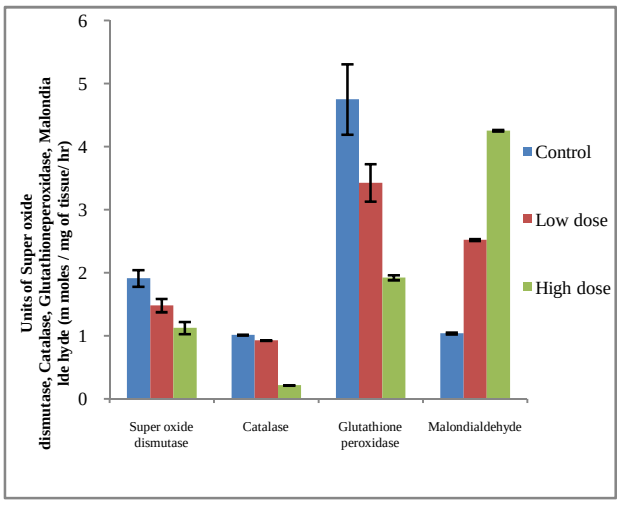

Fig-1 Superoxide dismutase, Catalase, Glutathione peroxidase, Malondialdehyde activity levels in different kidney tissues of albino rats exposed to BPA

when compared to control group. On treatment with BPA rats, showed significantly decreased super oxide dismutase, catalase, glutathione peroxidise activities, and significant elevated levels of malondialdehyde activity were found in the treated tissues, the gradual increased malondialdehyde activity was found in the tissues of higher dose, lower dose BPA treated groups.

Histological study

We found distinguishable degenerative changes in tissues of the hematoxylin-eosin stained tissue sections of treated rats (Figure: 2a-2c).

The tests of the BPA-treated animals exhibited morphological changes compared to the control group (Fig.2a). BPA at the low (50mg/kg/day) & high (500 mg/kg/day) doses cause dilation Bowman's space and hypercelluarity of glomerulus (Fig-2b & 2c). Also we observed that BPA induced degeneration of epithelial of proximal tube.

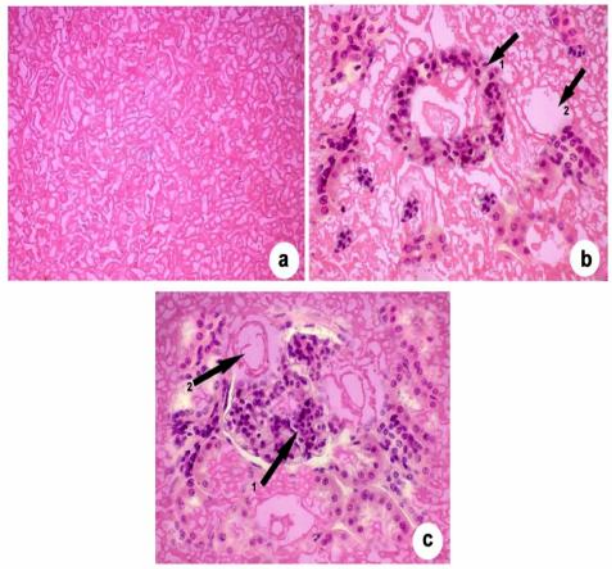

Fig-2 Morphology of kidney tissue: Photomicrographs of a rat kidney tissue (a) is control, (b)-50 mg/kg/d BPA & (c)-500 mg/kg/d BPA are injured kidney tissues, the arrow 1: showed degeneration of proximal tubule epithelium and symptoms of cell's nucleus piknosis, the arrow 2: showed glomerulus given to the dilation of Bowman's space and hypercelluarity (stained by hematoxylin - eosin, magnification × 400).

