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Hepatocellular Injury of Albino Rats Induced by Commonly Used Phenolic Plastic Additives

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ABSTRACT

The wide-spread use of plastic products induced noticeable hazards on human health that may be referred to the leakage of some plastic content in the food and drinks. The present study investigates effects of Bisphenol A (BPA) (25/ 100 mg/Kg (bw)) and 4-nonylphenol (NP) (25/ 100 mg/Kg (bw)) and their mixtures for two months on oxidant -antioxidant balance, liver biomarkers and liver tissue structure in albino rats. The obtained results revealed elevation in serum malondialdehyde (MDA), Protein Carbonyl (PC) and 8 hydroxyguanisin (8-OHG) oxidation markers. The elevation was dose-dependent in individuals of treated groups, and more obvious in mixture treated groups. Concurrent to the previous effects, reduction in antioxidant markers Superoxide Dismutase (SOD); catalase (CAT) and Total Antioxidant Capacity (TAC) were recorded. Remarkable changes in serum liver biomarkers Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total Protein (TP), Albumin (ALB), reflects the injury in liver tissues that leads to leakage in serum. The previous biochemical findings were confirmed by histopathological examination where damage in liver tissues architecture was represented in necrosis of the hepatocytes; congestion of the blood vessels and sinusoids and degeneration in hepatocytes in high dose treated animals in either individually or mixture groups. In conclusion, uses of plastic products especially in food should be limited as it forms a great hazard on the human health.

KEY WORDS: Bisphenol A; 4-Nonylphenol; Oxidative Stress Biomarkers; Antioxidants Biomarkers; Liver Biomarkers.

INTRODUCTION

Plastics usually comprise of organic polymers with high molecular masses, as well as other substances. They are largely synthetic and originate from petrochemicals, however many are considered to be partially natural (Verma et al., 2016). Plastic products contain varying types of constituents, such as Teflon, Polypropylene, Polyvinylchloride, Polystyrene, Polyethylene (Sati et al., 2012). Phenolic substances such as Bisphenol A(BPA) and Nonylphenol (NP) are used in high volumes worldwide (Su et al., 2018). The manufacture of polycarbonate plastic (for example water bottles and baby bottles) and epoxy resins employ environmental pollutants, including BPA and NP. Bisphenol A (2,2-bis(4-hydroxyphenyl)) propane is an instrumental chemical in the manufacture of plastic products, such as water bottles, bags, and containers as well as food utensils (Kharrazian et al., 2019). Extensive effects in biological systems haven't gone unnoticed and is subsequently characterized as an Endocrine Disrupting Chemical (EDC), which has garnered attention in various fields of toxicological and environmental research. As exogenous agents, EDCs are capable of impeding with the synthesis, metabolism, and action of endogenous hormones in animals and humans (Ola-Davies et al., 2018). Eating from canned food and drinking water from plastic are the most common sources of exposure to Bisphenol. It is filtered from the plastic lining of food and beverage cans and subsequently finds its way into their contents. Bisphenol is further filtered from plastic when washed with a strong detergent, when the plastic holds acidic liquids, or when it is placed in high temperature regions. The manufacture of epoxy gum, which is used for overlaying water tanks, also uses Bisphenol (Sabour, 2019).

BPA's effects the liver and kidney through the induction of reactive oxygen species (ROS) and oxidation of DNA in the liver. Presence of multinucleated giant cells in rat liver hepatocytes, modification of liver and kidney biochemical profiles, and degradation of renal tubules in kidneys of rats and mice were reported by Helal et al. (2019). Preceding research has concentrated on the effects of BPA on human health and concluded that the noxious impacts of BPA are consequences of amplified oxidative stress (Kim et al., 2016). In another study by Eweda et al. (2020) reported that BPA induced oxidative stress in liver cells and were also heightened as a result of amplified levels of hepatic MDA, lessened functionality of glutathione

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peroxidase/glutathione reductase (GPX/GR) system and SOD, and diminished quantities of GSH. Furthermore, mitochondrial-mediated apoptosis in the hepatic tissue and inflammatory cytokine dysregulation have also been observed as other effects of BPA (Abdelzaher et al., 2018).

Nonylphenol (NP) is a chemical greatly used industrially. NP has been observed in human adipose tissue. Various tissues, including but not limited to the liver, have been found to have elevated reactive oxygen species and oxidative stress was also induced as a result of exposure to NP (Yu et al., 2017). Effects of NP may be attributed to the amplified presence of reactive oxygen species (ROS) and participation of oxidative reactions (Kazemi et al., 2016). Catalase (CAT), superoxide dismutase (SOD) are vital antioxidant enzymes that aid the organism in alleviating external pollutants and complement the protective enzyme system of the organism that are reduced by NP (Faheem and Lone, 2017). The pro-oxidant/ antioxidant ratio of cells may be disrupted due to emerging contaminants, like NP and NP-9 (De la Parra-Guerra and Olivero-Verbel, 2020). Endocrine disrupting chemicals (EDCs), like BPA and 4-NP are hypothesized to be one of the reasons that lead to a heightened presence of nonalcoholic liver disease in animal models (Zhang et al., 2018).

The present study aimed to investigate the chronic impact of with different doses of each of Bisphenol A and Nonylphenol and their mixtures on oxidant antioxidant balance, liver biomarkers and liver tissue structure in albino rats.

MATERIALS AND MEHODS

Chemicals

1- Bisphenol A (2,2-Bis(4-hydroxyphenyl)propane; $C_{15}H_{16}O_2$) CASRN: 80-05-7,99% .
2- 4-Nonylphenol (4-Nonylphenol; $C_{15}H_{24}O$) CASRN 84852-15-3,98%.
All chemicals were obtained from Tokyo chemical Industry (TCI) CO., LTD and dissolved in ethanol.

Animals

70 male Albino rats aged between 3-5 months with weight (180-200g), were obtained from the animal House at King Fahd Center for Medical Research (KFMC), King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. All rats were kept in polycarbonate cages with stainless steel covers in a temperature-controlled room (23 ± 1ºC) and humidity (50 ± 10%) and fed with standard diet and free access of water. The rats were exposed to 12 hours of daily light for two months and handled with care as the recommended guidelines of the King Fahd Center for Medical Research Ethics committee. The animals were acclimatized to the laboratory for 1 week prior to the beginning of the experiment.

Experimental design

After acclimatization period, animals were divided into seven experimental groups, each group containing 10 rats. All animals were treated according to the standard procedures laid down by Organization of Economic Co-operation and Development (OECD) guidelines 2009 for combined chronic toxicity. The animals were treated orally by gavaging via stomach tube once daily for two months.
Animals were divided as follow:
Group (I): Control group rats were orally gavaged with 0.02% ethanol water and served as (+ve) control.
Group (II): (HBPA) rats were orally gavaged with a high dose of Bisphenol A (100mg/Kg (bw)) (Laws et al., 2000).
Group (III): (LBPA) rats were orally gavaged with a low dose of Bisphenol A (25mg/Kg (bw)) (Laws et al., 2000).
Group (IV): (HNP) rats were orally gavaged with a high dose of 4 Nonylphenol (100mg/Kg (bw)) (Jie et al., 2010).
Group (V): (LNP) rats were orally gavaged with a low dose of 4 Nonylphenol (25mg /Kg (bw)) (Jubendradass et al., 2012).
Group (VI): (HMIX of BPA & NP) rats were orally gavaged simultaneously with a mixture of high doses of each of BPA & NP (100mg /Kg(bw)).
Group (VII): (LMIX of BPA & NP) rats were orally gavaged simultaneously with a mixture of low doses of each of BPA & NP (25mg /Kg (bw)).

