



A Quantitative Study on the Protective Effects of Resveratrol against Bisphenol A-induced Hepatotoxicity in Rats: A Stereological Study

Hossein Bordbar^{1,2}, PhD;  Fatemeh Soleymani^{1,2}, MSc; Elham Nadimi^{1,3}, MSc; Seyedeh-Saeedeh Yahyavi², MSc; Khatereh Fazelian-Dehkordi², PhD 

¹Histomorphometry and Stereology Research Centre, Shiraz University of Medical Sciences, Shiraz, Iran;

²Department of Anatomical Sciences, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran;

³Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence:

Khatereh Fazelian-Dehkordi, PhD;
Department of Anatomical Sciences,
School of Medicine, Shiraz University
of Medical Sciences, Zand Blvd., Postal
code: 71348-45794, Shiraz, Iran
Tel: +98 9039203978

Email: kh.fazelian@gmail.com

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What's Known

- Bisphenol A is known to cause liver damage, with previous research indicating the impact of Bisphenol A levels on the oxidative injury, liver tissue apoptosis, inflammation, edema, degeneration, and hepatocyte necrosis. Additionally, Bisphenol A produces oxidative stress in the liver.

What's New

- This quantitative stereological study, conducted on the liver structure after Bisphenol A exposure, indicates the mitigating effects of resveratrol. Resveratrol protected the liver of the study rats against Bisphenol A by increasing the total liver volume, hepatocyte nucleus and cytoplasm volumes, and hepatocyte numbers.

Abstract

Background: Bisphenol A (BPA) is a widely used chemical with toxic effects on the liver. Resveratrol (RES) is an herbal compound with protective properties. This study aimed to investigate the protective effects of RES on the liver in rats exposed to BPA.

Methods: This study was conducted in 2018 in Shiraz, Iran. Thirty Sprague-Dawley male rats were divided into five groups: a control group (distilled water), a sham group (olive oil as a BPA solvent), a BPA group (50 mg/kg), an RES group (100 mg/kg), and a RES+BPA group (50 mg/kg+100 mg/kg). Olive oil, BPA, and RES were administered to the animals via gavage for eight weeks. After eight weeks, the animals' livers were removed, and stereological measurements were made to obtain the total liver volume, portal triad volumes, hepatocyte nucleus and cytoplasm volumes, hepatocyte numbers, sinusoidal space volumes and lengths, and Kupffer cell (KC) numbers. The data were analyzed using the one-way analysis of variance test.

Results: The hepatocyte number, the total liver volume, and hepatocyte nucleus and cytoplasm volumes in the BPA group decreased by 41% ($P<0.001$), 18% ($P<0.001$), 32% ($P=0.030$), and 37% ($P=0.014$), respectively. The number of KCs and the length of sinusoids in the BPA group were increased compared with all the other groups ($P<0.001$). Our histological study revealed vacuolization, sinusoidal space dilatation, and congestion in the BPA group.

Conclusion: In this study, the RES group, compared with the BPA group, exhibited a decrease in the total volume and length of sinusoids and the number of KCs. Additionally, the RES group showed an increase in the total liver volume, hepatocyte nucleus and cytoplasm volumes, portal triad volumes, and hepatocyte numbers after oral administration.

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Keywords • Antioxidants • Hepatocytes • Liver • Oxidative stress • Resveratrol

Introduction

Bisphenol A (BPA) is found in many artificial products such as plastics, food packages, paper receipts, medical devices, and dental materials.¹ Research suggests that BPA can be released from these materials independent of their temperature and pH

into food, air, skin, saliva, and blood.² Evidence showed that BPA-induced reactive oxygen species (ROS) significantly creates toxicity and carcinogenic potential.³ Exposure to BPA has harmful effects on various cells and tissues such as the liver. For instance, BPA can induce liver damage by affecting the oxidant/antioxidant balance in the rat liver.⁴ The BPA-glucuronide complex is the major metabolite of BPA in animals and humans and has a low estrogenic function. UDP-glucuronosyltransferase 2B2 (UGT2B) is a liver enzyme responsible for the glucuronidation of BPA and other estrogens.⁵

The liver is the main organ responsible for drug detoxification; a function that it serves by regulating toxins and hemostasis.⁶ A study on liver injury demonstrated that BPA led to increased levels of alanine aminotransferase and aspartate aminotransferase. These enzymes enter the bloodstream when hepatocyte cells are damaged, and injury to the mitochondrial cytoplasm of these cells begets impaired liver function.⁷ Liver dysfunction results in the increased absorption of toxins in the body and ultimately, liver toxicity.⁸ The volume of chemicals in the developed community has grown enormously, an instance of which is the disconcerting widespread use of plastic materials and their harmful constructive ingredients.⁹ Research on the deleterious effects of BPA on the liver and the resultant diseases has indicated a relationship between BPA levels and oxidative injury, liver tissue apoptosis, inflammation, edema, degeneration, and hepatocyte necrosis.¹⁰ It has also been shown that BPA produces oxidative stress in the liver.¹ Therefore, it is essential to create a new liver-protective agent that can be easily consumed by the general public. Resveratrol (RES) is an herbal compound¹¹ shown to exert protective effects on the nervous and cardiovascular systems¹² on the strength of its anti-inflammatory, antioxidant,¹³ and anticancer properties. More recent investigations have also demonstrated that RES has protective effects on the kidney,¹⁴ ovaries,¹⁵ and testes.¹⁶ The precise mechanism of the action of RES is unknown, but this herbal compound is likely to function through its antioxidant activity by inhibiting ribonucleotide reeducates and DNA polymerase enzymes.

Given the significant role that the liver plays in the body, we aimed to investigate the effects of RES on the liver structure in rats receiving BPA for eight weeks.