Effect of BPA on expression of proapoptotic protein caspase-3&anti apoptotic protein Bcl2 in kidney tissues

Various degrees of active caspase-3 & Bcl2 expressions in the kidney tissues of all groups (BPA treated and untreated control rats) were detected by Western blotting analysis (Fig-3). Image and statistical analyses showed that there was a significant difference in relative expression levels of active caspase-3&Bcl2 between the control and treated groups (Fig-4). In the low & high dose treated rat groups, expression levels of active caspase-3 were increased & decreased Bcl2 when compared to corresponding controls. Moreover, expression of active caspase-3 was significantly increased with the increasing BPA dose and normal level of expression in untreated rats.

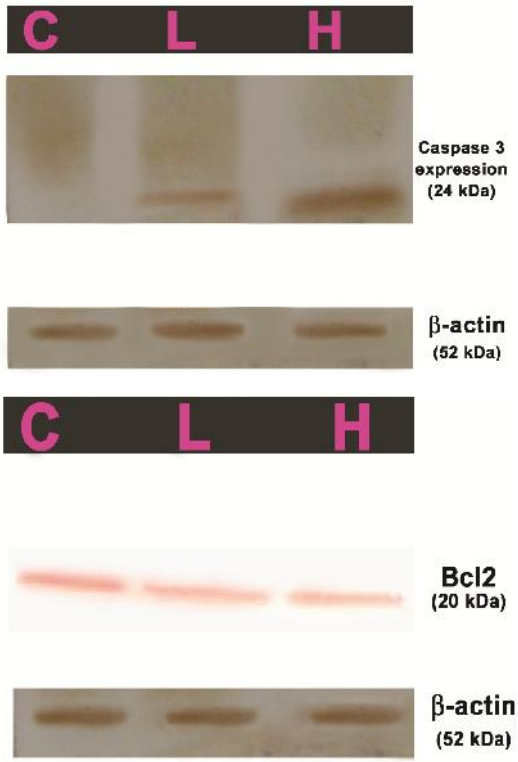

Fig-3 Caspase-3 and Bcl_2 activity levels in different kidney tissues of albino rats exposed to BPA
C: Control; L: Low dose (50 mg/kg/d BPA); H: High dose (500 mg/kg/d BPA)

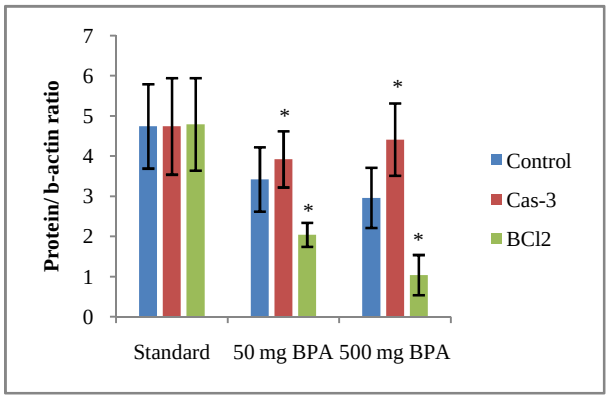

Fig-4 Densitometric analysis of the representative protein bands normalized to β-actin.

'*' indicates significant differences in BPA treated groups compared with the control.

DISCUSSION

Exposure to several chemicals and environmental contaminants has been reported to increase oxidative stress in body by disturbing the prooxidant/antioxidant balance of cells (M. Aydogan, *et al*., 2008). BPA was previously reported to induce oxidative damage in several tissues (A. Korkmaz *et al*., 2011). Some free radicals are generated as by product of metabolism and are extremely reactive. However, biological systems have evolved endogenous defense mechanisms against these free radical induced cell damage. SOD, CAT and GPX are the primary antioxidant enzymes which directly eliminate reactive oxygen species via hydroxyl radical, superoxide radical, hydrogen peroxide etc. The stress conditions depend on the degree of production or inactivation of these antioxidant enzymes (Krutz F, *et al*., 2002). Our aim was to study the effects of BPA on antioxidant defence mechanisms in kidney tissue. In the present study the activities of antioxidant enzymes like SOD, CAT, GPx, were decreased and the amount of malondialdehyde, a biomarker of lipid peroxidation, was increased in BPA treated rats compared to the respective vehicle control group of rats.

