Original Article

Effect of Different Doses of Bisphenol A on Albino Rats' Liver: Histological and Immunohistochemical Study

Heba M. Fawzy and Eman Ali Negm

Department of Histology, Faculty of Medicine, Ain Shams University, Egypt

ABSTRACT

Introduction: Bisphenol A is a chemical-compound widely utilised as an epoxy resin in everyday products.
Aim of the Work: Demonstration of the effects of different bisphenol-A dosages on the livers of adult male albino rats.
Materials and Methods: Thirty adult male albino rats were split into three groups (each 10 rats). The control group is placed first, followed by low BPA and high BPA. The exposure period lasted for a total of thirty days. The measurements of serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) have then been performed on blood samples. Also Samples of liver tissue were collected for immunohistochemical,oxidative and histological examinations.
Results: BPA administration orally at 50 and 150 mg/kg/day for 30 days caused a significant drop in glutathione peroxidase and superoxide dismutase levels. At low doses, hepatocytes had numerous bounded tiny vacuoles; at large doses, ballooning degeneration with Mallory bodies and an increase in kupffer cells and inflammatory infiltration were evident in both groups. A reduction in glycogen granules and hepatocyte apoptosis were seen in both groups, confirmed by more significant increase of Caspases 3 immunoreactivity in high dose group. Significant increases in collagen fibres in the portal tract (low dose) and persinusoidal (high dose). Vimentin expression significantly increased in both groups but more marked in the high dose group, confirming our results.
Conclusion: Bisphenol oral- exposure for 30 days is linked to a number of structural and biochemical changes that point to a serious liver injury which was more marked with high dose.

Received: 13 January 2024 **Accepted:** 20 January 2024

Key Words: Bisphenol A; caspase- 3; liver; vimentin.

Corresponding Author: Heba Mohamed Fawzy, MD, Department of Histology, Faculty of Medicine, Ain Shams University, Egypt, **Tel.**: +20 12 2400 3152, **E-mail:** hebafawzy@med.asu.edu.eg

ISSN: 1110-0559, Vol. 48, No. 1

INTRODUCTION

Bisphenol A (BPA) is currently widespread in our atmosphere. It is a chemical compound that is widely utilised as an epoxy resin in common products as cans, packaged foods, infant formula, bottles, and food storage containers found in homes. Furthermore, it may be included in oral prosthesis and thermal printed sales receipts[1].

Prior research revealed that BPA may be measured in the air, water, and dust, leading to widespread exposure[2].

BPA has been around for a long time since it was initially believed to be a harmless chemical which demonstrated limited chemical toxicity in everyday applications. However, BPA isn't a typical poison. Rather, it is an endocrine-disrupting chemical that acts in *vivo* similarly to oestrogen. Consequently, BPA hinders or interferes with the natural function of oestrogen in the human body. It's been suggested that it interferes with thyroid hormone receptors, androgen receptors, and other hormone-related signalling ways[3].

Prior research has shown that BPA has negative impacts on the neurological, immunological, and reproductive systems of humans. There was experimental evidence that BPA exposure negatively affected the reproductive and metabolic systems of rats. Obesity, issues with reproduction, and modifications to the growth and development plan are all caused by BPA. Furthermore, a high correlation has been observed between BPA and a variety of ailments, including as diabetes, cancer, cardiovascular disease, and reproductive issues[4].

The principal organ engaged in detoxifying and metabolism of xenobiotics, such as BPA is the liver. A particular oestrogen receptor is found in the liver, and interactions between oestrogens influence cellular responses[5].

At dosages exceeding 50 mg/kg body weight, certain investigations have demonstrated BPA could affect the liver, kidney, and body weight[6]. However, several investigated its impact on the reproductive system with insufficient investigations on other organs. Studying the impacts of BPA on diverse human subjects, especially at varying concentrations, poses significant challenges.

AIM OF THE WORK

This investigation aimed to demonstrate the effects of different BPA dosages on the livers of adult male albino rats.

DOI: 10.21608/ejh.2024.259809.1993

MATERIALS AND METHODS

Animals

Thirty mature male albino Wistar rats were used in this study, they weighed 150-200 gm and aged about eight weeks. Throughout the trial, the animals were resided in hygienic plastic-cages with mesh wire coverings, had no limit to tap water and a conventional diet of rat chow. They were housed in ideal humidity, temperature, and light levels. The experiment is conducted at the Medical Research Institute (MASRI) of Ain Shams University's Faculty of Medicine. All the experimental procedures were performed in accordance with the Animal Care Guidelines and the Scientific Research Ethical Committee of Ain Shams University's Faculty of Medicine. The current work received ethical agreement from the Research Ethics Committee of the Faculty of Medicine-Ain Shams University (FMASU R250/2023). It was carried out in compliance with the rules established by the US Office for Human Research Protections, the US Code of Federal Regulations, the International Council on Harmonisation (ICH), and the Islamic Organisation for Medical Science (IOMS). Federal Wide Assurance No. FWA 00017585 covers the study.

Three groups composed of ten rats each were created:

Group I (Control group): For 30 days, rats in this group received-orally 0.5 ml of olive oil.

Group II [Low doses BPA]: For 30 days, rats in the second group were orally administered 50 mg/kg/day of bisphenol A (BPA) dissolved in olive oil[7].

Group III [High doses BPA]: For 30 days, rats in the third group were administered 150 mg/kg/day of BPA dissolved in olive oil orally[8].

Bisphenol A [BPA]: obtained from [Sigma Pharmaceuticals Company].

Sample collection and preparation of tissues

After the experiment is over, Rats received 40 mg/kg body weight of thiopental sodium phosphate intraperitoneally[9] and were sacrificed by cervical dislocation. Through an anterior abdominal incision, the livers were cut aside and blood was collected via jugular vein. The right lobes of the livers were collected from all animals. They were promptly put in ten percent formalin over five days for fixation, then they were dehydrated in an increasing alcohol grades, cleared in xylene, and then paraffin was used for embedding. Sections of 5 µm thickness were cut serially and sections were then stained with Hematoxylin and Eosin stain (H&E), PAS for exhibition of glycogen and Masson′s trichrome stain for collagen fibers detection.

