J. Vet. Med. OH Res. (2019). 1 (1): 75-83 p-2664-2352 : **ISSN** : e-2664-2360
Website: www.lepvmbj.org DOI: https://doi.org/10.36111/jvmohr.2019.1(1).0007

EVALUATION OF BISPHENOL A INDUCED EFFECTS ON BLOOD BIO-CHEMICAL CONSTITUENTS AND HISTO-STRUCTURE OF LIVER IN SWISS ALBINO MICE AND ITS 'ONE HEALTH' PERSPECTIVES

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ABSTRACT

Background: Bisphenol A (BPA) is a plastic synthetic chemical ingredient produced widely in large quantities for use primarily in production of polycarbonate plastics and epoxy resins worldwide. The toxicity of BPA in human health is associated with enzymatic, androgenic, neurologic, liver and reproductive systems which have been reported but its toxicokinetics has not been established and even its status is not documented in Bangladesh.

Objectives: The aim of this study was to detect the BPA induced effects on blood bio-chemical constituents and histo-texture of liver in adult Swiss albino mice.

Materials and Methods: Fifteen mice, 6 to 8 weeks of age with an average bwt 27.1 ± 0.5g, were equally divided into three groups (n = 5). Group A (control) received only normal mouse pellet feed while groups B and C received pellet feed mixed with BPA (Sigma-Aldrich Co., USA) @ 50 mg and 100 mg / kg bwt daily for 12 weeks, respectively. Sera of all the mice were tested for biochemical constituents and liver tissues for histo-pathological studies.

Results: Results showed that cholesterol and LDL levels were elevated significantly ($p < 0.01$) in both the BPA treated groups (B and C) respectively in comparison to control group A. The HDL cholesterol and TG levels in mice of group C treated with BPA @100 mg / kg BW were significantly ($p < 0.01$) reduced in comparison to control group of mice. Serum glucose level was significantly ($p < 0.1$) decreased in both the BPA treated groups (B and C) whereas total serum protein level in mice of group C significantly ($p < 0.1$) increased in comparison to control group A. The liver enzymes (ALT, AST & ALP) were also significantly ($p < 0.01$) increased in BPA treated mice in comparison to control. Histo-pathological alterations were also detected in the liver of BPA-treated mice of both the groups.

Conclusions: These findings provide evidence of changes in the blood bio-chemical constituents and liver histo-texture induced by BPA and may have implications for understanding the toxicity of BPA in animals and humans. Further research may be performed on the status of level of BPA in food and beverage of plastic containers and their impact on human health in Bangladesh.

Keywords: Bisphenol A, Mice, Induced effects, Biochemical constituents, Histo-structure of liver, Public health importance

Article Info: Article Code No. © LEP: JVMOHR/0007/2019
Received: 10 May 2019 Revised: 18 May 2019 Accepted: 10 June 2019 Published: 30 June 2019

Citation: Hoque E, Sujan KM, Haque MI, Mustary A, Miah MA, Hossain MM and Islam MK (2019). Evaluation of Bisphenol A induced effects on blood biochemical constituents and histo-structure of liver in Swiss albino mice and its 'One Health' perspectives. *J. Vet. Med. OH Res.* 1 (1): 75-85

INTRODUCTION

BPA is a multi-purpose chemical compound used in combination with other chemicals in the manufacture of polycarbonate plastics and epoxy resins all over the world including Bangladesh.[1] Polycarbonate plastics are used in many consumer products, including food and beverage containers (food and drink packaging, e.g. water and infant bottles), tableware (plates and mugs), toys, eyeglasses, computers, kitchen appliances, impact resistant safety equipment and medical devices. Epoxy resins containing the chemicals are used in dental work, metal coatings for food cans, bottle tops, water supply pipes, cars, dairy and office equipment and other metal products.