Blood sample collection

After 24 hours of receiving the last dose, experimental rats were anesthetized with ether then blood samples were obtained from the retro-orbital plexus vein according to the method of Sorg and Buckner, (1964). Blood samples were left to coagulate at room temperature, then placed in the centrifuge at 3000 rpm and 4C^{o} for 15 minutes. The clear non-hemolyzed supernatant serum was quickly removed and kept at -20C^{o} for further biochemical analysis. Rats were sacrificed by dislocation and dissected for liver samples.

Biochemical analysis:

Malondialdehyde (MDA) was measured according to the method of Yoshioka et al. (1979), Protein Carbonyl (PC) was determined using the method of Cadenas et al. (1977) and Wakeyama et al. (1982). Superoxide Dismutase (SOD) was carried out using the method of Masayasu and Hiroshi, (1979), Catalase (CAT) was investigated using the method of Aebi, (1984) , Total Antioxidant Capacity (TAC) was carried out using the method of Koracevic et al. (2001) and 8-hydroxy-2-deoxyguanosine(8-OHdG) using the method of Valko et al. (2004). Aspartate aminotransferase (AST) and Alanine Transaminase (ALT) were investigated-according to Reitman and Frankel,(1957). Total protein in serum was measured according to Weichselbaum, (1946), Albumin (ALB) was a bromcresol green reagent (pH 4.2) by the method of Doumas et al. (1971).

Histopathology

The liver samples were preserved in 10% formalin for 24 hours. Livers were dehydrated, paraffinized and then cross-sectioned at 2-3 microns, followed by staining with hematoxylin and eosin for the light microscopic examination. The processing technique of the light microscopic examination (hematoxylin and eosin staining) was adapted from Carleton et al. (1980).

Statistical analysis

Gathered data from the biochemical studies were tabulated as Mean ± SE. Comparison between groups was calculated by one-way analysis of variance (ANOVA) followed by Duncan's test at $P < 0.05$ using the SPSS-PC computer software package version 22.

RESULTS

Oxidative stress biomarkers in serum

Lipid peroxidation biomarker malondialdehyde (MDA) marker of oxidative stress recorded a significant increase in high dose nonylphenol group (NH) versus Low dose Bisphenol group (BL) group at $p < 0.05$.

Meanwhile, mixture groups with both high (BH&NH) and low (BL&NL) doses induced remarkable significant elevation in (MDA) versus individually treated groups. The percentages of increase were 72.81 % and 22.81% from control as expressed in (*Figure 1-A*).

Protein oxidation biomarker, Protein Carbonyl (PC) had the same pattern of MDA results, where significant increase was recorded in all treated groups. The elevation in PC was dose-dependent where pronounced increase was reported in high dose bisphenol A (BH), high dose nonylphenol (NH) and their mixtures (BH&NH) (*Figure 1-B*). However, Low doses (BL&NL) and their mixture groups recorded an increase that is less than the previous effect as demonstrated in (*Figure 1-B*).

The shown data manifests that treatment with high doses of BPA and NP and their mixture induced an increase in serum 8 hydroxy guanisine (8-OHdG) marker of oxidative damage of DNA. A pronounced significant increase in high dose mixture treated group was recorded with 22.41% from control, and significant versus control and all other groups. Individually treated groups BL and NL showed significant decrease in 8-OHdG versus control and high dose groups at $P<0.05$. However, slight enhancement was recorded in low mixture group with a 4.83% increase from control (*Figure 1-C*).

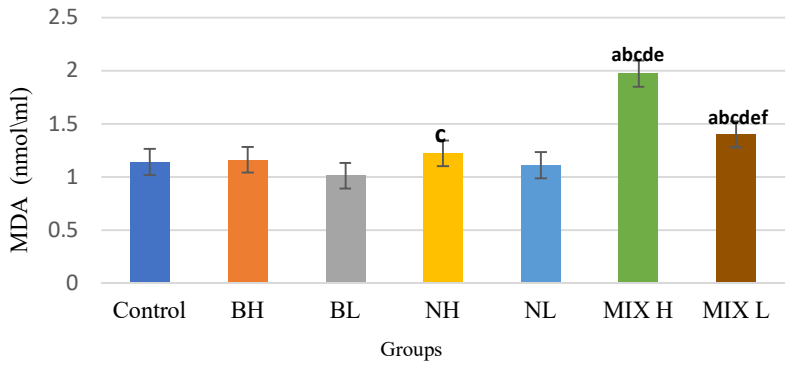


***Figure 1-A*: Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on Malondialdehyde (MDA)**

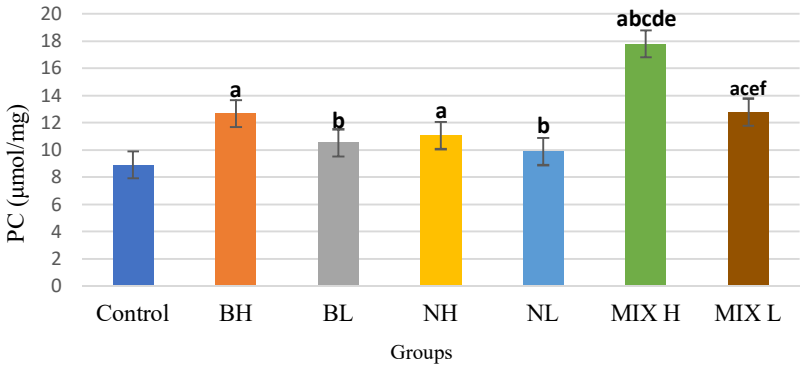


***Figure 1-B* : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on protein carbonyl (PC)**

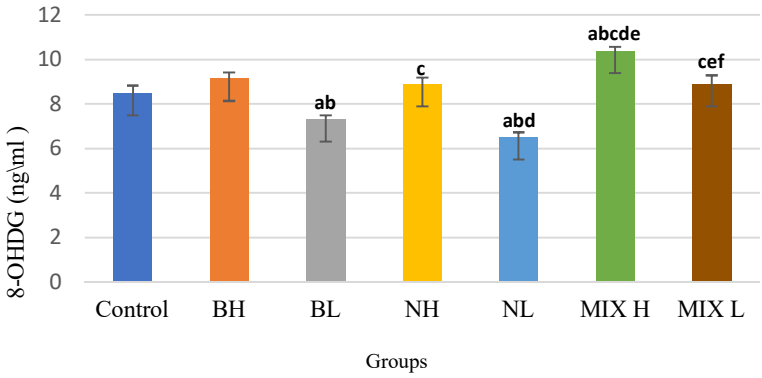


***Figure 1-C* : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on 8-hydroxy-2deoxyguanosine(8-OHdG)**

a: significant difference versus control at p<0.05. b: significant difference versus BH at p<0.05.
c: significant difference versus BL at p<0.5 d: significant difference versus NH at p<0.05.
e: significant difference versus NL at p<0.05. f: significant difference versus Mix H at p<0.05.