Materials and Methods

Animals

Thirty male Sprague-Dawley male rats weighing 200 to 250 g were purchased from

the Comparative and Experimental Medical Center of Shiraz University of Medical Sciences. The animals were kept in standard conditions, including a 12-hour light/12-hour dark cycle and free access to water and food, for eight weeks to maintain environmental compatibility.¹⁷ BPA was purchased from Sigma, Germany, and diluted with olive oil (Sabzdasht, Iran). RES was purchased in a commercially-available form of capsules and dissolved in ethanol (MERK, Germany). The research proposal was approved in accordance with the guidelines of the Animal Care¹⁷ and Ethics Committee (IR.SUMS.MED.REC.1398.031) by the Histomorphometry and Stereology Research Center, Shiraz University of Medical Sciences, in the year 2018.

Experimental Groups

The rats were randomly divided into the following five groups (n=6): a control group, which received distilled water; a sham group, which received olive oil as a BPA solvent; and three other groups that were respectively administered 50 mg/kg of BPA,¹⁸ 100 mg/kg of RES,¹⁹ and 50 mg/kg of BPA plus 100 mg/kg of RES. Olive oil, BPA, and RES were fed to the animals via gavage for eight weeks. At the end of the experiment, the rats were subjected to overnight fasting and on the day of euthanasia, they were anesthetized with an intraperitoneal injection of ketamine (Merck, Germany) at a dose of 100 µg/kg and xylazine (Merck, Germany) at a dose of 5 mg/kg. Liver samples were obtained and fixed in 10% formalin (Merck, Germany) in preparation for microscopic sections and tissue passaging. Subsequently, the slides were prepared and stained by hematoxylin and eosin (H&E) (Merck, Germany) staining for isotropic uniform random (IUR) sectioning. The pieces cut with a trocar were finally prepared for the calculation of tissue shrinkage.

Stereological Methods

Through unbiased stereology, possible liver stereological changes were estimated. In addition to the quantitative reporting of structural changes, stereological methods can be drawn upon to define the extent of alterations.^{20, 21}

Estimation of the Liver Volume

The livers were cleaned, and their total volume was estimated using the Scherle method.²² Briefly, a jar filled with isotonic saline was placed on a scale and weighed (V primary). Then, the liver was suspended with thin cotton in the jar, and the liver volume was estimated. Since IUR sectioning was required to determine the volume of the liver structures, the

orientation method was employed, whereby the whole liver was placed on one circle, a number was chosen randomly, and the liver was cut in that direction (here, it was six). Next, the other part of the liver was placed in another circle, and the second cut was made at another random number (here, it was three). The thickness of the slabs was chosen to guarantee that a total of 8 to 12 slabs were obtained (figure 1). Shrinkage was estimated by considering the radius of the trocar to be the area before (πr^2). After the passage of the tissue, cutting, and staining, the area after (πr^2) was used to calculate tissue shrinkage with a magnification microscope (31). The shrinkage volume in the liver of each rat was calculated via the following formula:²³

$$\text{Volume shrinkage} = 1 - (\text{area after} / \text{area before})^{1.5}$$

The tissue preparation steps were performed according to the usual method. After paraffin molding, 5- μm and 20- μm sections were cut to calculate their volume and number, correspondingly. Afterwards, the slides were stained with H&E and the Masson trichrome staining. Ultimately, the final volume was calculated via the following formula:^{24, 25}

$$V \text{ final liver} = V \text{ primary} \times (1 - \text{volume shrinkage})$$

Estimation of the Volume Density of the Liver Components

The volume density of the liver components was estimated using a microscope (Nikon, E200, Japan) and a software tool (Grid-Stereo Lite, Histomorphometry and Stereology Research Center, Shiraz University of Medical Sciences, Iran). Via systematic random sampling, in an average of 10 to 14 fields of 5- μm slides, hepatocyte nucleus and cytoplasm volume densities, sinusoidal space volume densities, nucleus-to-cytoplasm volume ratios, and portal triad volume densities were calculated. A point probe, composed of 25 points, was visualized by the monitor on the images of the tissue sections (figure 2A). The volume densities and total volumes of the liver components were obtained using the point-sampled intercept method. Then, the volume density of each component was measured, using the following formula:²⁴

$$V_v = \Sigma P \text{ structure} / \Sigma P \text{ reference}$$

where V_v is the volume density and ΣP is the total number of points composed on the structure and the reference.

Ultimately, the total volume associated with each component was estimated through the multiplication of the volume density by the total volume per animal:²³

$$V \text{ structure} = V \text{ final liver} \times V_v \text{ structure}$$

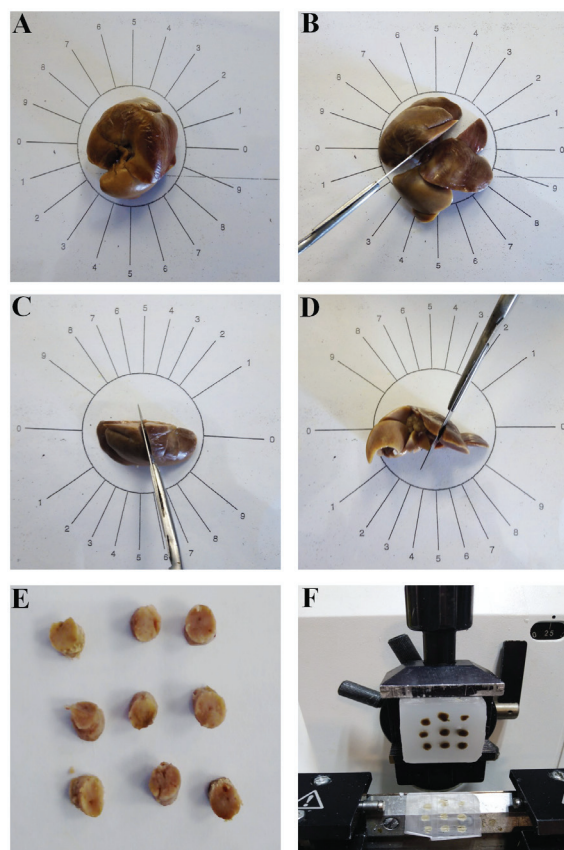


Figure 1: Orientated method is shown. (A) The liver tissue is placed on a uniformly divided circle. A random digit between 0 and 9 is selected. (B) The liver is sectioned into two parts in the orientation of the selected digit. (C) The cut surface of one part of the liver is placed bottom-down on the circle with cosine-weighted divisions. Again, a new random digit and orientation are selected and the tissue is cut along this angle. (D) The cut surface of the other part of the liver is then placed on the 0-0 direction of the cosine-weighted circle. A new random digit is selected and along the new angle, the tissue is cut. (E) Shrinkage is measured by punching a tissue cylinder from a liver piece with a trocar. The diameter of the cylindrical piece and the area of the circle are counted according to simple geometrical formula. (F) Sectioning is done on the block using a microtome.