On paraffin-embedded slices, the avidin biotin complex immunoperoxidase technique was used to identify caspase 3 and vimentin. After blocking deparaffinized slides with 1.75% methanolic hydrogen peroxide for twenty minutes, Biogenex Antigen Retrieval Citra solution was used to retrieve antigens, which was then done for thirty minutes in a 90°C bath of water. After allowing the slides to cool for twenty minutes, normal horse serum was used to block them for five minutes at 37°C. Sections were incubated with the anti-caspase-3 antibodies (rabbit polyclonal antibody (Ab-4) from Thermo Scientific, diluted 1:200) for an entire night at 4OC[10]. and Anti-Vimentin (mouse anti-human Purchased from (golden lab company), Swiss), secondary biotinylated goat anti-mouse IgG antiserum, and Streptavidin horseradish peroxidase conjugate). Next, the diaminobenzidine chromogen solution, or "DAB" (Sigma), was prepared and used to stain the immunohistochemistry reaction. Hematoxylin was used as a counterstain on the sections before DPX mounting was applied. The main antibody was substituted with PBS for the negative controls[11].

For microscopic inspection, a microscope (Leica, DM2500) was utilised, with a Canon EOS 1100D Digital SLR camera, with the magnification set to 10 (ocular) x 10 and 40 (object lens).

Histomorphometric Study and statistical analysis

Histomorphometric analysis was performed on animals in each group. It was conducted using the Leica QWin V.3 program, an image analyzer installed on a computer, that was linked to a Leica DM2500 microscope (Wetzlar, Germany). Measurements were obtained from 3 distinct slides per rat across all groups. From each slide, five independent fields(non-overlapped fields) were carefully examined to find the mean area % of collagen, mean area % of vimentin, and mean number of caspase positive cells (X20).

Every study's morphometric data was gathered and then statistically examined. The Statistical Package for the Social Sciences (SPSS) statistical tool class 21 (IBM Inc., Chicago, Illinois, USA) was utilised for determination of the standard deviation (SD) and mean value. of the measured parameters in each group. Comparison between studied groups was done using One-way analysis of variance (ANOVA). LSD post Hoc test was done to detect significance between groups. Values were shown as mean ± SD. The significance of the data was determined by probability of chance (*P- value*) where; *P value* < 0.05 was significant and *p*>0.05 was non-significant.

Biochemical assessment

Blood samples were allowed to coagulate at a room temperature and separated using centrifugation for twenty minutes at 3000 rpm., then kept between -18 and -20 OC. Using a commercial kit provided by Randox Co., the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were tested using Reitman and Frankel technique[12].

Assessment of oxidative stress

A homogenate containing 10 percent (w/v) was

produced in a PBS buffer (pH 7.4) ,centrifuged at 1520× g for twenty minutes at 4 ◦C to assess oxidative stress level in liver tissue. According to manufacturer's instructions, commercial-kits from Bio-Diagnostic, Egypt were used to detect the activities of glutathione peroxidase (GPx) (Cat# A006-1-1) and superoxide dismutase (SOD) (Cat# A001-3-2).Using the bicinchoninic acid (BCA) (Cat# P1513-1) protein test kit (Applygen Technologies Inc., Beijing, China).A measurement was made of the liver samples' protein content[13].

RESULTS

Histological and immunohistochemical results

Sections examination of the control group showed the hepatic lobules consist of hepatocyte cords that extend from the central vein. (CV). The polygonal hepatocytes appeared with vesicular nuclei and some of them were binucleated. Flat nuclei of endothelial cells lining blood sinusoids could be detected (Figures 1A,B) Portal regions were observed at the margins of the hepatic lobules .Hepatocytes in this region revealed acidophilic granular cytoplasm and vesicular nuclei The hepatic artery, portal vein, and bile-duct branches made up the portal area. (Figure 1C).

Examnation of H&E sections of group II showed apparent structural alterations. Most of hepatic lobules appeared disorganized, in which hepatocyte cords configuration were almost disrupted, many hepatocytes lost their polygonal form, their cytoplasm appeared faintly acidophilic with foamy vacuolations (mostly fatty vacuoles). Many apoptotic cells were detected with small shrunken nuclei, many central veins and blood sinusoids appeared dilated and congested. Cellular infiltration was well apparent surrounding central veins. Kupffer cells were markedly distributed (Figure 2).

Group III; was markedly more affected as compared to that of group II, with areas of cellular loss and variable affection of hepatocytes, cytolytic necrosis was detected in almost all of hepatic lobules, that was manifested histologically by ballooning degeneration mostly probably fatty degeneration, some hepatocytes appeared swollen and faintly stained. The cytoplasm appeared partially rarified and peripherally situated Mallory bodies formation were also detected, which appeared as eosiophilic structures. Cell membranes were usually unclear, and some remnants of cytoplasm appeared gathered around the nucleus. Other hepatocytes appeared with pyknotic nuclei. Some hepatic lobules showed granular acidophilic cytoplam with congested dilated sinusoids .Also cellular infiltration and Kupffer cells (KCS) were detected (Figure 3)

PAS-stained sections

Control group (Figures 4A,B) showed intense PAS positive reaction within hepatocytes cytoplasm. In group II (Figures 4C,D), an apparent reduction in PAS positive glycogen granules was seen in the cytoplasm of most of the hepatocytes especially around central veins. In group III (Figures 4E,F) as compared to that of control sections the cytoplasm appeared faint with weak PAS positive reaction in almost all hepatocytes.

Masson's trichrome stained sections

The stroma of very fine collagenous fibre meshwork appeared to support the liver's parenchyma in sections that have been Masson's trichrome stained. The portal regions and in-between the hepatocytes showed very little collagenous fibres. (Figure 5A). In group II, a notably increased quantity of the collagen fibers was noticed mainly surrounding the majority of portal tracts. (Figure5B). In group III, an apparent increased amount of collagen fibers was more marked in perisinusoidal areas (Figure 5C).

Immunohistochemical stains

Caspase-3-stained sections

Few hepatocytes in the control rats' liver sections displayed cytoplasmic faint brown staining of caspase-3 expression (Figure 6A). Rats treated with BPA in both groups II&III (Figure 6B,C) showed higher levels of caspase-3 immunoreactivity in their hepatocytes, which is more prominent in group III.

vimentin stained sections

Few positive brown cytoplasmic immunostaining reactions were observed in control group sections for anti-vimentin characteristic for HSCs, in the perisinusoidal spaces (Figure 7A), while both groups II&III showed apparent increase in anti-vimentin immune staining expression respectively (Figures 7B,C).

Biochemical and oxidative stress results

Following BPA therapy, serum levels of ALT and AST rose significantly (P<0.05) in groups II and III, respectively, compared to the control group. (Table 2). Moreover, hepatic SOD and GPx decreased significantly (P<0.05) after BPA therapy. When comparing Group III to the control group, this decline was more pronounced (Table 3).