Antioxidant biomarkers in serum

Superoxide dismutase is an enzyme that alternately catalyzes the dismutation of superoxide anion into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Significant decrease in SOD throughout the experimental groups versus control and between groups was recorded in *Figure* (*2-A*). Catalase (CAT) enzyme that catalyze decomposing of hydrogen peroxide to water and oxygen recorded significant reduction in all treated groups significant versus control and between groups. Slight elevation from control was recorded in NL treated group and statistically significant versus control, (BH), (BL) and (NH) groups (*Figure 2-B*). In regards to the total defense system total antioxidant capacity (TAC), the depicted data showed a significant reduction in serum all through the groups, significant versus control and between groups. It is worth expressing that the pronounced reduction was obvious in high mixture groups (BH & NH) more than the low mixture groups (BL & ML), and the effect was dose-dependent (*Figure 2-C*).

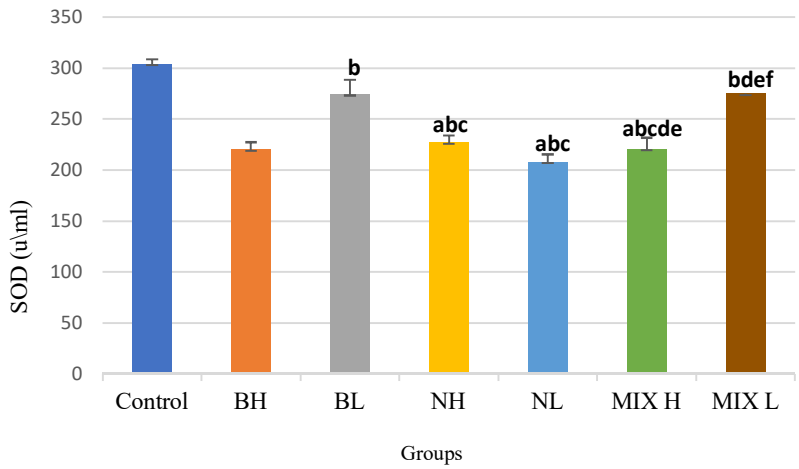


Figure 2-A :Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on Superoxide dismutase (SOD)

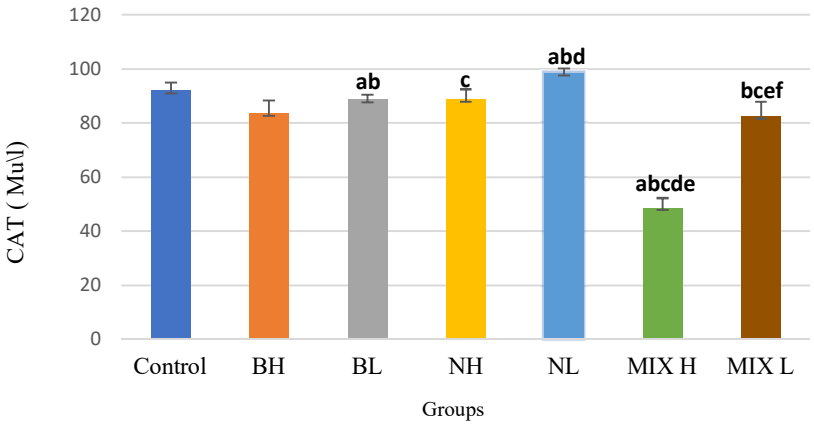


Figure 2-B : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on Catalase enzyme (CAT)

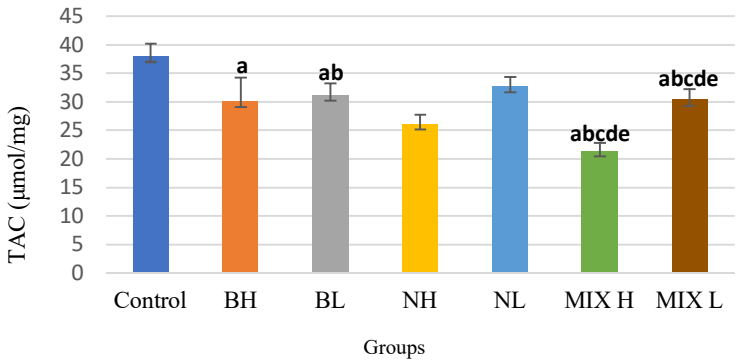


Figure 2-C : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on Total Antioxidant Capacity (TAC)

a: significant difference versus control at p<0.05. b: significant difference versus BH at p<0.05.
c: significant difference versus BL at p<0.5 d: significant difference versus NH at p<0.05.
e: significant difference versus NL at p<0.05. f: significant difference versus Mix H at p<0.05

Serum Liver biomarkers

Illustrated data in *Figure 3-A, 3-B, 3-C* and *4-D* expressed the effect of high and low doses of Bisphenol A (BH, BL) and Nonylphenol (NH, NL) in addition to their mixture on serum hepatospecific markers. Each of serum Alaninamino Transferase (ALT) and Aspartate amino transferase (AST) enzymes biomarker enzymes of liver recorded elevation in their activities, the increase was dose-dependent in all treatment groups compared to control in individually treated groups at p<0.05. Moreover, combination of BH & NH in mixture treated groups showed pronounced elevation in ALT and AST significant (P<0.05) versus other treated groups as shown in *Figure 3-A* and *Figure 3-B*.

Slight elevation in total serum protein (T.pro) was recorded in BH, NH and Mix (BH&NH) as expressed in *Figure 3-C*. Remarkable significant decrease in total protein was represented by -14.35%, -14.98% and -12.87% from control was recorded in low doses BL, NL and Mix L (*Figure 3-C*). Individual treatment with high and low doses of each of Bisphenol A and Nonylphenol as well as their mixtures induced pronounced significant (P<0.05) increase in serum albumin (ALB) level in all treated groups (*Figure 3-D*).

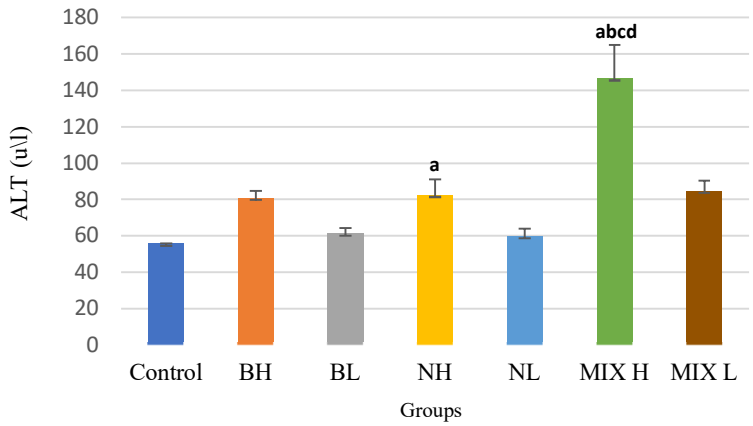


Figure 3-A : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on Alanine Transaminase (ALT)

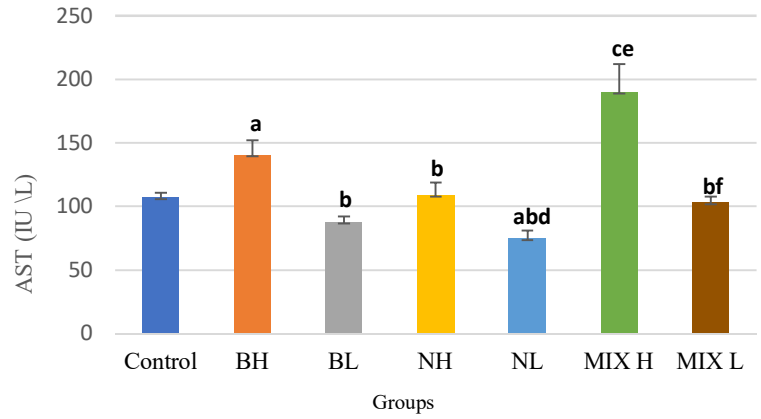


Figure 3-B : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on Aspartate Transaminase (AST)