The canned food and beverage industries appeared to grow rapidly due to heavy demand for packaged and convenience foods worldwide. Research results indicate that leaching of BPA from plastics and food cans lined with epoxy resins. BPA molecules linking with ester bond in polycarbonate and resins is subject to hydrolysis, resulting in leaking of PBA monomer even from polycarbonate and resins into the water at room temperature.[2] The ubiquity of BPA in the environment and in the human body has led to concerns about potential adverse health effects. The major adverse effects of BPA on human health have been reported as changes in neuronal development and reproductive system associated with decreased fertility, endocrine changes, metabolic, cardiovascular and immunological diseases, damage to genetic material and cancer with more sensitive populations are women during pregnancy and lactation, infants and children.[3] There is an increasing incidence of exposure to BPA worldwide, with an average increase of 2.5 to 3.0% per year.[4] The liver is the primary organ responsible for BPA metabolism in humans and animals.[5] Low doses of BPA show oxidative stress in liver of male rats.[6] The plastic sector in Bangladesh has around 3,000 manufacturing units that offer jobs to more than two million people with current market size is around US $ 1 billion but the negative image of this sector is the defective management of plastic wastes.[1] Moreover, the research works and even review reports on health risk assessment associated with toxic effects of plastic in human health and environment are very limited in Bangladesh.[7] Considering these facts, the present study was conducted to assess the BPA induced effects on blood bio-chemical constituents and histo-pathological changes in liver of adult Swiss albino mice.

MATERIALS AND METHODS

Laboratory mice and rats are the most commonly used biomedical laboratory animal models for studying potential BPA human health effects. This study was conducted on 15 apparently healthy female Swiss albino mice (*Mus musculus*), aged between 6 and 8 weeks with an average body weight of 27.10 ± 0.5g during the period from February to April 2018. These 15 mice were randomly divided into three groups, each consisted of five mice. Group A (control group) received only basal mouse pellet feed mixed with sunflower oil, while other two groups (groups B and C) received basal pellet feed mixed with formulated sunflower oil (as vehicle for BPA) containing two different doses of BPA (Sigma-Aldrich Co., USA) @ 50 mg and 100 mg / kg body weight daily for 12 weeks, respectively.

Serum biochemistry

Mice were sacrificed 24 hours following the last supplied dose of BPA. The mice were kept fasting overnight. Then the mice were placed one by one in an airtight container containing diethyl ether pre-soaked cotton. They were checked for unconsciousness. The mice were taken out from the airtight container and 1.0 to 1.5 ml blood samples were collected directly from heart by a sterile syringe. Serum was separated by centrifugation as per conventional method and stored at – 20 ^{0}C until tested.

Total serum protein (Total protein liquicolor), total cholesterol (Cholesterol CHOD-POD liquid), triglyceride (Triglycerides GPO-POD liquid), high density lipid (HDL) and low density lipid (LDL, HDL cholesterol-P precipitating reagents), Alanine transaminase (GPT (ALT) NADH. Kinetic UV. IFCC rec. Liquid), Aspartate aminotransferase (GOT (AST)-LQ. NADH. Kinetic UV. IFCC rec. Liquid) and Alkaline phosphatase (Alkaline Phosphatase p-Nitrophenylphosphate.kinetic. Liquid. DGKC) were measured by using the commercial reagents (Reactivous GPL, Barcelona, Spain) with the help of UV/VIS Spectrophotometer (Model: T80, PG Instrument, USA). The serum glucose was measured by using Glucose (GOD-PAP) reagent (Biocon Diagnostik, Germany).

Histopathology

Liver tissues were rapidly collected after completely removal of blood from heart and tissues were fixed in 10% neutral buffered formol-saline for 15 days. The well fixed tissues were processed, sectioned and stained with Hematoxylin and Eosin (H & E) for histo-pathological study.[8] The stained slides were observed under Optka Vision Lite 21 and photographs of the characteristic findings were recorded. Histologically, the degenerative lesions of liver in all groups of experimental mice were graded as mild (+), moderate (++) and severe (+++).

Statistical analysis

Data were continuous and normally distributed. One-way analysis of variance (ANOVA) was used to determine the effect of different parameters. The data was placed and stored in Microsoft Excel- 2007 and imported to the software IBM SPSS Statistics 20 for analysis. Descriptive statistics analysis was done to measure the mean, standard deviation and standard error and p value of different parameters. Because of using multiple comparisons, the corrected p value was calculated adjusted at 0.01 and 0.05 considered for level of significance.