Estimation of the Length of the Sinusoid

Length density is the length of the lumen of the sinusoid per unit volume of the liver tissue. The lumen density of the structure of the sinusoidal space was assessed by placing a counting frame on the live histological image of the liver tissue. The number of profiles of each lumen that was inside the count frame and did not touch the left and bottom borders of the frame was counted. Length (L) was estimated using the following formula:²⁴

$$L_v = 2 \times \Sigma Q / [\Sigma P \times \text{area (f)}] \times [1 - (\text{shrinkage})^{2/3}] \times V \text{ primary}$$

where L_v is the length density of the lumen, ΣQ is the total counted profiles of each lumen, area (f) is the area per frame (here 6908.93 μm^2), and Σp is the total number of the counted

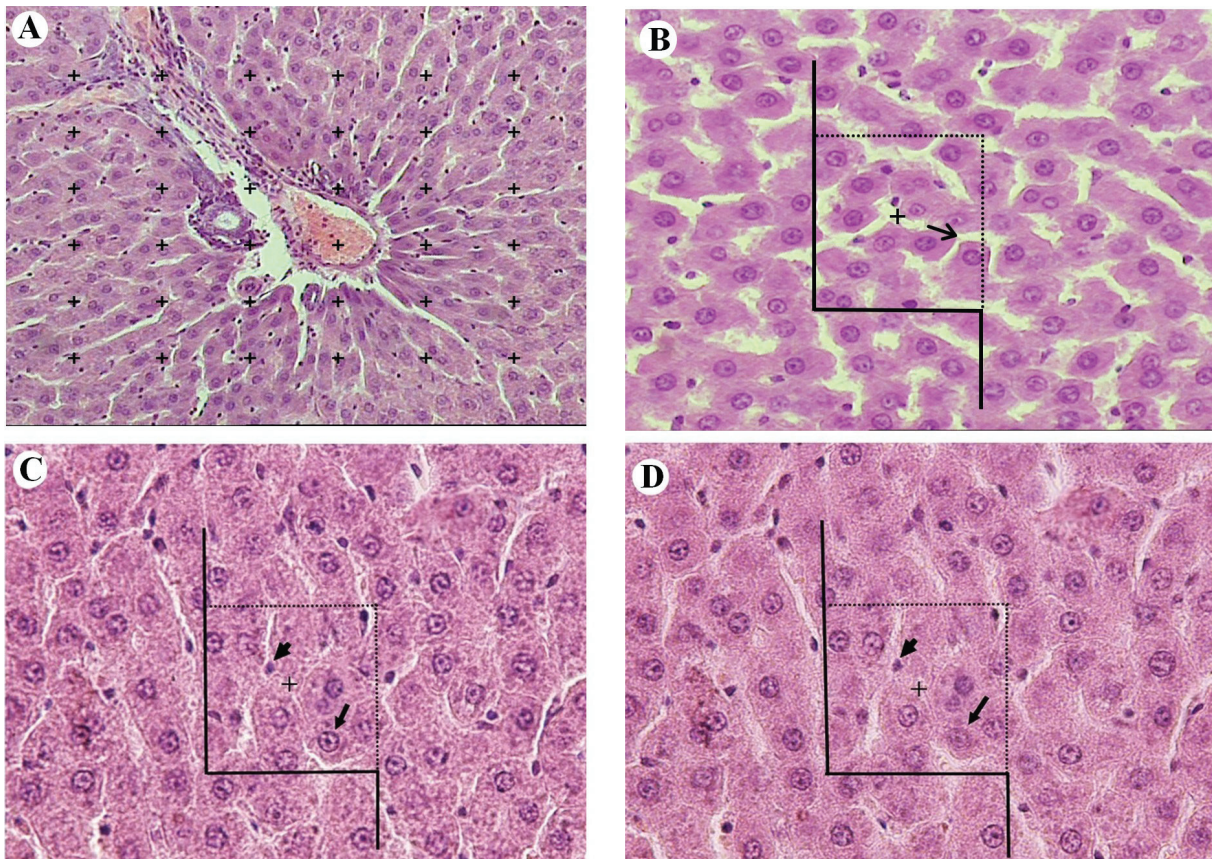


Figure 2: Volume estimation is shown using the point-sampled intercept method (A). The dissector technique is used to show the length of the sinusoid (arrow) in figure B, and the numbers of Kupffer cells (short arrow) and hepatocyte nuclei (long arrow) are illustrated in figure C and figure D.

frames in each animal.

To calculate the total volume of the parameters and the total length of the lumens and to avoid “reference trap”, the volume fraction or length density was multiplied by the final volume of the liver (figure 2B).

Estimation of the Number of Hepatocytes and KCs

The number of hepatocytes and KCs was estimated by using an optical dissector, an unbiased counting frame, and a microcator. Some fields were also randomly selected from the 20- μ m slides using a microscope with oil lenses (40X magnification, numerical aperture=1.30). The hepatocyte nuclei and KCs were counted without counting the nuclei on forbidden lines. The numerical densities of the hepatocytes were calculated using the following formula:^{14, 15}

$$N_v = \Sigma Q / (\Sigma P \times [\text{area (f)}] \times h)$$

where N_v is the numerical density in terms of number per unit volume (mm^3), ΣQ is the number of the cells counted, ΣP is the number of the frames (with the associated point) scrolled, area (f) is the area along each frame (mm^2), and “h” is the height of the dissector (figures 2 C and D).