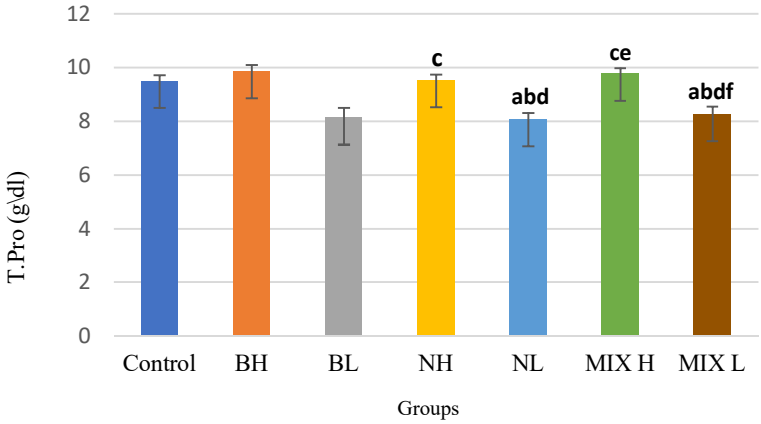


Figure 3-C : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol onTotal protein (Tp)

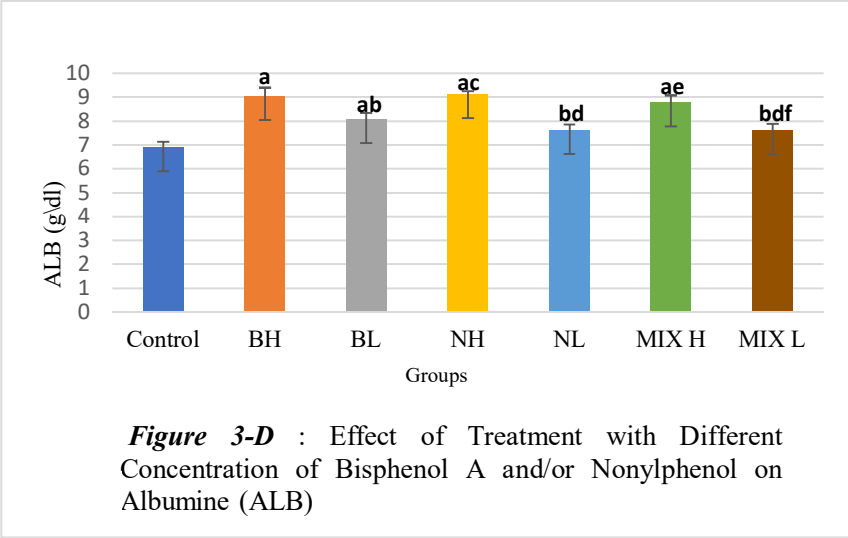
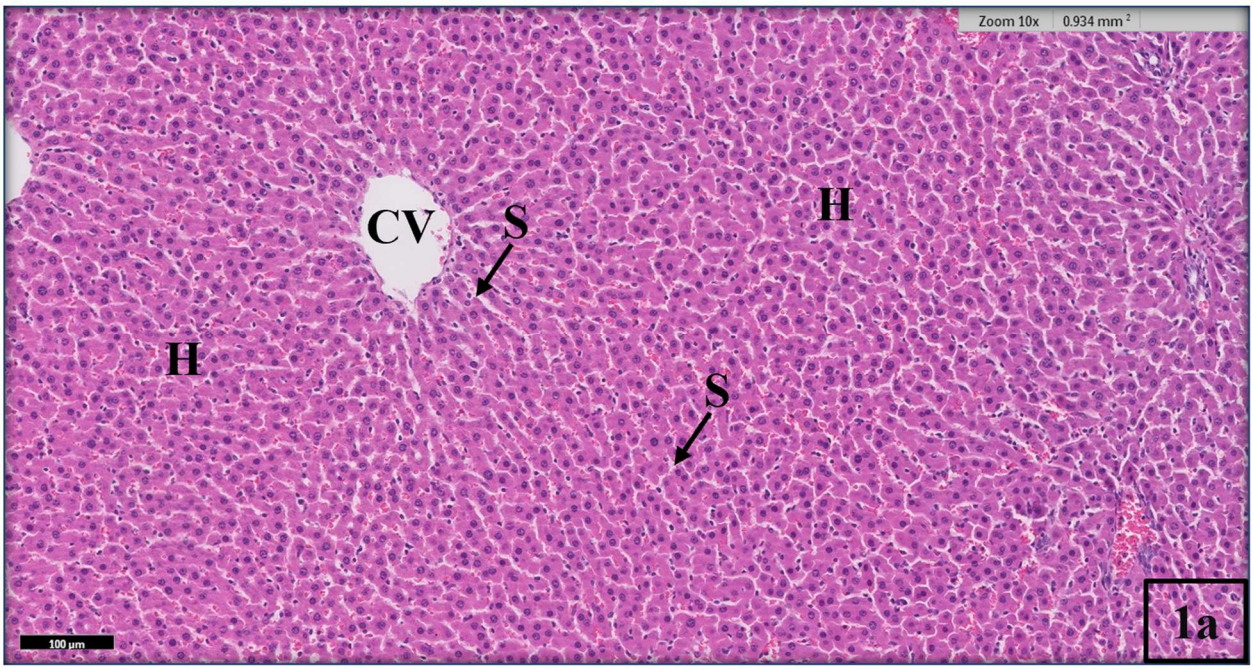


Figure 3-D : Effect of Treatment with Different Concentration of Bisphenol A and/or Nonylphenol on Albumine (ALB)

a: significant difference versus control at p<0.05. b: significant difference versus BH at p<0.05.
c: significant difference versus BL at p<0.5 d: significant difference versus NH at p<0.05.
e: significant difference versus NL at p<0.05. f: significant difference versus Mix H at p<0.05.