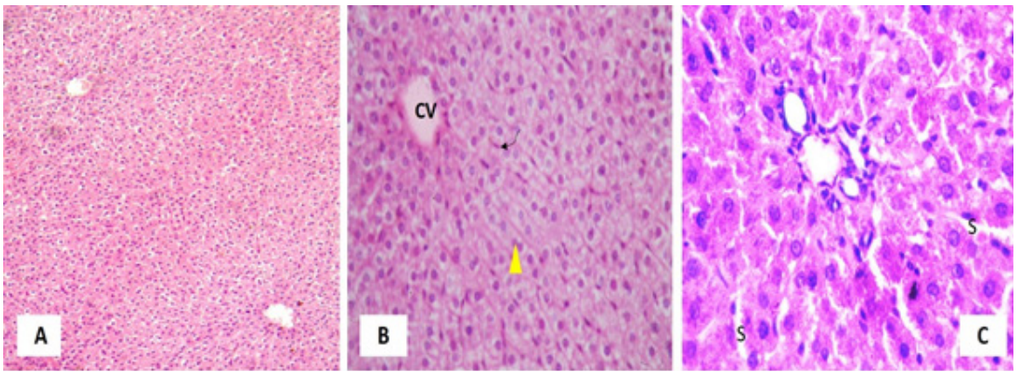

Fig. 1: (A,B,C) A photomicrograph of liver sections of control rats (group I), the central vein (CV), binucleated hepatocytes (yellow arrow head), endothelial cells (curved arrow), Blood sinusoids (S). H&E (AX100), (B,C X 400).

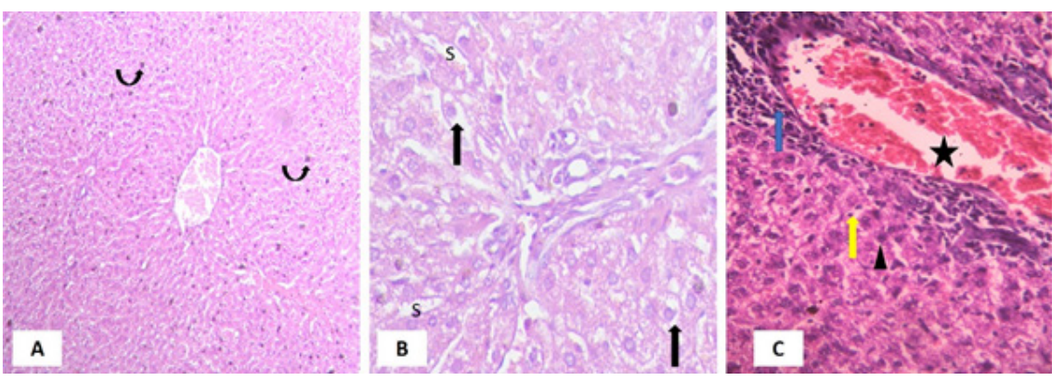

Fig. 2: (A,B,C) A photomicrograph of liver sections of group II; hemosidrin laden macrophage (curved arrow), hepatocytes with intracellular fatty vacuoles (↑); shrunken deeply stained nuclei (▲), cellular infiltration (blue↑), Kupffer cell (yellow↑), dilated congested vessel( ) H&E (AX100), (B,C X 400).

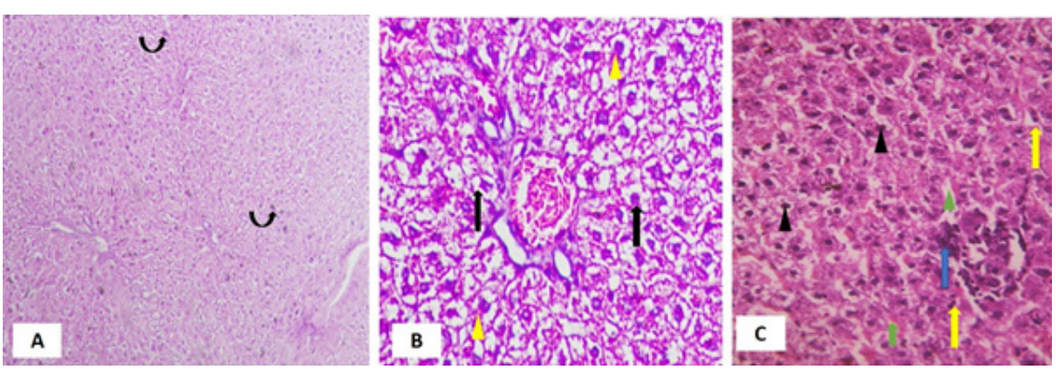

Fig. 3: (A,B,C) A photomicrograph of liver sections of group III; hemosidrin laden macrophage (curved arrow), hepatocytes with intracellular vacuoles (↑); Mallory body (yellow▲), shrunken deeply stained nuclei (▲), Kupffer cell (yellow↑), cellular infiltration (blue↑), congested blood sinusoide (green ↑) ,areas of cellular loss (green▲). H&E (AX100), (B,C X 400).