RESULTS AND DISCUSSION

Bisphenol A (BPA) is one of the most commonly industrial synthetic chemicals produced worldwide. BPA is used to make polycarbonate plastic and epoxy resins that are used in making plastic products ranging from bottles and food can linings to toys and water supply pipes, including many applications important to public health and food safety. BPA was synthesized for the first time in 1891 by the Russian chemist Alexander P. Dianin and commercial production began in 1957 for PC plastic application and with current production estimated at 4 billion kilograms[9] to 15 billion pounds in 2013 annually.[10,11] The global volume of BPA consumption was estimated at 7.7 million metric tons in 2015, is forecast to be 8

million metric tons in 2016 and is expected to reach 10.6 million metric tons in 2022 at a compound annual growth rate of 4.8% between 2016 and 2022.[3]

All types of plastic products degrades, BPA is released into the environment and routinely ingested by humans. Metabolic changes that take place once BPA is broken down inside the body that poses the greater health threat. BPA metabolites actually bind to the estrogen receptor much more strongly than BPA itself due to similar molecular structure with that of estradiol that disrupt the body's endocrine or hormone system. Accordingly, the BPA classified as endocrine disruptors include not only hormone-mimics or antagonists that act via binding receptors and interact with the thyroid receptor but also can interfere with hormone synthesis and clearance, as well as other aspects of tissue metabolism.[12]

Human exposure to BPA is mainly plastic industry workers at the work place, and consumption and drinking of plastic bottle and can foods, where polymers migrate into food and beverages. Once absorbed, BPA is conjugated in the liver by glucuronidation and excreted mainly through bile but also through urine. Consumption of BPA contaminated food and beverages are associated with endocrine disruptions to the rise of cancer, developmental problems, diabetes and possibly also obesity and the metabolic syndrome.[13] Animal and human research has associated BPA with many health problems including infertility, weight gain, behavioral changes, early onset of puberty, prostate and mammary gland cancers, cardiovascular effects, obesity and diabetes.[14]

The reference dose of BPA is defined as 50 μg/kg body weight / day by the Environmental Protection Agency with lower doses are considered safe but lower doses may increase risk of developing fetus and newborns. Early life exposure to low dose BPA may increase the risk of developing adult onset of disease and the biological changes can transmit to the 3rd or 4th generation. Therefore, European Food Safety Authority (EFSA) proposed the tolerable daily intake (TDI) levels of BPA from 50 to 4 μg/ kg body weight /day.

BPA induced serum biochemistry in mice

Effect of BPA toxicity study in animal models revealed that it interacts with steroid receptors and interferes with lipid, glycogen and other biochemical metabolism. Serum cholesterol is a term that includes the total level of cholesterol found in the blood stream. Measuring the level of total cholesterol includes identifying all types or classes of cholesterol that are found in the system. Intestinal cholesterol absorption plays a major role in maintaining total body cholesterol homeostasis, the present study is to investigate whether BPA affects cholesterol level.

The mice treated either with BPA @ 50 mg (group B) or 100 mg (group C) / kg body weight daily for 12 weeks exhibited a significant elevation in the total cholesterol and LDL cholesterol in both the treated groups at the end of experiment **(Table 1)**. On the other hand, TG and HDL levels were decreased in mice treated with 100 mg BPA where the degree of reduction was more prominent for serum TG ($p < 0.01$) compared to serum HDL ($p < 0.05$). This significant increase in serum total cholesterol and LDH cholesterol with concomitant decrease of TG and HDL cholesterol in mice is closely agreeable with the earlier reports.[15,16] The decreasing tendency of TG recorded in this study goes in parallel with earlier report made with BPA

Table 1. Effects of Bisphenol-A (BPA) on bio-chemical constituents in mice

SN Parameters	Control Group A (n=5) (BPA 0 mg / kg)	BPA-treated groups of mice Group B (n=5) (BPA 50 mg / kg)	Group C (n=5) (BPA 100 mg /kg)
① Cholesterol (mg/dl)	225.06 ± 4.65	244.88 ± 2.83**	256.42 ± 3.88**
② HDL (mg/dl)	44.29 ± 1.27	41.68 ± 1.45	39.68 ± 1.22*
③ LDL (mg/dl)	153.36 ± 4.35	177.42 ± 4.23**	192.60 ± 5.03**
④ TG (mg/dl)	137.04 ± 2.52	128.87 ± 2.94	120.72 ± 2.81**
⑤ SG (mg/dl)	185.25 ± 2.76	146.74 ± 1.80**	136.50 ± 3.25**
⑥ TSP (g/dl)	7.09 ± 0.59	8.20 ± 0.30	9.19 ± 0.57*
⑦ ALT (u/l)	3.07 ± 0.05	4.02 ± 2.20**	4.15 ± 0.20**
⑧ AST (u/l)	14.66 ± 1.33	21.89 ± 1.71**	29.86 ± 2.53**
⑨ ALP (u/l)	33.61 ± 3.44	42.32 ± 1.70*	48.29 ± 1.47**