After the calculation of the numerical density, it was multiplied by the total volume of the liver to estimate the total number of hepatocytes and KCs through the following formula:²⁶

$$N_{\text{total}} = N_v \times V \text{ (total volume of liver)}$$

Statistical Analysis

All the statistical analyses were performed using the GraphPad Prism statistical software, version 6.07 (IBM, Armonk, NY, USA). The data were analyzed using the one-way analysis of variance (ANOVA) with the *post hoc* least significant difference (LSD). P values of equal to or less than 0.05 were considered to be statistically significant.

Results

Quantitative Changes

Estimation of the Total Volume of the Liver

The quantitative parameters are shown in figure 3. The total volume of the liver in the control group was 18% higher than that in the BPA group ($P=0.001$). However, this volume was not significantly different between the RES group and the control group. Additionally, the volume was increased by 11% in the RES+BPA group in comparison with the BPA group ($P=0.019$) (figure 3A).

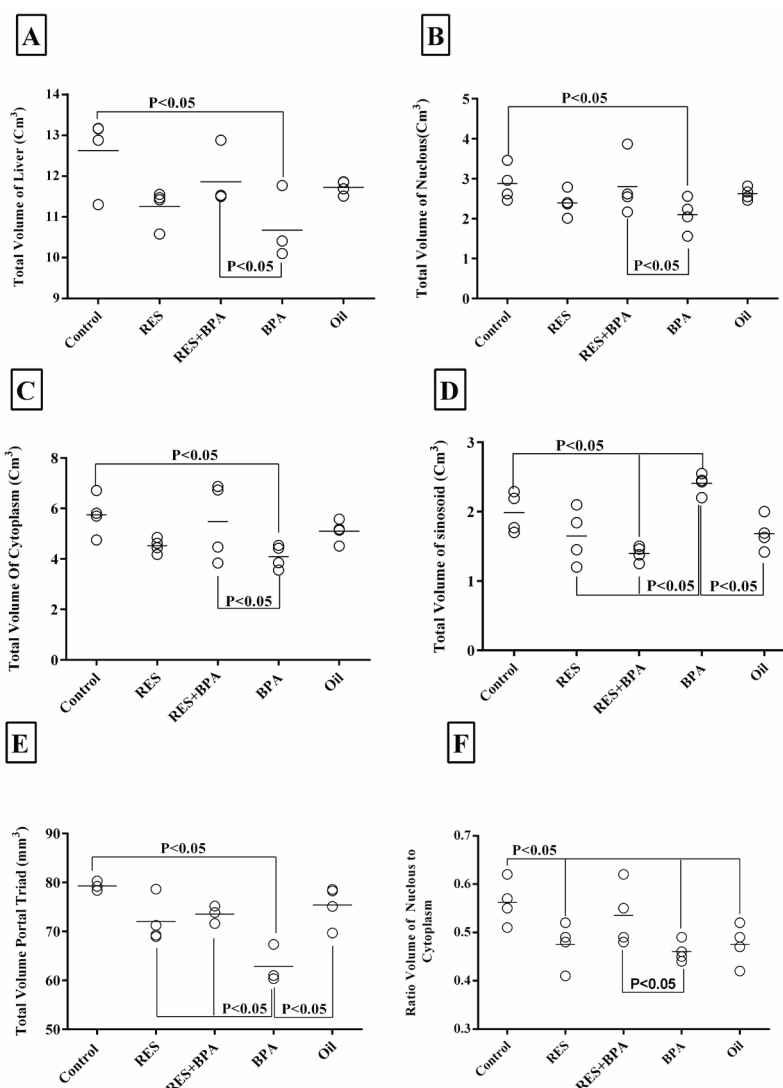


Figure 3: The aligned dot plots of quantitative changes are depicted. The total volume of the liver (A), the total volume of nuclei (B), the total volume of cytoplasm (C), the total volume of the sinusoidal space (D), the total volume of the portal triad (E), and the nucleus-to-cytoplasm volume ratio (F) are illustrated. $P \leq 0.005$ was considered significant. The one-way analysis of variance (ANOVA) was done to analyze the data of the different groups. RES: Resveratrol; RES+BPA: Resveratrol+Bisphenol A; BPA: Bisphenol A

Estimation of the Total Volume of the Liver Components

The total nucleus volume of the hepatocytes in the BPA group, compared with the control group, was decreased by 32%. Moreover, the nucleus volume of the hepatocytes in the RES+BPA group, compared with the BPA group, was increased by 13% ($P=0.046$) (figure 3B).

The total cytoplasm volume of the hepatocytes in the BPA group, in comparison with the control group, was decreased by 37% ($P=0.014$). This volume in the RES+BPA group was increased by 21% in comparison with the BPA group ($P=0.034$) (figure 3C). Additionally, the sinusoidal space showed increases of 17% ($P=0.037$), 42% ($P<0.001$), 31% ($P=0.001$), and 30% ($P=0.001$) in the BPA group compared with the control, RES+BPA, RES, and olive oil groups, respectively. Furthermore, the total sinusoidal

space in the RES+BPA group, compared with the control group, was decreased by 30% ($P=0.005$) (figure 3D). The portal triad volume in the BPA group was decreased by 20% ($P=0.001$), 13% ($P=0.036$), 14% ($P=0.021$), and 17% ($P=0.004$) in comparison with the control, RES, RES+BPA, and olive oil groups, respectively (figure 3E). The nucleus-to-cytoplasm volume ratio was reduced in the RES, BPA, and olive oil groups by 19% ($P=0.017$), 24% ($P=0.006$), and 19% ($P=0.017$) compared with the control group, respectively. Furthermore, this volume ratio in the RES+BPA group, in comparison with the BPA group, indicated an 18% increase ($P=0.036$) (figure 3F).