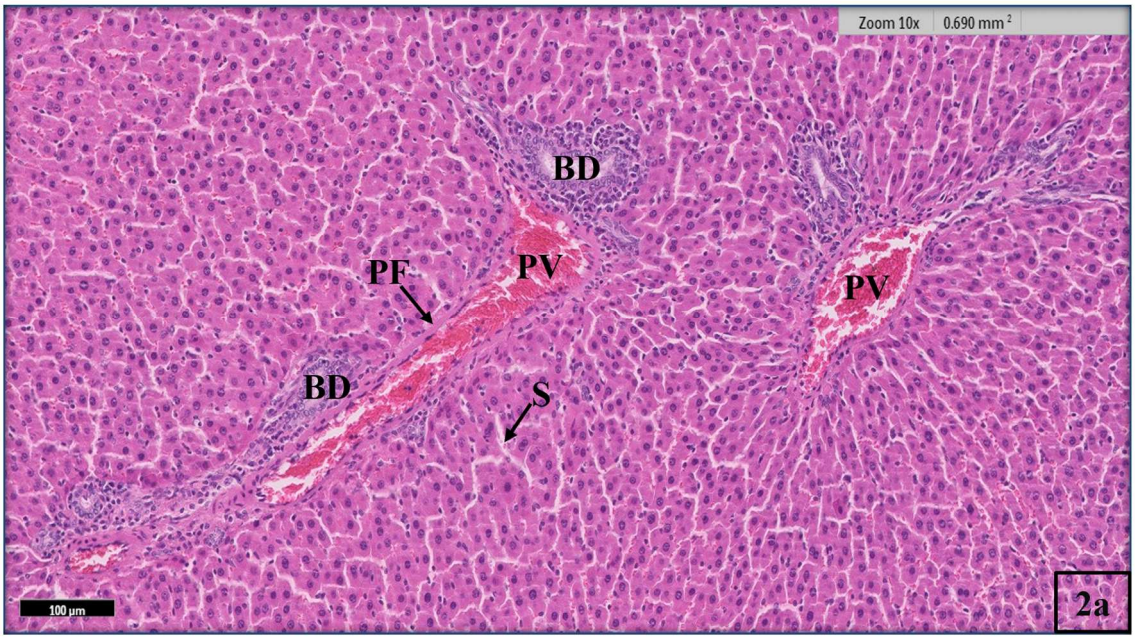
Histopathological Results

Histological examination of liver of rats in the control group revealed a distinguishable normal histoarchitecture and the central vein and stripes of hepatocytes with round nuclei as demonstrated in *photomicrograph (1a)*.

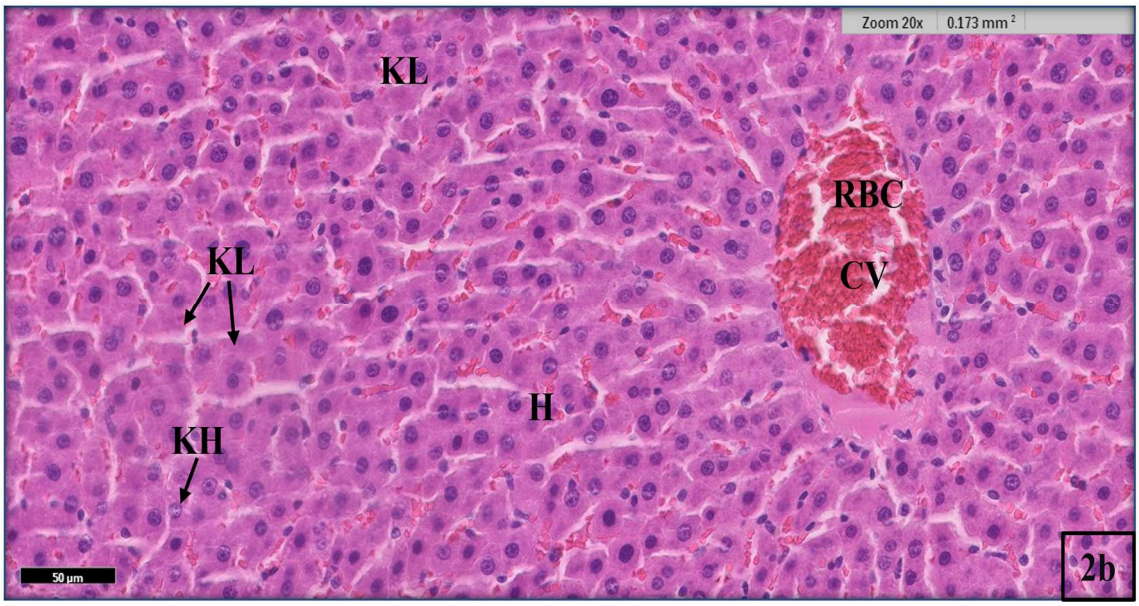


Photomicrograph 1a. **light micrograph of Group 1 control showing:** the central vein (CV) surrounded by plates of hepatocytes (H) separated by sinusoids (S)**.** H&E (×100µm).

Group (BL) of rats exposed to 25 mg/kg of Bisphenol A demonstrated histopathological changes represented in severe disruption in the portal area. Dilation and congestion of blood vessels with blood appeared along with an unusual proliferation of the bile ducts with an increase in the connective tissue surrounding the portal area, and inflammatory cellular infiltration around the blood vessels as shown in *photomicrograph (2a)*. In addition, dilation and congestion of the sinusoids was also evident and vacuolar degeneration was noticed in some hepatocytes where nuclei appeared karyolytic. Some cells appeared necrotic with atrophic and dark nuclei (pyknosis) (*photomicrograph 2b*).



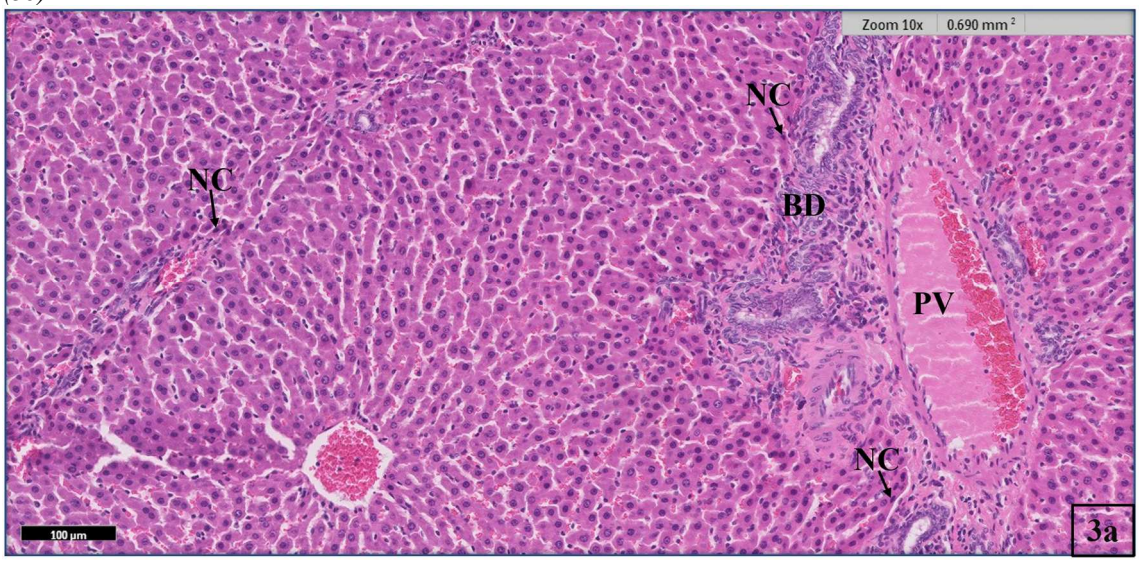
Photomicrograph (2a). **light micrograph of rats exposed to 25 mg/Kg (bw) concentration of Bisphenol A showing:** severe congestion of portal vein (PV) and moderate portal fibrosis (PF). Notice: bile duct proliferation (BD) and widened sinusoid (S). H&E (×100µm).