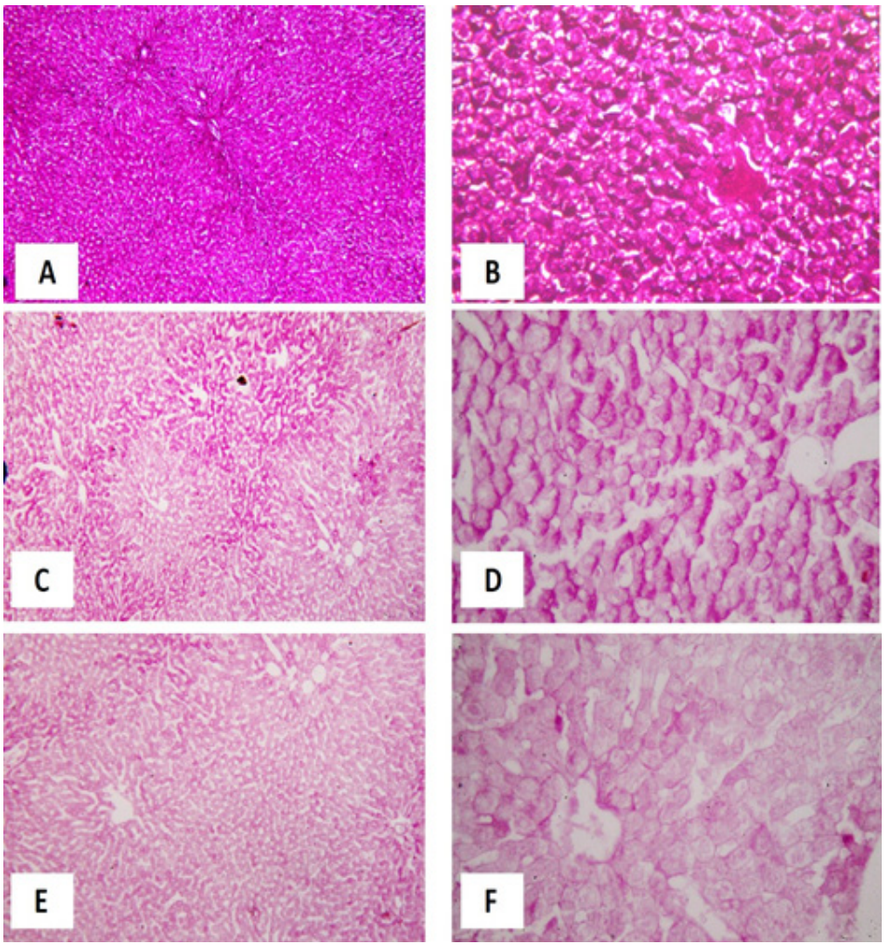

Fig .4: Photomicrographs of liver sections from various groups. (A,B) Control group (group I), (C,D) (group II); (E,F) (group III). Most of the hepatocytes are studded with PAS positive granules in control group and gradually decreased PAS granules in groups II and III respectively. PAS (A, C, E,X100), (B, D, F X 400).

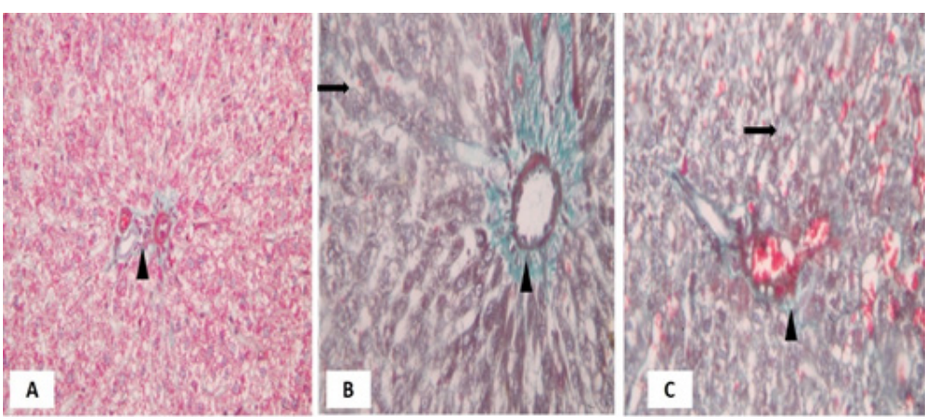

Fig. 5: (A,B,C) Photomicrographs of liver sections from different groups. (A) Control group (group I), (B) (group II); (C) (group III); Perisinusoidal collagen fibers (↑); and in the portal tract (▲). Masson`s trichrome stain X 400

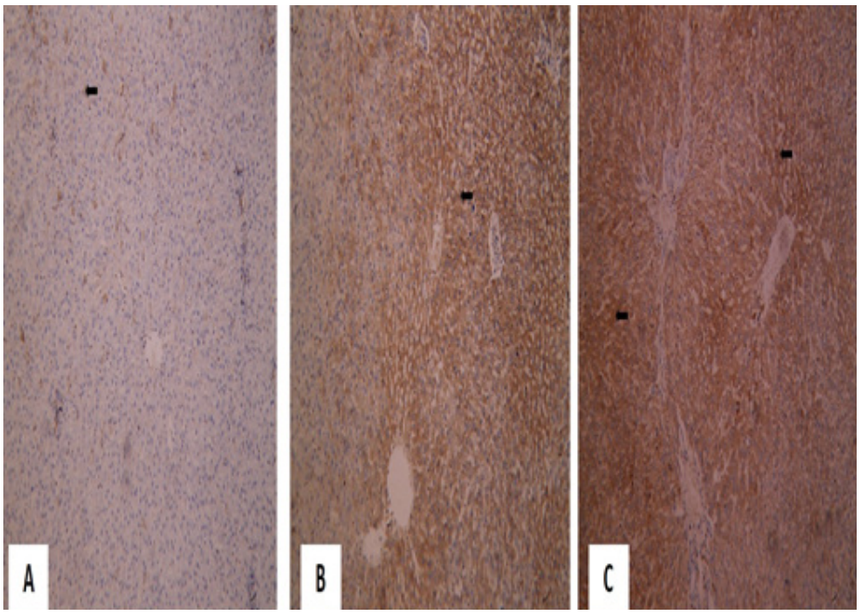

Fig. 6: Photomicrographs of liver sections from different groups. (A)] Control group (group I), (B) (group II); (C) (group III); Hepatocytes with increased caspase 3 cytoplasmic reaction (↑). (Immunostaining for caspase 3 X100).

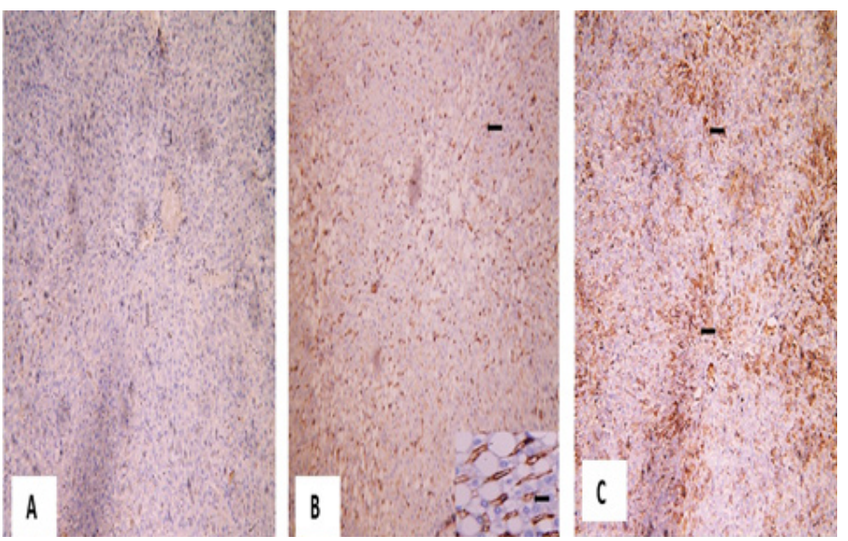

Fig. 7: Photomicrographs of liver sections from different groups. (A) Control group (group I), (B) (group II); (C) (group III); cells with increased positive anti-vimentin reaction in perisinusoidal spaces (↑). (Streptavidin-biotin peroxidase for anti-vimentin x100) Insets X400

Table 1: Showing the mean area percentage of collagen, the mean number of caspase positive cells and the mean area percentage of Vimentin in different groups

Parameter \ Groups	Control group	Group II	Group III
Mean of area percentage of collagen fibers	8.73 ± 1.82	18.37 ± 4.32[a]	25.82 ± 3.83[ab]
Mean number of caspase positive cells	2.9 + 1.01	57.6 + 15.35[a]	80.6 +19.88[ab]
Mean area percentage of vimentin	5.3+ 1.12	19.7 + 3.6[a]	24.2 + 4.21[ab]

Values represent mean ± SD
a= significance calculated by LSD from control group (P<0.05).
b= significance calculated by LSD from group II (P<0.05).