Estimation of the Total Number of Hepatocyte Nuclei and KCs in the Liver

The number of hepatocyte nuclei in the BPA, RES+BPA, and RES groups was decreased

by 41% ($P < 0.001$), 45% ($P = 0.001$), and 21% ($P = 0.004$) by comparison with the control group, respectively. Nonetheless, there was no significant difference in the number of hepatocytes between the RES group and the control group. This parameter in the BPA and RES+BPA groups was decreased by 50% ($P = 0.001$) and 27% ($P = 0.009$) compared with the olive oil group, respectively (figure 4A). The number of KCs in the BPA group was increased by 38% ($P < 0.001$), 45% ($P < 0.001$), 50% ($P < 0.001$), and 42% ($P < 0.001$) compared with the control, RES, RES+BPA, and olive oil groups, respectively (figure 4B).

Estimation of the Length of the Sinusoids in the Liver

The length of the sinusoids in the RES+BPA group was decreased by 10% ($P = 0.014$) compared with the control group. Furthermore, the sinusoidal space length in the BPA group was increased by 13% ($P = 0.001$), 14% ($P = 0.001$), 21% ($P < 0.001$), and 16% ($P < 0.001$) in comparison with the control, RES, RES+BPA, and olive oil groups, respectively (figure 4C).

Qualitative Changes

Histological studies of the liver in H&E-stained slides in the different groups showed that the structure of the liver in the control, RES, and olive oil groups was normal (figure 5A, B, & F). The RES+BPA group had a relatively normal liver tissue structure (figure 5C). Vacuolization, sinusoidal dilatation (figure 5D), and congestion were observed in the BPA group (figure 5E).

Discussion

The present quantitative stereological study was conducted on the structure of the liver after BPA exposure, and the results indicated the mitigating effects of RES. We found that the use of BPA caused several structural changes in the liver of rats by decreasing total liver and portal triad volumes, diminishing nucleus and cytoplasm volumes, truncating hepatocyte numbers, augmenting sinusoidal space volumes and lengths, and increasing KC numbers.

Han C and colleagues showed that BPA exerted substantial destructive effects on the liver tissue.²⁷ Recently, oxidative stress has been proposed as another harmful cellular effect of BPA on the liver.²⁸ The manufacture of ROS is influenced by the activity of nicotinamide adenine dinucleotide phosphate oxidase in hepatic stellate cells, KCs, and hepatocytes.²⁹ ROS released by KCs and hepatocytes can increase oxidative stress in hepatocytes and

thus, their apoptosis.³⁰ Macrophages that enter the liver tissue when it is exposed to the toxic effects of BPA, cause the release of oxygen free radicals (OFRs).³¹ Hassan and colleagues demonstrated that BPA caused hepatotoxicity by inducing oxidative stress in the liver.⁷ Hence, BPA-induced ROS and oxidative stress may bring about changes in the liver structure.

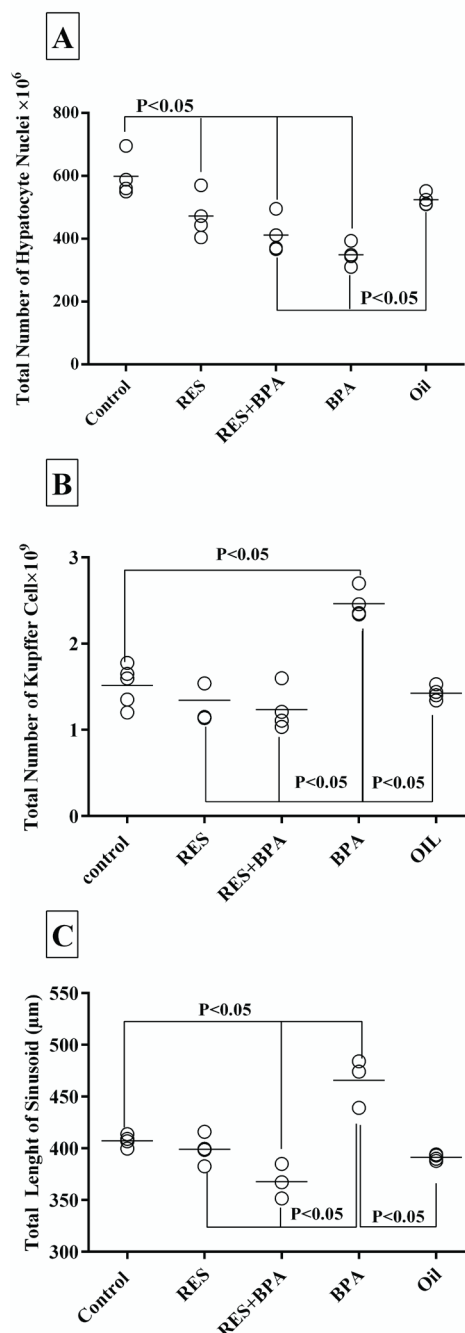


Figure 4: The aligned dot plots of quantitative changes are shown. The total number of hepatocyte nuclei (A), the total number of Kupffer cells (B), and the total length of the sinusoidal space (C) are depicted. $P \leq 0.005$ was considered significant. The one-way analysis of variance (ANOVA) was done to analyze the data of the different groups. RES: Resveratrol; RES+BPA: Resveratrol+Bisphenol A; BPA: Bisphenol A