From our study it is suggested that BPA might induce the oxidative stress in renal tissue by inhibiting the activities of antioxidant enzymes as a result of the accumulation of reactive oxygen species (ROS) in the heart. The accumulated ROS in turn induces lipid peroxidation in the biological membranes (Sato M, *et al*., 1996) as revealed by the increase in MDA level in our study. In order to study the oxidative stress induced kidney tissue damage we have studied the degenerative changes in the tissue in histological sections of kidney in BPA treated animals. The sizes of the nuclei were enlarged and the shapes were rounded &degeneration of proximal tubule epithelium in BPA treated rats. This result suggests that BPA inhibits the function of kidney presumably by inducing the tissue damage through elevation of ROS levels in cells as a result of inhibition of antioxidant enzymes. Oxidative stress plays an important role in kidney pathogenesis (parlakpine *et al*., 2005). The aberrant level of antioxidant enzyme activities in kidneys in the BPA group exposed to low & high doses showed that BPA may cause nephrotoxicity via free radical generation.

The present study results revealed that the relation between BPA concentration and cell death pathways in kidney tissue of pregnant rats, proapoptotic protein caspase-3 & antiapoptotic protein Bcl2. The caspase-3 protein expression levels were significantly increased & decreased Bcl2 activity in low & high BPA concentration group of rats, which may be activate of apoptotic path ways and alter the Bcl2 activity. This is in agreement with the previous study of Lin *et al*, (2013) that found BPA suppressed cell viability and disturbed insulin secretion in a dose dependent manner. BPA also has cytotoxic actions on various cells and tissues through multiple signaling pathways (Wetherill *et al*., 2007). Kluck *et al*., (1997) observed that, an alteration in the ratio of anti-apoptotic protein-Bcl2, resided on the outer membrane of mitochondria, may modulate the release of apoptogenic proteins. BPA-induced apoptosis has been demonstrated in cultured liver cells reported by Nakagawa *et al*., (2000).

In conclusion, the results of this study revealed that oral BPA administration induced adverse oxidative effects on the exposed animals as evidenced by the recorded abnormalities in the investigated biochemical parameters. In addition, there were histopathological alter-ations and expression of apoptotic protein in the investigated organs.

References

1. Korkmaz, M. Aydogan, D. Kolankaya, N. Barlas. (2011). Vitamin C coad-ministration augments bisphenol A, nonylphenol, and octylphenolinduced

