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Bisphenol A Exerts a Transient Perturbation of Liver Function in Wistar Albino Rats at Acute and Sub-Chronic Exposure Doses

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ABSTRACT:

Bisphenol A (BPA) is a well-known endocrine-disrupting chemical found in the environment. Oral exposure from food is generally considered the major source of BPA exposure for all age groups for non-occupationally exposed individuals. Apart from its oestrogenic property there are uncertainties about its effects on liver functions at very low doses over short time exposure periods. This study was carried out to determine the acute and sub-chronic exposure effects of BPA on the liver functions and plasma proteins of albino rats following oral administration. To five experimental groups each containing five (5) female rats was administered 50, 100, 150, 200, and 250 µg BPA/kgbw/day. To the sixth control group was given water. A replicate group of experimental rats were similarly treated for seven (7) days to ascertain possible sub-chronic effects. Animals were sacrificed at the end of the respective studies and sample specimens analyzed by routine diagnostic procedures for plasma protein profile and liver functions using Chemwell Chemical Analyzer. The result reveals significant decreases in serum total protein and albumin and elevated values of AST and ALT (suggesting liver damage) as well as alkaline phosphatase and bilirubin (suggestive of jaundice).It is clear from the results that low doses of BPA exert adverse effects on the liver functions in the Wistar rats even for a short exposure period. Histological evaluations of the livers did not reveal any gross lesion and microscopic analysis show normal hepatic parenchymal-vascular relationship with no fatty changes, apoptotic bodies nor hepatic necrosis.

KEY WORDS: acute, sub chronic, toxicity,graded doses, bisphenol A, liver and functions

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INTRODUCTION:

Bisphenol A (BPA) with the IUPAC nomenclature 4, 4'-(propane-2, 2-diyl)diphenol is a carbon-based synthetic compound with the chemical formula $(CH_3)_2C(C_6H_4OH)_2$. It belongs to the group of diphenylmethane derivatives and bisphenols, with two hydroxyphenyl groups. It is a monomer used in the manufacture of polycarbonate plastics and resins. Given the wide range of products made of polycarbonate plastics and epoxy resins, BPA could easily be said to be ubiquitous in our environment. Low levels of BPA may be ingested routinely by humans as the compound leaches from the lining of tins and cans into food[1] (Noon*et al* 2011), from dental sealant into saliva[2] (Rathee et al, 2012), and from polycarbonate bottles into their contents[3] (Hoekstra and Simoneau, 2013).

BPA was initially considered to be a weak environmental estrogen with known endocrine disruptor activity. More studies have since revealed that BPA can stimulate cellular response at very low concentrations and that BPA is equipotent to estradiol in some of its effects[4] (Hugo et al 2008). Multiple cellular sites in addition to the nucleus and membrane have been proposed as target of BPA action[5] (Ropero et al 2006). Studies have demonstrated a wide array of BPA activity such as neurochemical alteration, abnormalities in sperm and oocyte maturation, disruption

of fertility, changes in growth rate and immune dysfunctions[6] (Richter et al 2007). BPA is also associated with potential organ perturbations at acute, short-term and sub chronic exposures[7] [Tyl 2008].

Though certified to be toxic in laboratory animals there is still a lot of controversy about the nature of its toxic effects and the dose at which these occur[8] (WHO, 2011). The aim of this study is to establish the type and extent of plasma proteins and liver functionperturbations induced by acute and sub chronic exposure to bisphenol A in albino rats at above human exposure doses.

Materials and methods

Seventy two (72) non-pregnant female rats of age 9 weeks were acclimatized in the laboratory for seven days and randomly divided into two equal groups of 36 rats each. Each group was further subdivided into six experimental groups each containing six (6) rats and respectively administered 50, 100, 150, 200, and 250 µgBPA/kgbw/day. The sixth group which served as control did not receive any treatment.Respective concentrations of BPA (obtained from E. Merck Laboratory Darmstadt, Germany) were dissolved in double distilled water and administered by oral gavage using intubation canular. Blood were obtained from the first groups by cardiac puncture one hour after BPA administration and the animals sacrificed by suffocation with formalin soaked in cotton wool in a glass jar. Animals were subsequently dissected and their liver obtained for histological analysis. Blood samples were processed for clinical assay while the liver were fixed in 10% neutral formalin, trimmed dehydrated in a series of graded ethanol concentration, cleared in xylene and embedded in paraffin wax. Thin sections of 4-5 microns thick were made using a rotary microtone and stained with haematoxylin and eosin for light microscope. The slides were viewed at x100 objective lens to check the architecture of the cell.