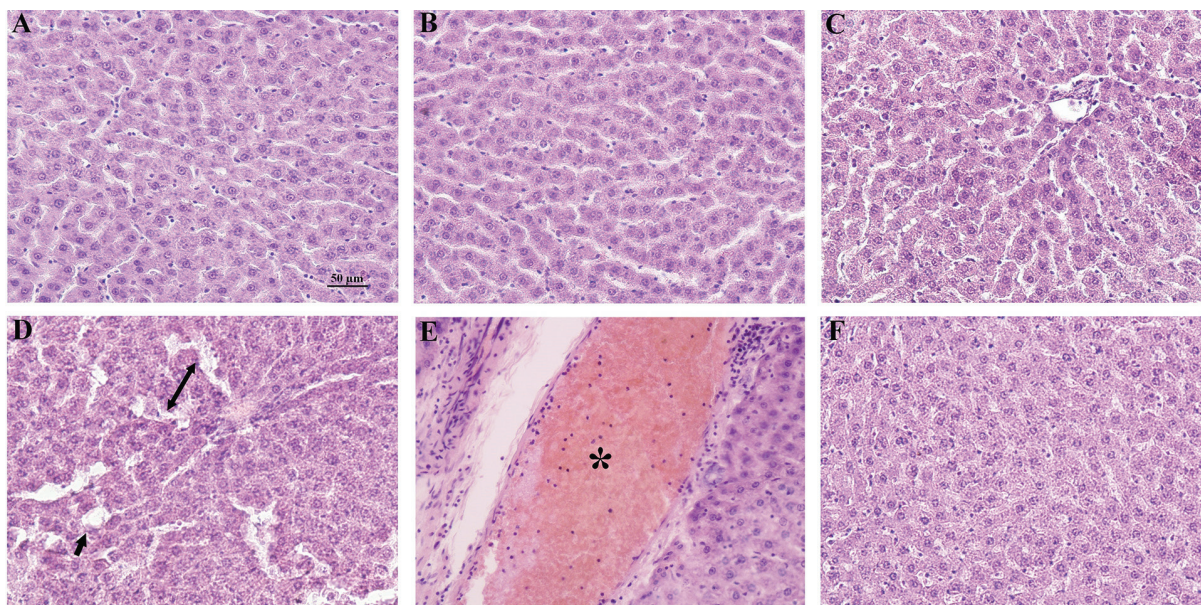


Figure 5: Photomicrographs of the rats' livers are shown. The control (A), RES (B), RES+BP (C), BPA (D&E), and olive oil (F) groups were stained with hematoxylin and eosin (H&E $\times 20$). Comparisons of the liver tissue between the different groups show a dilated sinusoidal space, a decreased hepatocyte cytoplasm volume, vacuolated foamy hepatocytes filled with a lipid type (arrow), and congestion (star sign) in the BPA group. No structural changes are identified in the rats treated with RES. RES: Resveratrol; RES+BPA: Resveratrol+Bisphenol A; BPA: Bisphenol A

KCs are hepatic macrophages set in the lumen of the liver sinusoids. When activated, they release several cytokines and play a key role in the pathogenesis of various liver diseases.³² KCs have been posited as the source of the inflammatory response, because they are implicated in the production of pro-inflammatory cytokines.³³ The oral administration of BPA induces inflammatory cellular infiltration, increases the number of KCs, and causes other histopathological changes in the mouse liver.³⁴ A study demonstrated that treatment with BPA led to vacuolated hepatocytes, dilated sinusoids, and increased KC numbers.³⁵ In the same line, previous research indicated that BPA causes cell inflammation,³⁵ hepatocyte vacuolation, and liver damage.³⁶ Our quantitative microscopic examination confirms these findings.

In the current investigation, we also focused on identifying the effects of RES on the liver exposed to BPA and observed the protective effects of this herbal compound on the liver manifested as diminished KC numbers, augmented total liver volume, enhanced hepatocyte nucleus and cytoplasm volumes, and increased hepatocyte numbers. Gong and colleagues reported that RES prevented the increase in oxidative enzymes, thus preventing liver oxidation.³⁷ In a previous study, investigators induced liver damage by the acute administration of carbon tetrachloride (CCl₄), and observed that the transferase enzymes in the liver tissue were increased significantly, while RES completely prevented the increase in

catalytic enzymes.³⁸ Another study showed that RES conferred protection against methotrexate-induced hepatic injury in rats.³⁹ The results of another investigation on RES suggested that it lowered mortality and liver damage in mice.⁴⁰ Kawada and others showed that RES selectively decreased the level of cell-cycle protein in stellate cells in the liver and concluded that the generation of nitric oxide (NO) and tumor necrosis factor- α (TNF- α) by lipopolysaccharide-stimulated KCs was strongly inhibited by RES.⁴¹

In the present study, BPA consumption resulted in increased KC numbers, sinusoidal space lengths and volumes, and tissue vacuolation, while it led to diminished hepatocyte numbers, hepatocyte nucleus and cytoplasm volumes, and portal triad volumes, all of which affected a reduction in the total volume of the liver tissue. We observed that the use of 50 mg/kg of BPA to rats, in comparison with their control counterparts, created histologically altered liver tissue, inflammatory factor release, macrophage accumulation, sinusoidal dilatation, and vacuolated hepatocytes, followed by increased KC numbers, diminished liver and portal triad volumes, and decreased hepatocyte numbers. In other words, BPA caused severe injury to the liver, and it appears that BPA increased NO and led to sinusoidal dilatation.⁴²

Our findings appear to confirm the notion that RES protects the liver against damage by decreasing the total number of KCs and probably reducing ROS. Moreover, our results demonstrated that RES conferred an increase

in the volume of the liver and the total number of hepatocytes and decreased the sinusoidal space. The major limitation of the current study was, however, the currently inevitable practice of animal euthanasia.

Conclusion

In the present stereological study, rats exposed to BPA were treated with different combinations of RES. Complications caused by BPA to the liver structure included reduced hepatocyte numbers, decreased hepatocyte nucleus and cytoplasm volumes, and increased sinusoidal spaces. The results demonstrated that while BPA led to oxidative damage to the liver by augmenting KC numbers, the consumption of RES conferred liver protection. We recommend that future studies on RES measure such factors as liver enzymes to obtain more robust results.

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Conflict of Interest: None declared.