Table 2: Showing changes in biochemical measurement (ALT, AST) in different groups

	Group I	Group II	Group III
ALT (IU/L)	26.2± 6.14	42.1±3.75[a]	63.3±5.67[ab]
AST (IU/L)	22.1±4.12	53.6±4.63[a]	72.3±9.87[ab]

Table 3: Showing changes in biochemical measurement of Tissue SOD and GPx in different groups

	Group I	Group II	Group III
SOD (U/mg)	4.44±0.19	2.28±0.27[a]	1.89±0.23[ab]
Tissue GPx (U/g)	645.33+152.3541	254.437+61.159[a]	183.121+22.146[ab]

DISCUSSION

There is more and more proof that BPA is a hazardous substance. However, dose, duration, frequency, individual variability, and the age of the exposure all affect how harmful the exposure is. It was also added that the majority of BPA metabolism occurs in the liver[14].

Our study used two distinct concentrations to evaluate the harmful impact of BPA on male Wister Albino rats due to the paucity of data regarding the toxic effect of various dosages on liver.

The results of our study demonstrated that adult rats' liver injury was influenced by BPA in a dose-dependent way. Some hepatocytes in group II contain well circumscribed small vacuoles (mostly fat vacuoles),other hepatocytes were apoptotic .Proliferation of Kupffer cells, cellular infiltration (most probably inflammatory cells) and sinusoidal dilatation were also noticed. While, in group III affection was more marked, hepatocytes became more ballooned that contained intracellular vacuoles as well as Mallory bodies. Similarly, Sangai *et al*,[15] reported that, Oral BPA treatment for thirty days was linked to hepatic cell necrosis, cytoplasmic vacuolization, centrilobular hepatocyte enlargement, and a decreased hepatocellular compactness.

Also EWEDA *et al*.[16] documented, BPA treatment resulted in hepatic sinusoidal expansion, a central venous haemorrhage, disappearance of the typical hepatocyte structure, degradation and vacuolation of hepatocytes with dark nuclei, and lymphocyte aggregation.

BPA interacts with oestrogen receptors because of its phenolic structure, which is similar to that of diethylstilbestrol (DES). However, due to its weaker estrogenic properties than DES, BPA has a dual behaviour: it can act as an agonist, mimic endogenous hormones, magnify their effects, and, in certain situations, inhibit the binding of natural hormones to their receptors[17]. The main mediator of oestrogen signalling to prevent hepatic steatosis is believed to be oestrogen receptor α (ERα). Serum lipoprotein levels and hepatic lipid metabolism are two of the numerous organ systems that oestrogens affect. An essential location for the metabolism of fatty acids, triglycerides, and cholesterol is the liver[18].

Moghaddam,[19] found that, 30 days of exposure to BPA at 50, 500, and 5000 mg/kg BPA altered the energy balance, increased blood triglyceride and cholesterol levels, and accelerated adipogenesis and lipid synthesis.

Also Ke *et al*[20] found that male mice treated by 0.5 micrograms of BPA—a level comparable to that of humans—exposed their livers to marked triglyceride and cholesterol accumulation.

Mahdavinia *et al*[21]. demonstrated that administering BPA at a dosage of 50 mg/kg for a duration of 30 days resulted in elevated triglyceride (TG) levels. This treatment had no impact on cholesterol, LDL-C, or HDL-C levels but did lead to microvesicular steatosis in liver tissue.

Increased KCs together with cellular infiltration detected in our study which were more marked in group III was detected as well by Hussein &Eid[22]. study who documented that KCs, are key to liver disorders and proinflammatory response. Increased KCs, infiltration of inflammatory cells, and liver changes are all brought on by oral BPA exposure. They added, after liver damage induced by BPA, hepatocytes secret proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumour necrosis factor-alpha, which generated by the liver and upon an insult they might activate the macrophages and lead to inflammation. It was demonstrated that up on exposure to BPA ,mRNA amounts of those pro-inflammatory cytokines increased[22].

Furthermore, in another study BPA treatment induced a 38% increase in Kupffer cell (KC) count due to BPA induced oxidative damage[7].

With higher doses of administered BPA, cellular infiltration and the number of KCs steadily increase. It has been established that hepatic macrophage KCs is crucial in the spread of acute hepatic injury. As a result of their simultaneous pro- and antifibrotic characteristics[23].

The statement from Abdulhameed *et al*.[24] argues that nitric oxide may rise as a result of being exposed to bisphenol A (BPA), resulting in sinusoidal dilatation and causing notable damage to the liver.

The observed significant rise in Mean number of caspase positive cells in groups II could be due to ROS generated by KCs and hepatocytes that cause increased oxidative stress in liver cells, which may ultimately lead to apoptosis[25].

Also Caspases 3 expression significantly increased in group III,Kourouma *et al*[14] discovered that a high BPA dosage both increases the production of free radicals and decreases the ability of the body to eliminate reactive oxygen species (ROS). Because of this, excessive BPA dosages produce superoxide radicals, and tissue damage brought on by peroxynitrite may cause LPO levels to rise. In liver tissue, activated caspases produce apoptotic signals that cause apoptosis and hepatotoxicity.

The current investigation discovered that giving BPA considerably raised the concentrations of blood AST and ALT and significantly reduced SOD and GPx in a dose dependant way. It has been discovered that BPA increases oxidative stress and inhibits cellular antioxidant capacity[26]. Based on other research, it is hypothesised that BPA induces oxidative stress, which cause hepatotoxicity. This hypothesis is supported by the increased expression of caspase-3 in liver cells, as well as increasing serum levels of AST and ALT[27].

Antioxidant indicators, namely SOD and GPx, are crucial for cell defence against ROS-induced oxidative stress[28]. According to Stinghen *et al*.[29] SOD functions as the initial line of defence against the free radicals, breaking down the free radical superoxide into less harmful hydrogen peroxide. GPx is a selenoenzyme that uses glutathione (GSH) to catalyse the conversion of hydrogen peroxide to water, which can protect mammalian cells from oxidative damage[30].