The second groups of experimental rats were similarly treated like the previous group except that treatment was sustained daily for seven (7) days to ascertain sub-chronic effects. Animals were housed in aluminum wire-mesh cages in a well-ventilated animal laboratory with a 12 h dark/light cycle and at room temperature and were provided commercial rat pellets (Vital feed from Vital group of Company, Nigeria) and water *ad libitum*. The body weights of all animals were determined daily.

At the end of the experiments liver function activities were assayed using Chemwell 2910 Auotanalyser. All reagents were commercially obtained as already prepared kits. The kits for total protein, and albumin were purchased from Egyptian Company for Biotechnology (SAE), Cairo, Egypt; while the kits for aspartate amino transaminase (AST), alanine amino transaminase (ALT), bilirubin, andalkaline phosphatase (ALP)were obtained from Randox Laboratories Ltd Co Antrim, United Kingdom. Individual tests were carried out according to the kit specifications

Data obtained were subjected to one-way analysis of variance using SPSS software version 17.0. Significant values were further subjected topost hoc analysis using Turkey and Borferroni tests.

RESULTS

Biochemical assays:

The serum protein and albumin contents of the experimental rats following acute and sub chronic administration of graded doses of BPA are shown in figures 1(a) and 1(b) respectively. A low significant difference in total protein levels was observed when test groups are compared with the control group for both the acute and sub chronic exposure. Albumin was significantly low at acute exposure but high following the sub-chronic exposure.

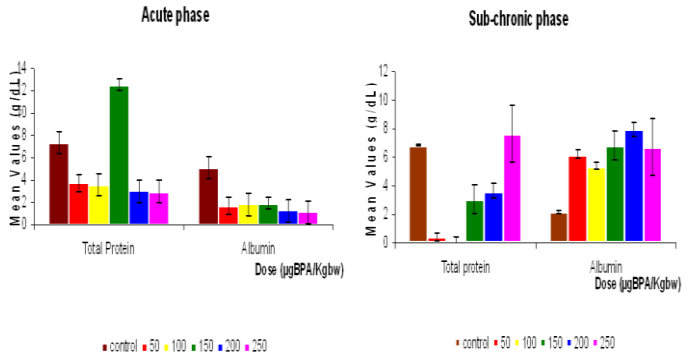

Fig. 1 Total serum protein and albumin following (a) acute and (b) sub chronic administration of BPA

Figures 2a and 2b show the bilirubin pattern following acute administration and sustained sub chronic administration. The result reveal a significant increase of both conjugated and unconjugated bilirubin levels in test animals. This increase is more pronounced at the acute phase of administration and the effect does not appear to be dose dependent. The very high levels of total bilirubin suggests that BPA may induce haemolytic aneamia while the consistent higher levels of unconjugated bilirubin points to a possible interference in the liver conjugation activity.

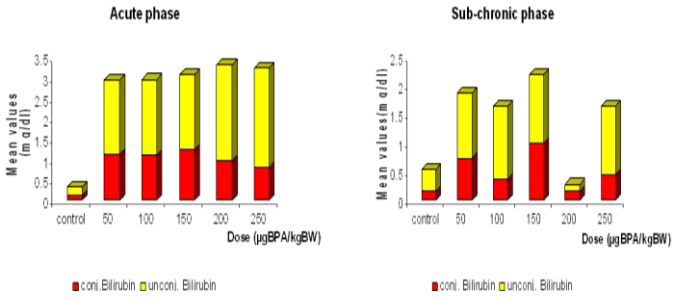

Fig. 2. Total bilirubin profile of rats exposed to (a) acute and (b) sub chronic doses of BPA

The results of serum ALT and AST activities are shown in figures 3a and 3b while that of ALP activity is shown in figures 4a and 4b. BPA administration lowered AST activity at acute phase but has an opposite effect when administration is sustained for a few more days. ALT activities are increased at both acute and sub chronic phases of administration. The differences in AST and ALT activities in the two instances are statistically significant but not dose dependent.

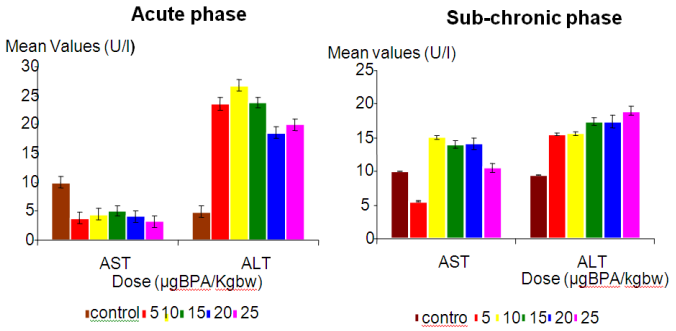

Fig.3. AST and ALT enzyme activities of rats livers exposed to (a) acute and (b) sub chronic doses of BPA

ALP activity is severely and significantly altered at both acute and sub chronic phases of BPA administration. The alteration in ALP activity is more pronounced at acute than at sub chronic phase. The alteration appear to be dose dependent but of no special order.