References

- Rochester JR, Bolden AL. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. *Environ Health Perspect.* 2015;123:643-50. doi: 10.1289/ehp.1408989. PubMed PMID: 25775505; PubMed Central PMCID: PMC4492270.
- Acconcia F, Pallottini V, Marino M. Molecular Mechanisms of Action of BPA. *Dose Response.* 2015;13:1559325815610582. doi: 10.1177/1559325815610582. PubMed PMID: 26740804; PubMed Central PMCID: PMC4679188.
- Gassman NR. Induction of oxidative stress by bisphenol A and its pleiotropic effects. *Environ Mol Mutagen.* 2017;58:60-71. doi: 10.1002/em.22072. PubMed PMID: 28181297; PubMed Central PMCID: PMC45458620.
- Korkmaz A, Ahabab MA, Kolankaya D, Barlas N. Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. *Food Chem Toxicol.* 2010;48:2865-71. doi: 10.1016/j.fct.2010.07.019. PubMed PMID: 20643179.
- Hanioka N, Naito T, Narimatsu S. Human UDP-glucuronosyltransferase isoforms involved in bisphenol A glucuronidation. *Chemosphere.* 2008;74:33-6. doi: 10.1016/j.chemosphere.2008.09.053. PubMed PMID: 18990428.
- Grijalva J, Vakili K. Neonatal liver physiology. *Semin Pediatr Surg.* 2013;22:185-9. doi: 10.1053/j.sempedsurg.2013.10.006. PubMed PMID: 24331092.
- Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH, et al. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. *Oxid Med Cell Longev.* 2012;2012:194829. doi: 10.1155/2012/194829. PubMed PMID: 22888396; PubMed Central PMCID: PMC409570.
- Bjornsson ES. Hepatotoxicity by Drugs: The Most Common Implicated Agents. *Int J Mol Sci.* 2016;17:224. doi: 10.3390/ijms17020224. PubMed PMID: 26861310; PubMed Central PMCID: PMC4783956.
- Yang CZ, Yaniger SI, Jordan VC, Klein DJ, Bittner GD. Most plastic products release estrogenic chemicals: a potential health problem that can be solved. *Environ Health Perspect.* 2011;119:989-96. doi: 10.1289/ehp.1003220. PubMed PMID: 21367689; PubMed Central PMCID: PMC3222987.
- Faheem M, Jahan N, Lone K. Histopathological effects of bisphenol-A on liver, kidneys and gills of Indian major carp, *Catla catla* (Hamilton, 1822). *J Anim Plant Sci.* 2016;26:514-22. doi: 10.29261/pakvetj/2019.003.
- Burns J, Yokota T, Ashihara H, Lean ME, Crozier A. Plant foods and herbal sources of resveratrol. *J Agric Food Chem.* 2002;50:3337-40. doi: 10.1021/jf0112973. PubMed PMID: 12010007.
- Breuss JM, Atanasov AG, Uhrin P. Resveratrol and Its Effects on the Vascular System. *Int J Mol Sci.* 2019;20. doi: 10.3390/ijms20071523. PubMed PMID: 30934670; PubMed Central PMCID: PMC6479680.
- Bo S, Ciccone G, Castiglione A, Gambino R, De Michieli F, Villosio P, et al. Anti-inflammatory and antioxidant effects of resveratrol in healthy smokers a randomized, double-blind, placebo-controlled, cross-over trial. *Curr Med Chem.* 2013;20:1323-31. doi: 10.2174/0929867311320100009. PubMed PMID: 23298135.
- Kyselova V, Peknicova J, Buckiova D, Boubelik M. Effects of p-nonylphenol and resveratrol on body and organ weight and in vivo fertility of outbred CD-1 mice. *Reprod Biol Endocrinol.* 2003;1:30. doi: 10.1186/1477-7827-1-30.