oxidative damage on kidney of rats, Environ. Toxicol. 26 (4), 325–337.
2. Al-Shobaili, A. Al-robaee, A. Alzolibani and Z. Rasheed. (2013). Immunological studies of reactive oxygen species damaged catalase in patients with systemic lupus erythematosus: correlation with disease activity index. Immunol. Invest. 42, 191-203.
3. Burridge E. (2008). Chemical profile: bisphenol A. ICIS Chem Business 274:48.
4. Bindhumol V, Chitra KC and Mathur PP. (2003). Bisphenol A induces reactive oxygen species generation in the liver of male rats, Toxicol, 188(2-3): 117-124.
5. Chitra KC, Rao KR and Mathur P. (1995). Effect of bisphenol A and co -administration of bisphenol A and vitamin C on epididymis of adult rats: a histopathological and biochemical study, Asian J Andro, 5: 203-208.
6. Dekant W and W. Volkel. (2008). human exposure to bisphenol a by biomonitering: methods, results and assessment of environmental exposures. Toxicol. Appl. Pharmacol., 228,114-134.
7. Hernandez-Rodriguez, G., Zumbado, M., Luzardo, O.P., Monterde, J.G., Blanco, A. and Boada, L.D. (2007). Multigenerational study of the hepatic effects exerted by the consumption of Haniokanonylphenol and 4-octylphenol contaminated drinking water in Sprague-Dawley rats. Environ. Toxicol. Pharmacol. 23: 73-81.
8. Huang YQ, Wong CK, Zheng JS, Bouwman H, Barra R, Wahlstrom B, Neretin Land Wong MH. (2011). Bisphenol-A (BPA) in China: A review of sources, environmental levels, and potential human health impacts. Environ Int. vol.May 17, In press.
9. Kabuto H, Amakawa M and Shishibori T.(2004).Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice, Life Sci, 74: 2931-2940.
10. Kluck, R.M., Bossy-Wetzel, E., Green, D.R. and Newmeyer, D.D. (1997). The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science. 275: 1132–1136.
11. Lin, Y., X .Sun, L. Qiu, J. Wei, Q. Huang, C. Fang T. Ye, M. Kang, H. Shen, and S.Dong. (2013). Exposure to bisphenol A induces disfunction of insulin secretion and apoptosis through the damage of mitochondria in rat insulinoma(INS-1) cells. Cell death dis., 4, e460.
12. Lessene, G., Czabotor,P. and P.Colman. (2008). Bcl-2 family antagonists for cancer therapy. nat. rev. Drug. Discov, 7, 989-1000.
13. M. Aydogan, A. Korkmaz, N. Barlas, D. Kolankaya.(2008). The effect of vita-min C on bisphenol A, nonylphenol and octylphenol induced braindamages of male rats, Toxicology 249 ,35–39.
14. Marklund S and Marklund G. (1974).Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, European J Biochem, 47: 469-474.
15. Nakagawa, Y. and Tayama, S. (2000). Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. Arch. Toxicol. 74: 99-105.
16. Parlakpinar, H., S.Tasdemir, A. Polat, A. Bay-Karabulut, N.Vardi, M.Ucar and A. Acet. (2007). Protective role of caffeic acid phenethyl ester on gentamicin – induced acute renal toxicity in rats. Toxicology, 207,169-77.
17. Pio., J., Z. Cui, Y. Furusawa, K. Ahmed , M. Rehman, Y.Tabhuchi, M.Kadowaki and T.Kondo. (2013).The molecular mechanisms and gene expression profiling for shikonin induced apoptotic and necroptotic cell death in U937 cells. Chemi. boil. Interact., 205,119-127.
18. Prins, G., W. Tang, J. Belmonte and S. Ho: (2008). Developmental exposure to bisphenol a increases prostate cancer susceptibility in adult rats: epigenetic mode of action is implicated. Fertile. Sertil., 89, e 41.
19. Rotruct JT, Pope AL, Ganther HE and Swanson AB, Selenium. (1973). Biochemical roles as a component of glutathione peroxidase, Science, 179: 588-590.
20. Roy, N., S. Bagchi and P. Roychoudari. (2012). Damaged DNA binding protein 2 in reactive oxygen species regulation and premature senescence. int. j. mol. sci., 13, 11012-26.
21. Sato M, Ramarathnam N, Suzuki Y, Ohkubo T, Takeuchi M and Ochi H. (1996).Variental differences in the phenolic content and superoxide radical scavenging potential of wines from different sources, J Agr Food Chem, 44: 37-40.
22. Sinha KA, Colorimetric assay of catalase, Anal Biochem, 47: 389-394, (1972).
23. Vandenberg, L.N., Chahoud, I., Padmanabhan, V.,Paumgartten, F. J., and Schoenfelder, G.(2010). Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A. Journal of Environmental Health Perspectives, vol. 118, no. 8, pp. 1051-1054.
24. Wetherill, Y.B., Akingbemi, B.T, Kanno, J., McLachlan, J.A., Nadal, A., Sonnenschein, C., Watson, C.S., Zoeller, R.T. and Belcher, S.M. (2007). In vitro molecular mechanisms of bisphenol A action. Reproductive Toxicology (Elmsford, N.Y.). 24: 178-98.

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