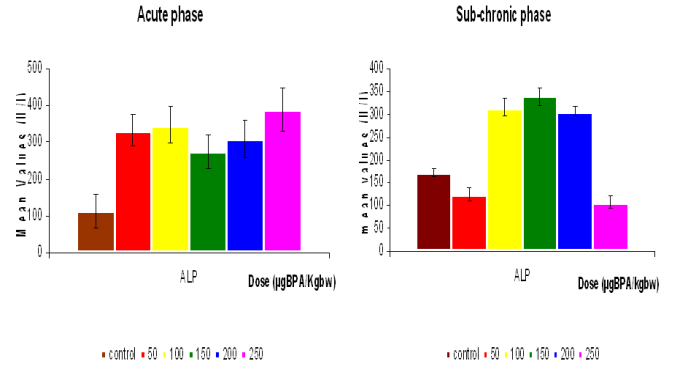

Fig. 4. Liver alkaline phosphatase activity of rats exposed to (a) acute and (b) sub chronic doses of BPA

Histological features

The photomicrographs of the architecture of the liver cells of the experimental animals are shown in plate 1. The histological evaluations of the micrographs were obtained from an expert histologist. According to the expert report the macroscopy of the livers did not reveal any gross lesion. Microscopic analysis of the liver cells obtained from different experimental animals are similar showing normal hepatic parenchymal – vascular relationship. Neither fatty changes, apoptotic bodies nor hepatic necrosis were seen.

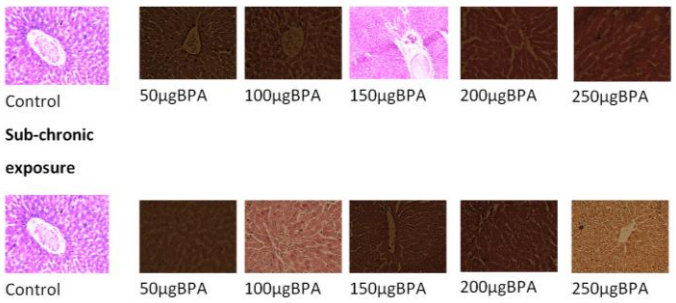

Plate 1- Photomicrographs of the architecture of the liver cells of the experimental animals

DISCUSSION

A significant decrease ($p < 0.05$) was observed in the serum protein of BPA treated rats following acute and sub chronic exposures except for rats administered 150µgBPA in the acute and 250µgBPA in the sub chronic phases respectively (see fig.1).This result is consistent with the earlier findings of Sangai and Verma[9] (2012) who also reported a similar but dose-dependent decrease in the serum proteins of mice administered BPA for 30 days. Decreased serum protein biosynthesis in the liver of rats treated with BPA may be attributed to the formation of BPA adducts. De Flora *et al*[10](2009) had reported the ability of BPA to form DNA adducts *in vitro* in an acellular system and *in vivo* in rodent liver. Atkinson and Roy[11] (1995) had found that BPA is converted to bisphenol o-quinone, which might bind to DNA. When this occurs the transcription of DNA to mRNA will be impaired resulting ultimately in the inhibition of protein synthesis.

The reduced synthetic capacity of the liver may also account for the significantly decreased ($p < 0.05$) value of albumin observed in rats after acute administration of BPA. Yamasaki et al[12] (2002) reported similar decreases in albumin levels in female rats. In contrast significantly higher values of albumin were detected in instances of sub chronic administration. While the mechanism for this is not clear it is certain that exposure to BPA within the experimental dose range

interfered with the albumin balance in experimental animals.

Total bilirubin was found to be significantly ($p< 0.05$) high in all instances suggesting that BPA induces haemolytic anaemia in the albino rats. Both conjugated and unconjugated bilirubin were exceptionally high indicating impairment of both conjugative and excretory functions.

The significant changes in the activities of AST, ALT and ALP (Figs. 3 and 4) points to the alteration effect of BPA on the liver. The aspartate amino transaminase(AST) activity is significantly ($p < 0.05$) lower at acute phase and higher at sub chronic phase.Both alanine amino transaminase (ALT) and alkaline phosphatase (ALP) activities increased significantly at both acute and sub chronic phases. While there is paucity of information liver enzyme responses immediately after a one-shot exposure, the observed elevated values are consistent with literature. Korkmaz *et al*[13] (2010) reported a significant increase in AST and ALT activities in rats treated with 25 mg/kg BPA for 50 days. Iman and Yasser[14](2012) reportedsimilar significant elevation in AST and ALT over control values in rats treated daily with 25 mg/kg for 6 weeks. Sangai and Verma[9] 2012 reported BPA treatment in mice for 30 days caused significant ($p < 0.05$) elevation in ALT, AST and ALP. Melzer and his team[15] (2010) found that low-dose levels of BPA concentrations were also associated with abnormally elevated levels of the three liver enzymes.