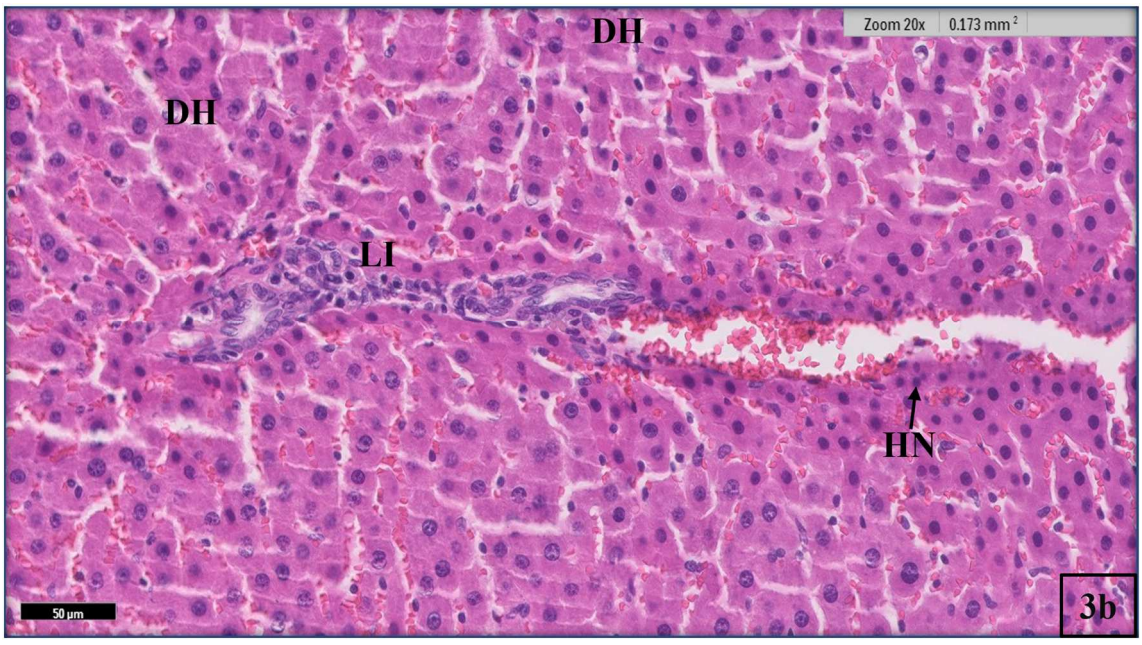
Photomicrograph (2b). **light micrograph of rats exposed to 25 mg/Kg (bw) concentration of Bisphenol A showing:** Congestion of central vein (CV) and sinusoid (S) with red blood cells necrosis (RBC) and degeneration of hepatocytes (H). Notice karyolysis (KL) and karyorrhexis (KH). H&E (×50µm).

Whereas, rats exposed to 100 mg/kg Bisphenol A (BH) group revealed nonspecific portal hepatitis which is a nonspecific response of the liver to various groups of abnormal histopathological changes as demonstrated in *photomicrograph (3a)*. Severe dilation and congestion of the portal vein was evident

surrounded by highly fibrosis connective tissue and proliferation of bile ducts. A row of necrotic cells was noted surrounding the portal area and the presence of proliferated Kupffer cells in the hepatic tissue and macrophage cells proliferated in the portal area. These changes were accompanied by the presence of cells surrounding the dilated sinusoids with inflammatory cellular infiltration as in *photomicrograph (3b)*.

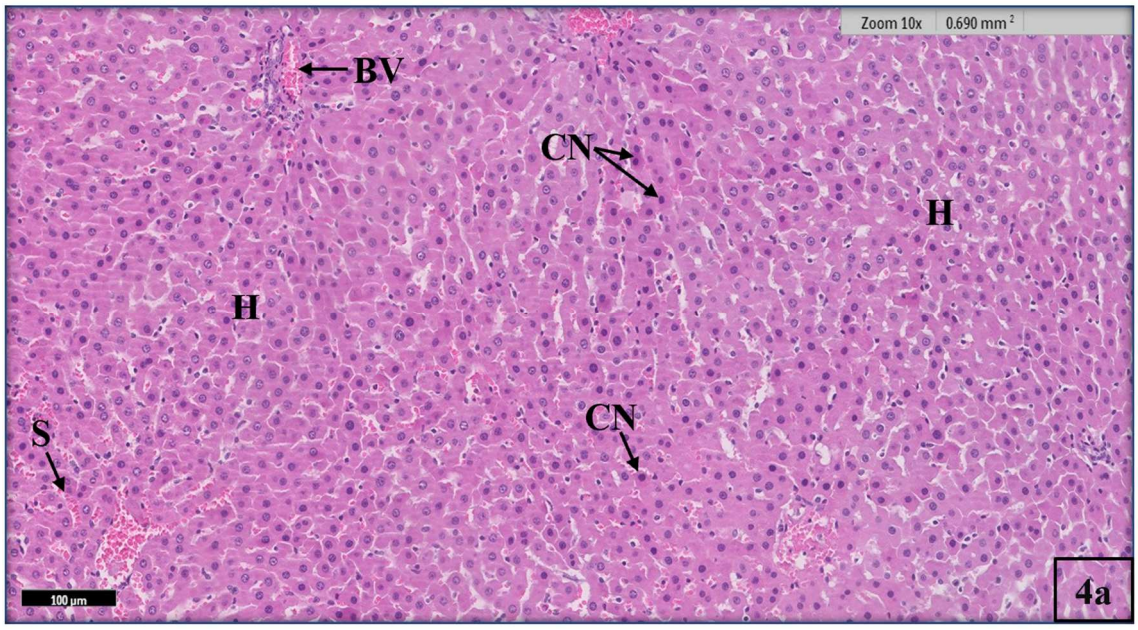


Photomicrograph (3a). **light micrograph of rats exposed to 100 mg/Kg (bw) concentration of Bisphenol A showing:** nonspecific portal hepatitis, severe dilated and chronic congested portal vein (PV) surrounded by a highly fibrous tissue and bile duct degeneration (BD). Notice: proliferation of necrotic cell (NC) around the fibrous tissue. H&E (×100µm).

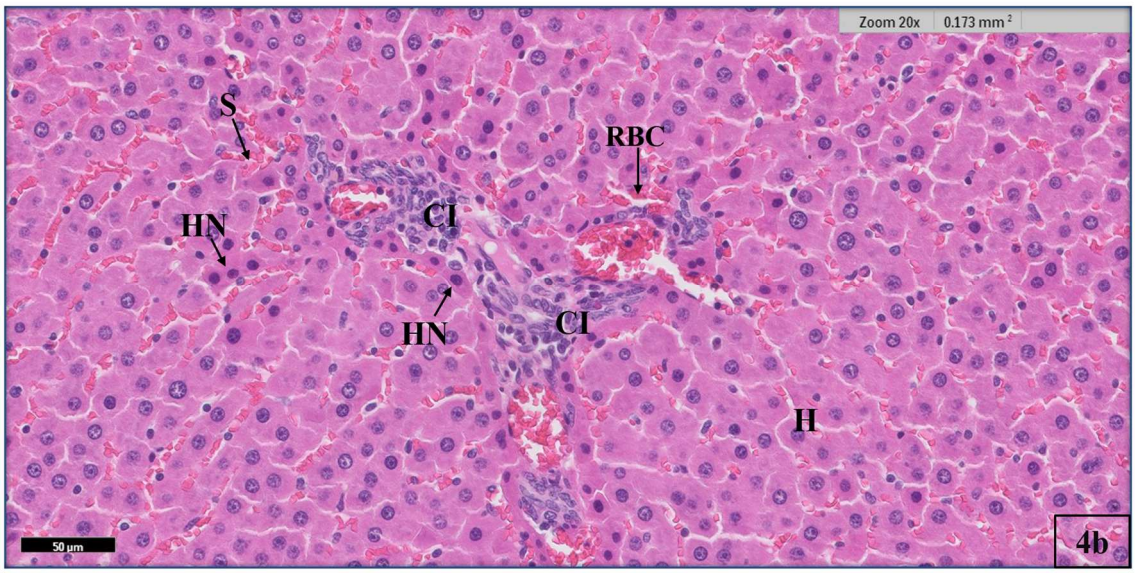


Photomicrograph (3b). **light micrograph of rats exposed to 100 mg/Kg (bw) concentration of Bisphenol A showing:** Hepatocellular necrosis (HN) surrounding the widened sinusoid and lymphocytes infiltration (LI). Notice: degeneration of hepatocytes (DH). H&E (×50µm).