Abdel-Wahab,[31] showed that Rat livers produced antioxidant defence enzymes, but BPA reduced their activity and enhanced lipid peroxidation.

Li *et al*[32] added that ,When the liver becomes injured, inflammation and necrosis are followed by the release of lactate dehydrogenase (LDH), alkaline phosphatase (ALP), AST, and ALT from the liver into the blood.

Mačczak *et al*[33] investigation showed that BPA compromised the integrity and function of the secretory liver and decrease GPx and SOD activity.

Eweda *et al*[16], revealed comparable outcomes as a result of increased liver damage and decreased cell membrane integrity, which caused hepatic fluid to leak enzymes in the cytoplasm. Further investigations have found that both doses of BPA (high and low) exposure result in genomic damage and alterations in liver enzyme levels[34].

In our study, we found significant increase in vimentin expression in both groups II and III. This was in accordance with Huang *et al*[35] who discovered that, in comparison to the control group, vimentin expression steadily increased with the increase in dose.

Vimentin is an intermediate filament protein primarily found in cells of mesodermal origin, such as the white blood cells, endothelial cells, and fibroblasts[36].

The current investigation revealed a positive correlation for the vimentin area % of positive immune response and the collagen area percentage. The rise in the area % of vimentin immune reaction may be accompanied by increased collagen deposition. This was in accordance with a study[37] that discovered a direct link between the onset of fibrosis and the presence of vimentin-positive cells.

The pivotal element in liver fibrogenesis is the stimulation of hepatic stellate cells (HSCs), marked by migration & proliferation. A crucial component in this process is the vimentin intermediate filament network, essential for controlling the growth, shape, and mobility of cells, as shown by Wang *et al*[38].

In our work, BPA administration orally in group III-caused significant increase of collagen deposition .This was in agreement with Elswefy *et al*[39] who found extensive collagen deposition manifested by elevation in Hyp content (marker of fibrosis) in the hepatic tissues of the rats received BPA. Cohen-Naftaly & Friedman[40] Referred occurrence of fibrosis to the stimultion of HSCs, which produce MMP-2, MMP-9, and MMP-3 causing liver fibrosis and damage the basement membrane, that attract inflammatory cells to the damage location. This explained our finding of presence of perisinusoidal fibrosis in high dose group due to marked activation of hepatic stellate cells present mainly in those areas.

In our study, PAS stain of groups II and III showed apparent reduction in PAS positive cytoplasmic glycogen granules of most of the liver cells respectively. Similarly,Pinafo, *et al*.[41] discovered that BPA-treated groups reacted negatively to PAS, indicating that these animals' livers showed glycogen depletion.

Zaulet *et al*[42]. demonstrated that the diminished phosphorylation of protin kinase B, leading to reduced glycogen synthesis, is likely responsible for the decreased concentration of glycogen induced by BPA. This observation aligns with our own research findings.

CONCLUSION

In summary, the current investigation demonstrated that BPA-induced liver damage and fibrosis associated with inflammation, increased vimentin expression, and apoptosis manifested by increased caspase 3. These findings clearly imply that exposure to BPA is hazardous to the liver in a dose dependant manner. Future research should focus on clinical and experimental studies to identify preventative measures for those who have been exposed to BPA on a regular basis.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Besaratinia A. The State of Research and Weight of Evidence on the Epigenetic Effects of Bisphenol A. International Journal of Molecular Sciences. 2023; 24(9):7951. https://doi.org/10.3390/ijms24097951
2. Asimakopoulos AG, Thomaidis NS, Koupparis MA. Recent trends in biomonitoring of bisphenol A,toctylphenol, and 4-nonylphenol. Toxicol Lett. 2012;(210) 141-154. [DOI: 10.1016/j. toxlet.2011.07.032].
3. Vandenberg, L.N.; Ehrlich, S.; Belcher, S.M.; Ben-Jonathan, N.; Dolinoy, D.C.; Hugo, E.R.; Hunt, P.A.; Newbold, R.R.; Rubin, B.S.; Saili, K.S.; *et al*. Low dose effects of bisphenol A. Endocr. Disruptors .2013; (1) e26490. [CrossRef]
4. Gassman N.R., Coskun E., Stefanick D.F., Horton J.K., Jaruga P., Dizdaroglu M., Wilson S.H., Bisphenol a promotes cell survival following oxidative DNA damage in mouse fibroblasts, PLoS One .2015;(10) e0118819.
5. Linillos-Pradillo B, Rancan L, Paredes SD, Schlumpf M, Lichtensteiger W, Vara E, Tresguerres JÁF. Low Dose of BPA Induces Liver Injury through Oxidative Stress, Inflammation and Apoptosis in Long-Evans Lactating Rats and Its Perinatal Effect on Female PND6 Offspring. Int J Mol Sci. 2023 Feb 26;24(5):4585. doi: 10.3390/ijms24054585. PMID: 36902016; PMCID: PMC10002922.
6. Tyl R. W., "Commentary to the CERHR expert panel report on bisphenol A," Birth Defects Research B.2008;(83) 3:152.
7. Bordbar H, Soleymani F, Nadimi E, Yahyavi SS, Fazelian-Dehkordi K. A Quantitative Study on the Protective Effects of Resveratrol against Bisphenol A-induced Hepatotoxicity in Rats: A Stereological Study. Iran J Med Sci. 2021;46(3):218-227. doi: 10.30476/ijms.2020.83308.1233. PMID: 34083854; PMCID: PMC8163701.
8. Abbas MA, Samir AFE, Abo-Elfotoh MAM. Effect of Oral Exposure to Bisphenol A on the Liver and Kidney of Adult Male Albino Rats IJMA 2021; 3[1]: 930-937. DOI: 10.21608/IJMA.2020.37970.1152
9. El-Kashef, Dalia H., and Rehab S. Abdelrahman. "Montelukast Ameliorates Concanavalin A-Induced Autoimmune Hepatitis in Mice via Inhibiting TNF-α/ JNK Signaling Pathway." Toxicology and Applied Pharmacology. 2020. ; (393): 114931. https://doi.org/10.1016/J.TAAP.2020.114931.
10. Suvarna, S. Kim, Christopher Layton, and John D. Bancroft. 2018. "Bancroft's Theory and Practice of Histological Techniques, Eighth Edition." Bancroft's Theory and Practice of Histological Techniques, Eighth Edition 1–557. doi: 10.1016/C2015-0-00143-5.
11. Bancroft JD and Gamble M. Theory and Practice of Histological Techniques. Edinburgh: Churchill-Livingston. 2007: 427-437.
12. Itam Ako Hogan, Yu-Cheng Kuo, Asmau N. Abubakar, Bashir Lawal, Abdulhakeem R. Agboola, Halimat Yusuf Lukman, Sunday Amos Onikanni, Femi Olawale, Adewale Oluwaseun Fadaka, Yunusa O. Ibrahim, Shukurat B. Babalola, Gaber El-Saber Batiha, Sarah M. Albogami, Mohammed Alorabi, Michel De Waard, Hsu-Shan Huang,Attenuation of hyperglycemia-associated dyslipidemic, oxidative, cognitive, and inflammatory crises via modulation of neuronal ChEs/NF-κB/COX-2/NOx, and hepatorenal functional deficits by the Tridax procumbens extract,Biomedicine & Pharmacotherapy,Volume 158,2023,114114,ISSN 0753-3322,https://doi.org/10.1016/j.biopha.2022.114114.
13. Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 2010; 5[1]:51-66. doi: 10.1038/nprot.2009.197.
14. Kourouma Ansoumane , Chao Quan, Peng Duan, Suqin Qi, Tingting Yu, Yinan Wang, and Kedi Yang. Bisphenol A Induces Apoptosis in Liver Cells through Induction of ROS .Advances in Toxicology . 2015; Article ID 901983, 10 pages
15. Sangai Neha P , Ramtej J Verma1 and Mrugesh H Trivedi. Testing the efficacy of quercetin in mitigating bisphenol A toxicity in liver and kidney of mice. Toxicology and Industrial Health .2014; 30(7): 581–597
16. Eweda SM, Newairy AA, Abdou HM, Gaber AS. Bisphenol A-induced oxidative damage in the hepatic and cardiac tissues of rats: The modulatory role of sesame lignans. Exp Ther Med. 2020; 19 (1): 33–44. doi: 10.3892/etm.2019.8193.
17. Snyder RW, Maness SC, Gaido K, Welsch F, Sumner SC, Fennell TR. Metabolism and disposition of bisphenol A in female rats. Toxicol Appl Pharmacol. 2000;(168): 225- 234.
18. Palmisano BT, Zhu L, Stafford JM. Role of Estrogens in the Regulation of Liver Lipid Metabolism. Adv Exp Med Biol. 2017;(1043):227-256. doi: 10.1007/978-3-319-70178-3_12. PMID: 29224098; PMCID: PMC5763482.
19. Moghaddam, HS, Samarghandian S, Farkhondeh T. Effect of bisphenol A on blood glucose, lipid profile and oxidative stress indices in adult male mice. Toxicol Mech Method. 2015; (25): 507-513.
20. Ke ZH, Pan JX, Jin LY, Xu HY, Yu TT, Ullah K, Rahman TU, Ren J, Cheng Y, Dong XY, Sheng JZ, Huang HF. Bisphenol A Exposure May Induce Hepatic Lipid Accumulation via Reprogramming the DNA Methylation Patterns of Genes Involved in Lipid Metabolism. Sci Rep. 2016 ; (9)6:31331. doi: 10.1038/srep31331. PMID: 27502578; PMCID: PMC4977563.