Elevated levels of serum enzymes, aspartate amino transaminase and alanine amino transaminase, are suggestive of liver damage while elevated values of alkaline phosphatase and bilirubin is suggestive of jaundice.

Data obtained from this study reveal that BPA altered the biochemical functions of the liver following acute and sub chronic exposure.Interestingly, the overall inference from the histology report suggests that the liver of all exposed animals are microscopically normal. The implication is that despite perceived liver dysfunction there are no morphological changes caused by this short time exposure to low doses of BPA.

CONCLUSION

This study examined the impact of acute and sub chronic exposure of varying concentrations of bisphenol A on plasma proteins and liver functions of Wistar albino rats. The aim was to establish the type and extent of perturbations induced at such exposure doses. The results of the study suggest that BPA exposure at both acute and sub chronic exposure doses interfered with serum protein levels, induced haemolytic jaundice and impaired normal liver functions in Wistar albino rats. A histological examination of the liver cells of exposed animals did not reveal any morphological changes suggesting that the BPA effect on liver function was transient and reversible.

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REFERENCES

1. Noon Gregory O., Luke K Ackerman and Timothy H Begley. Concentration of bisphenol A in highly consumed canned foods on the U.S. Market. Journal of Agriculture and Food Chemistry, 2011; 59(13): 7178 – 7185
2. Rathee Manu, Poonam Malik and Jyotirmay Singh. Bisphenol A in dental sealants and its estrogen effect. Indian Journal of Endocrinology and Metababolism, 2012; 16(3):339 -342.
3. Hoekstra Eddo J. and Catherine Simoneau. Release of bisphenol A from polycarbonate –a review. Critical Reviews in Food Sciences and Nutrition, 2013; 53(4): 386 - 402
4. Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander, JW, Ben-Jonathan N. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose explants and adipocytes. Environmental Health Perspectives, 2008; 116: 1642–1647
5. Ropero AB, Alonso-Magdalena P, Ripoll C, Fuentes E, and Nadal A. Rapid endocrine disruption: environmental estrogen actions triggered outside the nucleus. J. Steroid Biochemistry and Molecular Biology,2006; 102:163–169.
6. Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, Vandenbergh JG, Walser-Kuntz DR, and Vom Saal FS. In vivo effects of bisphenol A in laboratory rodent studies. Reproductive Toxicolcology,2007; 24:199–224
7. Tyl R, Myers CB, and Marr MC. Abbreviated one-generation study of dietary bisphenol A (BPA) in CD-1[s] (Swiss) mice. Research Triangle Park, NC: RTI (sponsored by the Society of the Plastics Industry, Inc.). Report nr 65C.07036.312, 2002.

8. WHO. Toxicological and health aspects of bisphenol A. Report of Joint FAO/WHO expert meeting 2nd – 5th November, Ottawa, Canada, 2011.

9. Sangai NP, and Verma RJ. Quercetin ameliorates bsiphenol - induced toxicity in mice. Acta Poloniae Pharmaceutical n Drug Research, 2012; 69(3): 557-563.

10. De Flora S, Izzotti A, and Kantiz S. Direct evidence revealingstructural elements essentialfor the high binding ability of bisphenol A. Mutatation Research, 2009; 679: 28

11. Atkinson A and Roy D. In vivo DNA adduct formation by bisphenol A. Environmental Molecular Mutagen, 1995; 26: 60-66.

12. Yamasaki K, Sawaki M, Noda S, Imatanaka N, and Takatsuki M. Subacute oral toxicity study of ethynylestradiol and bisphenol A, based on the draft protocol for the "Enhanced OECD Test Guideline no. 407" Archives of Toxicology, 2002; 76:65–74.

13. Korkmaz A, Aydogan M, Kolankaya D and Barlas N. Influence of vitamin E on bisphenol A, nonylphenol and octyl-phenol induced oxidative damage in liver of male rats. Food and Chemical Toxicology, 2010; 48:2865-2871.

14. Iman M, and Yasser AK. The sensitivity of liver, kidney and testis of rats to oxidative stress induced by different doses of bisphenol A. International Journal of Life Science and Pharmaceutical Research, 2012; 2(2): 2453-2459

15. Melzer D, Rice NE, Lewis C, Henley WE, and Galloway TS. Association of urinary bisphenol A concentration with heart disease: evidence from NHANES 2003/06. PLoS One 5(1):e8673.doi:10.1371/journal.pone.0008673, 2010.