On the other hand, rats treated with 25 mg/kg of NP (NL) group still held its hepatic histoarchitecture with moderate to severe changes represented in necrosis of the hepatocytes and slight congestion of the blood vessels and sinusoids as demonstrated in *photomicrograph (4a)*. Inflammatory cellular infiltration was also apparent and detailed around the congested blood vessels with a few proliferations of necrotic cells in the hepatic tissue, with dark and atrophic nuclei and the presence of vacuolar degeneration of the hepatocytes (*Photomicrograph 4b*).

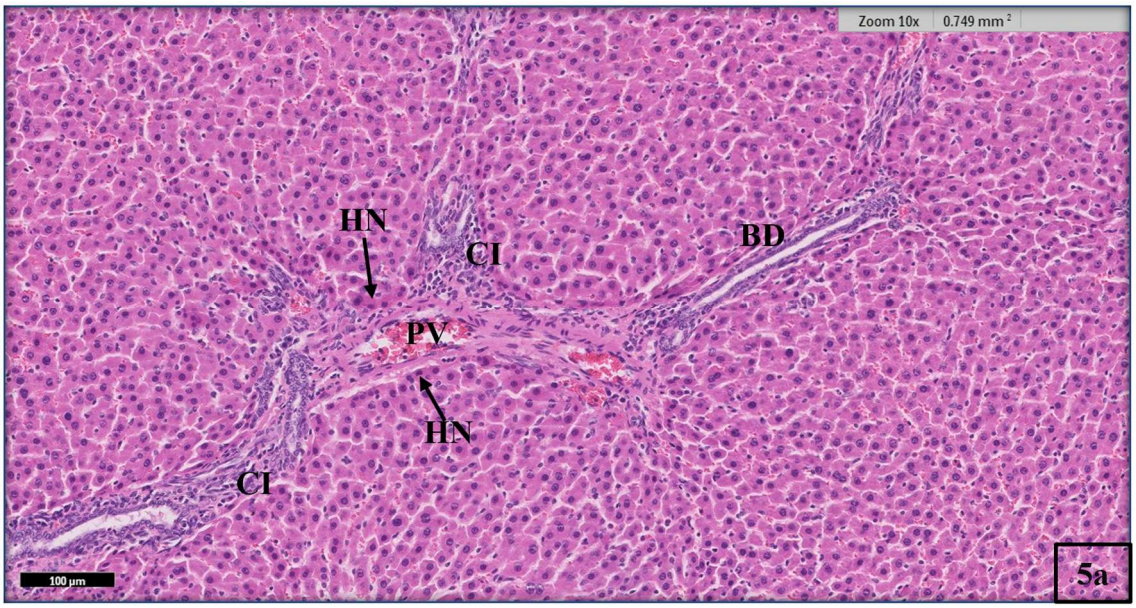


Photomicrograph (4a). **light micrograph of rats exposed to 25 mg/Kg (bw) concentration of Nonylphenol showing:** Nearly normal hepatocytes structure (H) with moderate liver cellular necrosis (CN) and a few congestions of blood vessels (BV) and sinusoids (S). H&E (×100µm).

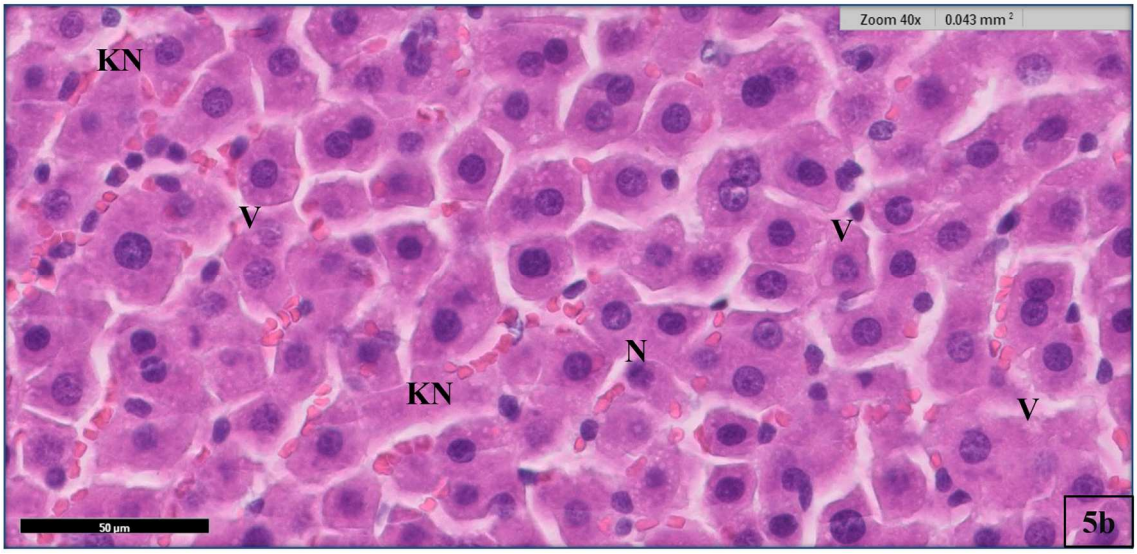


Photomicrograph (4b). **light micrograph of rats exposed to 25 mg/Kg (bw) concentration of Nonylphenol showing:** Focal inflammatory cellular infiltration (CI) and converging blood sinusoids (S) with red blood cells (RBC). Notice: few hepatocellular necrosis (HN) and lysis of Hepatocytes (H). H&E (×50µm)

However, the apparent affects in the liver of rats treated with high dose of 100 mg/kg of NP (NH group) increased, where hepatic tissue showed loss in the hepatic histoarchitecture where severe fibrosis appeared surrounding the portal area, with rupture of the portal vein's wall and the accumulation of substances inside it. In addition, inflammatory cellular infiltration and aggregation of macrophage cells were apparent as in *Photomicrograph (5a)*. Hepatocytes appeared with stored lipid droplets of different sizes, where they appeared vacuolated in the micrographs as a result of the lipids dissolving during the tissue sections preparation. In addition to vacuolar degeneration in many cells as demonstrated in *Photomicrograph (5b)*.