21. Mahdavinia Masoud , Layasadat Khorsandi , Soheila Alboghobeish , Azin Samimi , Mohammad Amin Dehghani , Leila Zeidooni. Liver histopathological alteration and dysfunction after bisphenol A administration in male rats and protective effects of naringin. AJP.2021;(11)p No. 4.

22. Hussein RM, Eid JI. Pathological mechanisms of liver injury caused by oral administration of bisphenol A. Life Science Journal. 2013;(10):663.

23. Zimmermann, H.W., Tacke, F. Modification of chemokine pathways and immune cell infiltration as a novel therapeutic approach in liver inflammation and fibrosis. Inflammation & Allergy Drug Targets .2011; (10): 509- 536

24. Abdulhameed AAR, Lim V, Bahari H, Khoo BY, Abdullah MNH, Tan JJ, Yong YK. Adverse Effects of Bisphenol A on the Liver and Its Underlying Mechanisms: Evidence from In *Vivo* and In *Vitro* Studies. Biomed Res Int. 2022 Aug 16;2022:8227314. doi: 10.1155/2022/8227314. PMID: 36017387; PMCID: PMC9398799.

25. Kisseleva T, Brenner DA. Role of hepatic stellate cells in fibrogenesis and the reversal of fibrosis. J Gastroenterol Hepatol. 2007;(22) Suppl 1:S73-8. doi: 10.1111/j.1440- 1746.2006.04658.x. PubMed PMID: 17567473.

26. Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH, AlOlayan EM .Bisphenol a induces hepatotoxicity through oxidative stress in a rat model. Oxid Med Cell Longev., 2012: 1-6.

27. Abdel Samie, H., Nassar, S., Hussein, Y. Ameliorative Potential of Selenium against Bisphenol A- Induced Hepatotoxicity in Rats. The Egyptian Journal of Hospital Medicine. 2018; 67(1): 444-454.

28. Uzunhisarcikli M, Aslanturk A, Kalender S, Apaydin FG, Bas H (2016) Mercuric chloride induced hepatotoxic and hematologic changes in rats: the protective effects of sodium selenite and vitamin E. Toxicol Ind Health 32:1651–1662. https://doi.org/10.1177/ 0748233715572561

29. Stinghen AEM, Chillon JM, Massy ZA, Boullier A (2014) Differential effects of indoxyl sulfate and inorganic phosphate in a murine cerebral endothelial cell line (bEnd.3). Toxins 6:1742–1760. https://doi.org/10.3390/toxins6061742

30. Bhattacharjee A, Basu A, Ghosh P, Biswas J, Bhattacharya S (2014) Protective effect of selenium nanoparticle against cyclophosphamide induced hepatotoxicity and genotoxicity in Swiss albino mice. J Biomater Appl 29:303–317. https://doi.org/10.1177/0885328214523323

31. Abdel-Wahab WM: Thymoquinone attenuates toxicity and oxidative stress induced by bisphenol A in liver of male rats. Pak J Biol Sci .2014;(17): 1152-1160.

32. Li, Q.; Zhang, H.; Zou, J.; Mai, H.; Su, D.; Feng, X.; Feng, D. Bisphenol A exposure induces cholesterol synthesis and hepatic steatosis in C57BL/6 mice by down-regulating the DNA methylation levels of SREBP-2. Food Chem. Toxicol. 2019; (133): 110786. [CrossRef] [PubMed]

33. Maćczak A, Cyrkler M, Bukowska B and Michałowicz J: Bisphenol A, bisphenol S, bisphenol F and bisphenol AF induce different oxidative stress and damage in human red blood cells (in *vitro* study). Toxicol In *Vitro*. 2017;(41): 143-149.