HDL = High density lipoprotein LDL = Low density lipoprotein TG = Triglycerides
SG = Serum glucose TSP = Total serum protein n = No. of mice
ALT = Alanine transaminase AST = Aspartate aminotransferase ALP = Alkaline phosphatase
*Significant at 5% level ($p < 0.05$) **Significant at 1% level ($p < 0.01$)

related compound @100 and 300 mg/kg bodyweight.[17] A decrease in HDL with increased LDL might be manifested by the probable disruption of estrogenic signaling pathway by BPA because estrogen is known to play an important role in reducing serum cholesterol.[18] BPA can induce estrogenic activity where estrogens have a significant effect on plasma cholesterol. The effect of cholesterol is probably due to an action of the hormone on the lipoproteins associated with cholesterol in the circulation.[19,20]

BPA treated mice of both the groups B and C showed significantly ($p < 0.01$) decreased blood glucose levels **(Table 1)**. This finding supports the earlier reports on rat model treated with BPA @ 10 µg / kg body weight that produced an increase in the pancreatic insulin content, a rapid increase in plasma insulin and hence a decrease in blood glucose. [21,22] These results also indirectly support that the supply of BPA by oral gavage causes anorexia which lead to decreased feed consumption and so on to decreased body weight gain in rats.[23]

Mice of group C treated with higher dose of BPA @ 100 mg / kg body weight showed significantly ($p < 0.05$) increased total serum protein in comparison to mice of control group A **(Table 1)**. The elevated level of total serum protein in mice treated with higher dose of PBA could be a consequence of the destruction of cellular organelles and subsequent release of proteins into the circulation. [21,22]

Treatment with BPA either low (50 mg/kg bwt) or high (100 mg/kg bwt) doses significantly ($P<0.01$) increased the liver enzyme activities (ALT, AST and ALP) as compared to the control group (Table 1). Whereas, ALP significantly increased with a degree of ($p<0.05$) and ($p<0.01$) in BPA 50 mg and BPA 100 mg treated mice respectively (Table 1). These findings are in support with the earlier observation that BPA @ 100 µg/kg bwt had significant increased effects on ALT and AST values in rats.[24] However, low concentrations of BPA had no

significant effect on liver tissue and enzymes but its increased concentration can cause damage to liver tissue and increase the serum levels of liver enzymes.

The physiological and biochemical activities in the albino mice were completely disturbed after the oral administration of BPA. The overall protein turnover in animal is the dynamic equilibrium between synthesis and degradation rates. BPA induced oxidative stress in cells are known to damage protein and showed decreased protein content in BPA administered animal. The high dose of BPA induces damage affecting blood 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.[25]

BPA induced liver histo-structure in mice

The liver is the primary organ responsible for BPA metabolism in humans and animals.[5] The liver is reported to be affected by BPA in animal studies and has been also reported to possess hepatic toxicity. Low doses of BPA show oxidative stress in liver of male rats.[6] Histological findings of liver in mice treated with BPA were compared with the findings of untreated control mice. Control group of mice showed normal histological structure with mild (+) vacuolar degeneration **(Photo 1)**. Experimentally 50 mg BPA inoculated mice showed mild (+) vacuolar degeneration with mild (+) degeneration of hepatocytes characterized by acidophilic cytoplasm **(Photo 2)**, while 100 mg BPA inoculated mice showed almost similar degree of vacuolar degeneration (+) with diffuse and severe (++) acidophilic cytoplasm **(Photo 3)**. However, the changes in liver induced with BPA in both the experimental groups of mice were found variables. The hepatotoxicity in rats exposed to BPA @ 5 mg / kg body weight / day have been reported.[26]

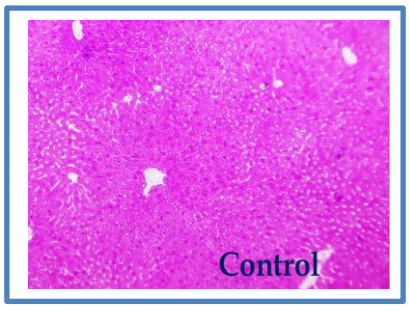

Photo 1. Normal histology of liver of mice with vacuolar degeneration (+) of control group (H & E, 10 ×)