- PubMed PMID: 12749770; PubMed Central PMCID: PMCPMC155686.
- 15 Opipari AW, Jr., Tan L, Boitano AE, Sorenson DR, Aurora A, Liu JR. Resveratrol-induced autophagocytosis in ovarian cancer cells. *Cancer Res.* 2004;64:696-703. doi: 10.1158/0008-5472.can-03-2404. PubMed PMID: 14744787.
 - 16 Guo Y, Wang A, Liu X, Li E. Effects of resveratrol on reducing spermatogenic dysfunction caused by high-intensity exercise. *Reprod Biol Endocrinol.* 2019;17:42. doi: 10.1186/s12958-019-0486-7. PubMed PMID: 31060552; PubMed Central PMCID: PMCPMC6503383.
 - 17 Olsson AS, Robinson P, Pritchett K, Sandøe P. Animal research ethics. Hau J, Van Hoosier Jr GL, Handbook of Laboratory Animal Science. Boca Raton: CRC Press; 2002. p. 13-31.
 - 18 Ballesteros-Gomez A, Rubio S, Perez-Bendito D. Analytical methods for the determination of bisphenol A in food. *J Chromatogr A.* 2009;1216:449-69. doi: 10.1016/j.chroma.2008.06.037. PubMed PMID: 18635192.
 - 19 Karabekir SC, Ozgorgulu A. Possible protective effects of resveratrol in hepatocellular carcinoma. *Iran J Basic Med Sci.* 2020;23:71-8. doi: 10.22038/IJBMS.2019.36821.8774. PubMed PMID: 32405350; PubMed Central PMCID: PMCPMC7206832.
 - 20 Boyce RW, Dorph-Petersen KA, Lyck L, Gundersen HJ. Design-based stereology: introduction to basic concepts and practical approaches for estimation of cell number. *Toxicol Pathol.* 2010;38:1011-25. doi: 10.1177/0192623310385140. PubMed PMID: 21030683.
 - 21 Tang Y, Nyengaard JR, Andersen JB, Baandrup U, Gundersen HJ. The application of stereological methods for estimating structural parameters in the human heart. *Anat Rec (Hoboken).* 2009;292:1630-47. doi: 10.1002/ar.20952. PubMed PMID: 19714657.
 - 22 Weibel ER, Kistler GS, Scherle WF. Practical stereological methods for morphometric cytology. *J Cell Biol.* 1966;30:23-38. doi: 10.1083/jcb.30.1.23. PubMed PMID: 5338131; PubMed Central PMCID: PMCPMC2106982.
 - 23 Nyengaard JR. Stereologic methods and their application in kidney research. *J Am Soc Nephrol.* 1999;10:1100-23. PubMed PMID: 10232698.
 - 24 Karbalay-Doust S, Noorafshan A. Stereological study of the effects of nandrolone decanoate on the mouse liver. *Micron.* 2009;40:471-5. doi: 10.1016/j.micron.2008.12.006. PubMed PMID: 19188074.
 - 25 Marcos R, Monteiro RA, Rocha E. Design-based stereological estimation of hepatocyte number, by combining the smooth optical fractionator and immunocytochemistry with anti-carcinoembryonic antigen polyclonal antibodies. *Liver Int.* 2006;26:116-24. doi: 10.1111/j.1478-3231.2005.01201.x. PubMed PMID: 16420517.
 - 26 Rafati A, Hoseini L, Babai A, Noorafshan A, Haghbin H, Karbalay-Doust S. Mitigating Effect of Resveratrol on the Structural Changes of Mice Liver and Kidney Induced by Cadmium; A Stereological Study. *Prev Nutr Food Sci.* 2015;20:266-75. doi: 10.3746/pnf.2015.20.4.266. PubMed PMID: 26770914; PubMed Central PMCID: PMCPMC4700916.
 - 27 Han C, Hong YC. Bisphenol A, Hypertension, and Cardiovascular Diseases: Epidemiological, Laboratory, and Clinical Trial Evidence. *Curr Hypertens Rep.* 2016;18:11. doi: 10.1007/s11906-015-0617-2. PubMed PMID: 26781251.
 - 28 Bindhumol V, Chitra KC, Mathur PP. Bisphenol A induces reactive oxygen species generation in the liver of male rats. *Toxicology.* 2003;188:117-24. doi: 10.1016/s0300-483x(03)00056-8. PubMed PMID: 12767684.
 - 29 Iwakiri Y. Nitric oxide in liver fibrosis: The role of inducible nitric oxide synthase. *Clin Mol Hepatol.* 2015;21:319-25. doi: 10.3350/cmh.2015.21.4.319. PubMed PMID: 26770919; PubMed Central PMCID: PMCPMC4712158.
 - 30 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.
 - 31 Nakagawa Y, Tayama S. Metabolism and cytotoxicity of bisphenol A and other bisphenols in isolated rat hepatocytes. *Arch Toxicol.* 2000;74:99-105. doi: 10.1007/s002040050659. PubMed PMID: 10839477.
 - 32 Wu LM, Yang Z, Zhou L, Zhang F, Xie HY, Feng XW, et al. Identification of histone deacetylase 3 as a biomarker for tumor recurrence following liver transplantation in HBV-associated hepatocellular carcinoma. *PLoS One.* 2010;5:e14460. doi: 10.1371/journal.pone.0014460. PubMed PMID: 21206745; PubMed Central PMCID: PMCPMC3012077.
 - 33 Kopf M, Bachmann MF, Marsland BJ. Averting inflammation by targeting the cytokine environment. *Nat Rev Drug Discov.*

- 2010;9:703-18. doi: 10.1038/nrd2805. PubMed PMID: 20811382.
- 34 Hussein RM, Eid JI. Pathological mechanisms of liver injury caused by oral administration of bisphenol A. *Life Science Journal*. 2013;10:663.
 - 35 Mourad IM, Khadrawy YA. The sensitivity of liver, kidney and testis of rats to oxidative stress induced by different doses of bisphenol A. *Life*. 2012;50:19.
 - 36 Hassan N, Loon LL. Goal programming with utility function for funding allocation of a university library. *Applied Mathematical Sciences*. 2012;6:5487-93.
 - 37 Li F, Gong Q, Dong H, Shi J. Resveratrol, a neuroprotective supplement for Alzheimer's disease. *Curr Pharm Des*. 2012;18:27-33. doi: 10.2174/138161212798919075. PubMed PMID: 22211686.
 - 38 Rivera H, Shibayama M, Tsutsumi V, Perez-Alvarez V, Muriel P. Resveratrol and trimethylated resveratrol protect from acute liver damage induced by CCl₄ in the rat. *J Appl Toxicol*. 2008;28:147-55. doi: 10.1002/jat.1260. PubMed PMID: 17541932.
 - 39 Tunali-Akbay T, Sehirli O, Ercan F, Sener G. Resveratrol protects against methotrexate-induced hepatic injury in rats. *J Pharm Pharm Sci*. 2010;13:303-10. doi: 10.18433/j30k5q. PubMed PMID: 20816014.
 - 40 Bujanda L, Garcia-Barcina M, Gutierrez-de Juan V, Bidaurrezaga J, de Luco MF, Gutierrez-Stampa M, et al. Effect of resveratrol on alcohol-induced mortality and liver lesions in mice. *BMC Gastroenterol*. 2006;6:35. doi: 10.1186/1471-230X-6-35. PubMed PMID: 17105669; PubMed Central PMCID: PMC1657014.
 - 41 Kawada N, Seki S, Inoue M, Kuroki T. Effect of antioxidants, resveratrol, quercetin, and N-acetylcysteine, on the functions of cultured rat hepatic stellate cells and Kupffer cells. *Hepatology*. 1998;27:1265-74. doi: 10.1002/hep.510270512. PubMed PMID: 9581680.
 - 42 Sanz-Cameno P, Medina J, Garcia-Buey L, Garcia-Sanchez A, Borque MJ, Martin-Vilchez S, et al. Enhanced intrahepatic inducible nitric oxide synthase expression and nitrotyrosine accumulation in primary biliary cirrhosis and autoimmune hepatitis. *J Hepatol*. 2002;37:723-9. doi: 10.1016/s0168-8278(02)00266-0. PubMed PMID: 12445411.