34. Kim S, Mun GI, Choi E, Kim M, Jeong JS, Kang KW, *et al*. Submicromolar bisphenol A induces proliferation and DNA damage in human hepato-cyte cell lines in *vitro* and in juvenile rats in *vivo*. Food Chem Toxicol. 2018; (111): 125–132. doi: 10. 1016/j. fct.2017. 11.010.

35. Huang DY, Zheng CC, Pan Q, Wu SS, Su X, Li L, Wu JH, Sun ZY. Oral exposure of low-dose bisphenol A promotes proliferation of dorsolateral prostate and induces epithelial-mesenchymal transition in aged rats. Sci Rep. 2018 ; 11;8(1):490. doi: 10.1038/s41598-017-18869-8. PMID: 29323181; PMCID: PMC5764998.

36. Ridge KM, Eriksson JE, Pekny M, Goldman RD. Roles of vimentin in health and disease. Genes Dev. 2022; 1;36(7-8):391-407. doi: 10.1101/gad.349358.122. PMID: 35487686; PMCID: PMC9067405.

37. Abd El-Haleem, Manal Reda; Abass, Marwa A. Effect of bisphenol A on the myocardium of adult male albino rats and the possible role of lycopene: a histological and biochemical study. The Egyptian Journal of Histology. 2012; 35(2): 326-339, | DOI: 10.1097/01.EHX.0000414921.94640.de

38. Wang PW, Wu TH, Lin TY, Chen MH, Yeh CT, Pan TL. Characterization of the Roles of Vimentin in Regulating the Proliferation and Migration of HSCs during Hepatic Fibrogenesis. Cells. 2019;1;8(10):1184. doi: 10.3390/cells8101184. PMID: 31581522; PMCID: PMC6830351.

39. Elswefy SE, Abdallah FR, Atteia HH, Wahba AS, Hasan RA. Inflammation, oxidative stress and apoptosis cascade implications in bisphenol A-induced liver fibrosis in male rats. Int J Exp Pathol. 2016;97(5):369-379. doi: 10.1111/iep.12207. Epub 2016 Dec 7. PMID: 27925325; PMCID: PMC5206814.

40. Cohen-Naftaly M. & Friedman S.L. Current status of novel antifibrotic therapies in patients with chronic liver disease. Therap. Adv. Gastroenterol. 2011;(4), 391–417.

41. Pinafoa Mariane S , Philippe R. Benedettia , Letícia B. Gaiottea , Fabiano G. Costaa , João Paulo F. Schoffena , Glaura S.A. Fernandesb , Luiz Gustavo A. Chuffac , Fábio R.F. Seiva Pinafo. 2019. Effects of Bauhinia forficata on glycaemia, lipid profile, hepatic glycogen content and oxidative stress in rats exposed to Bisphenol A.Toxicology Reports. 2019;(6): 244–252

42. Zaulet M., S.E.M. Kevorkian, S. Dinescu, C. Cotoraci, M. Suciu, H. Herman, L. Buburuzan, L. Badulescu, A. Ardelean, A. Hermenean, Protective effects of silymarin against bisphenol A-induced hepatotoxicity in mouse liver, Exp. Ther. Med. 2017; 13 (3): 821–828, https://doi.org/10.3892/etm.2017.4066

الملخص العربى

تأثير جرعات مختلفة من ثنائى الفينول أ على كبد الجرذان البيضاء: دراسة نسيجية وكيميائية مناعية

هبه محمد فوزى وايمان نجم

قسم الأنسجة وبيولوجيا الخلية، كلية الطب، جامعة عين شمس، القاهرة، مصر

المقدمة: ثنائى الفينول أ هو مركب كيميائي يستخدم على نطاق واسع كرانجات الايبوكسي في المنتجات اليومية.
الهدف من العمل: عرض آثار جرعات مختلفة من ثنائي الفينول أ على كبد ذكور الجرذان البيضاء البالغة.
المواد والطرق: تم تقسيم ثلاثين من ذكور الجرذان البيضاء البالغة إلى ثلاث مجموعات [كل ١٠ فئران]. يتم وضع المجموعة الضابطة أولا ، تليها مجموعة ثنائي الفينول أ منخفضة الجرعة ثم مجموعة ثنائي الفينول أ عالية الجرعة . استمرت فترة التعرض لمدة ثلاثين يوما. ثم تم إجراء قياسات مصل ألانين أمينوترانسفيراز [ALT] وأسبارتات أمينوترانسفيراز [AST] على عينات الدم. كما تم جمع عينات من أنسجة الكبد للفحوصات المناعية والكيميائية والأكسدة والنسيجية.
تسبب إعطاء BPA عن طريق الفم عند ٥٠ و ١٥٠ مجم / كجم / يوم لمدة ٣٠ يوما في انخفاض كبير في مستويات الجلوتاثيون بيروكسيديز وديسموتاز الفائق الأكسيد. في الجرعات المنخفضة ، كان لدى خلايا الكبد العديد من الفجوات الصغيرة المحدودة. عند الجرعات الكبيرة ، كان التنكس المتضخم مع أجسام مالوري وزيادة خلايا كوبفر والتسلل الالتهابي واضحا في كلا المجموعتين
النتائج: تسبب إعطاء ثنائي الفينول أ عن طريق الفم عند ٥٠ و ١٥٠ مجم / كجم / يوم لمدة ٣٠ يوما في انخفاض كبير في مستويات الجلوتاثيون بيروكسيديز وديسموتاز الفائق . بدت زيادة خلايا كوبفر والتسلل الالتهابي واضحة في كلا المجموعتين ،عند الجرعات المنخفضة ، كان لدى خلايا الكبد العديد من الفجوات الصغيرة المحدودة. عند الجرعات الكبيرة ، كان التنكس المتضخم مع أجسام مالوري. ولوحظ انخفاض في حبيبات الجليكوجين في كلا المجموعتين ، لوحظ موت الخلايا المبرمج في خلايا الكبدمن خلال زيادة النشاط المناعي Caspases ٣ بشكل كبير . كانت الزيادات الكبيرة في ألياف الكولاجين في القناة البابية (جرعة منخفضة) و الاحياز المحيطة بالجيبانيات (جرعة عالية).كما زاد تعبير Vimentin بشكل ملحوظ في مجموعة الجرعات العالية ، مما يؤكد نتائجنا.
الخلاصة: يرتبط التعرض عن طريق الفم للبيسفينول لمدة ٣٠ يوما بعدد من التغيرات الهيكلية والكيميائية الحيوية التي تشير إلى إصابة خطيرة في الكبد والتي كانت أكثر وضوحا فى الجرعة عالية.