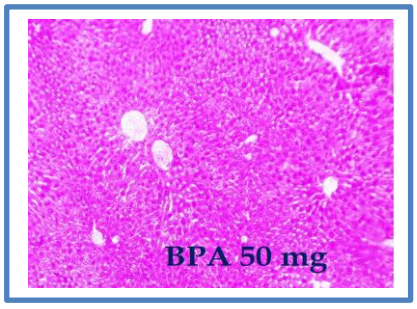

Photo 2. Degeneration of hepatocytes (+) with acidophilic cytoplasm and vacuolar degeneration (+) in mice received 50 mg BPA (H & E, 10 ×)

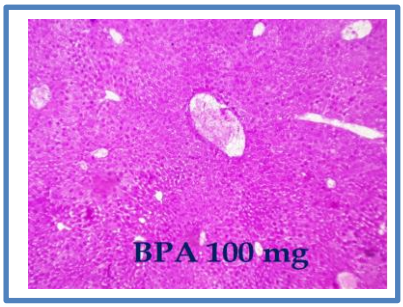

Photo 3. Vacuolar degeneration (+) with diffuse acidophilic cytoplasm (++) in mice treated with 100 mg BPA (H & E. 10 ×)

The high dose of BPA @ 50 mg / kg induces liver damage, affecting oxidant / antioxidant balance, as a result of reactive oxygen species in rat liver and the dose @ 10 mg / kg could cause damage and must be taken in consideration.[26] Bi-nucleated liver cells, hyper-chromatic nuclei, karyomegaly, extensive bile duct proliferation with dysplasia and proliferation of

endothelial cells in BPA (500 mg and 250mg/kg bwt) treated groups of rats have also been reported.[27] Hepatic damage induced by BPA may be due to accumulation of BPA toxic metabolites and ability of the generation of ROS in the liver. The vascular degeneration, necrosis, widening of blood sinusoids, vacuolization swelling in hepatocytes and pyknosis in nuclei with increased number of Kupffer cells have also been reported due to induced BPA in rats.[5] BPA only @ 100μg/kg bwt caused inflammation and vacuolization in liver tissue and its low concentrations had no significant effect on the liver tissue.[24]

In addition to liver, other internal organs have also been reported to be affected with induced BPA in laboratory animals. Exposure to BPA resulted in structural anomalies in the rat myocardium in the form of disarrangement of myofibers, hypertrophy of myocytes, myocardial fibrosis and dilatation of intramyocardial arterioles.[28] The sub-acute toxicity of BPA caused a reduction of the epididymal sperm count, sperm motility, head and tail abnormalities, and affect the germ cells leading to impairment in the spermatogenesis and also suppress the bone marrow functioning which lead to normocytic hypochromic anemia in rats.[2,29]

Considering the potential public health risks posed by BPA,[30] there are about 40 countries that have adopted restrictive policies on BPA use in food contact plastics especially intended for growing children and pregnant women.[31] Therefore, it is the high time for Bangladesh to investigate the status of BPA sources and toxicity in the environment and humans through laboratory animal model considering 'One Health' perspective to reduce the toxic effects of the widely used plastic on human health and environment.

CONCLUSIONS

This study showed that exposure to the two doses of BPA (50 mg and 100 mg / kg BW orally for 12 weeks) induces dysfunction in the blood bio-chemistry and histopathological structure of the liver in mice. Increased cholesterol and LDL levels due to BPA exposure is a risk key factor in the development of cardiac diseases. Abnormally higher pancreatic secretion caused by BPA is manifested by the reduction in glucose level which could eventually lead the individual to suffer from type 1 diabetes mellitus and cardiovascular problems as well. Results of this study indicate that the BPA driven damage at cellular level which demands further extensive research on this aspect. Moreover, identifying populations that are highly exposed to environmental chemicals and their patterns of exposure in populations are important for possible source of exposure and protecting public health and preventing health equalities. Application of proper rules and regulations for the production and use of plastics can reduce toxic effects of plastics on human health and environment.

CONFLICT OF INTEREST

There is no conflict of interests of this article.

ACKNOWLEDGEMENTS

The authors thank the authority of the BAU Central Laboratory and Department of Pathology, Mymensingh Medical College for analysis of blood biochemical constituents and histo-pathological studies of liver, respectively. This research work was supported by the NST

fellowship of Bangladesh for MS degree to the first author of this article (Grant No. 2091/311/2017-2018).

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