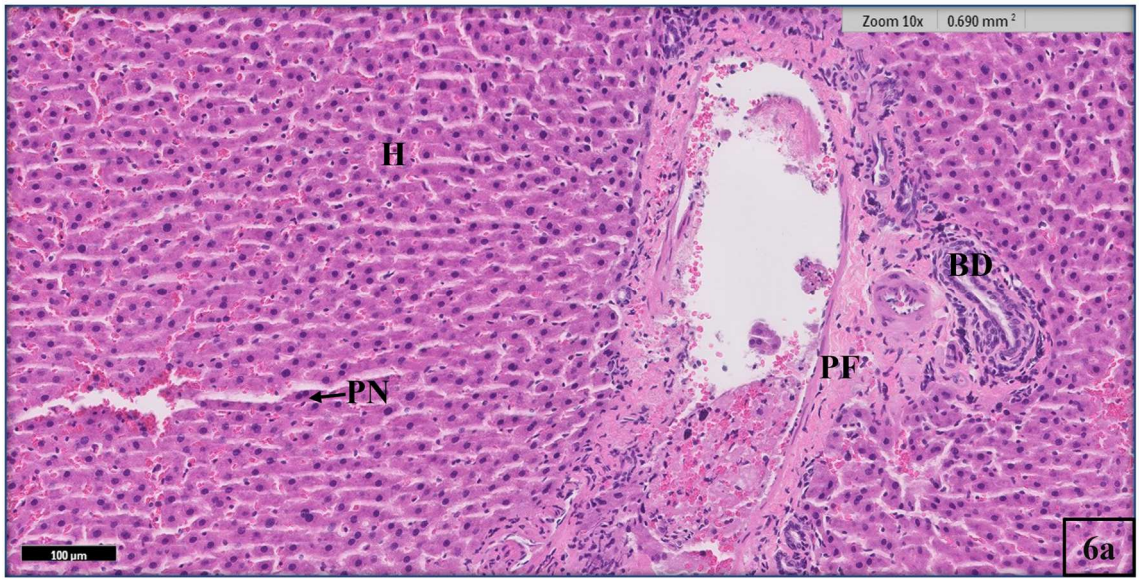


Photomicrograph (5a). **light micrograph of rats exposed to 100 mg/Kg (bw) concentration of Nonylphenol showing:** Loss of the normal architecture and degenerated hepatocytes (H) with pyknosis nuclei (PN) and severe portal fibrosis (PF) with chronic liver congestion and bile duct proliferation (BD). H&E (×100µm).

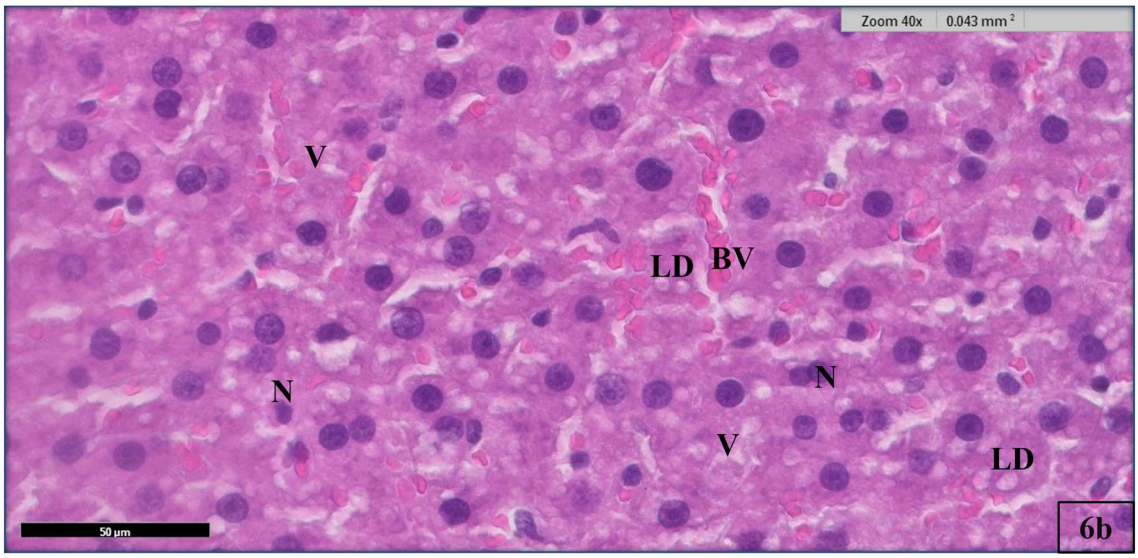


Photomicrograph (5b).**light micrograph of rats exposed to 100 mg/Kg (bw) concentration of Nonylphenol showing:** marked blood vessels congestion (BV) and lipid droplets (LD) of different sizes in hepatocytes. Notice: marginal nuclei of hepatocytes (N) and vacuolation in hepatocytes (V). H&E (×50µm).

Regarding the low dose mixture group (BL & NL mix), a dilation in the bile ducts appeared with congestion in the portal vein. Infiltration of inflammatory cells was found proliferated as a result of circulatory disturbance in the portal area and surrounded with a row of necrotic cells with an increase in the proliferation of Kupffer cells as shown in *Photomicrograph (6a)*. Additionally, a few hepatocytes were degenerated and necrotic in some places with the appearance of cytoplasmic vacuolations in the hepatocytes (*Photomicrograph 6b*).

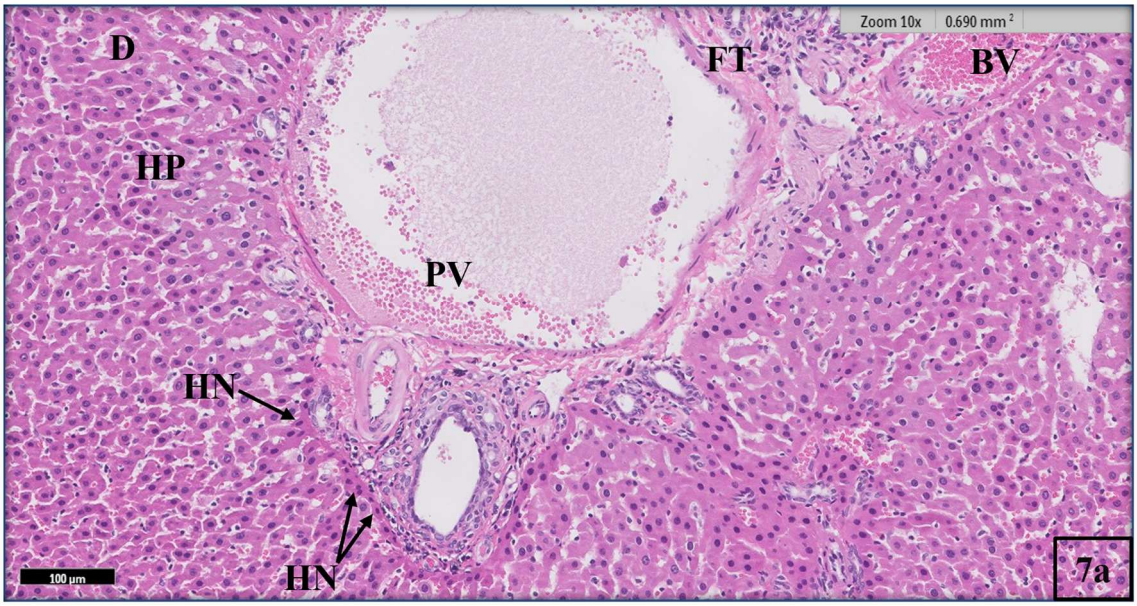


***Photomicrograph (6a)*. light micrograph of rats exposed to 25 mg/Kg (bw) of mixed concentrations of Bisphenol A & Nonylphenol showing:** Congestion of portal vein (PV) and diffused inflammatory cellular infiltration (CI), severe dilation of Bile duct (BD) surrounded by hepatocellular necrosis (HN). H&E (×100µm).

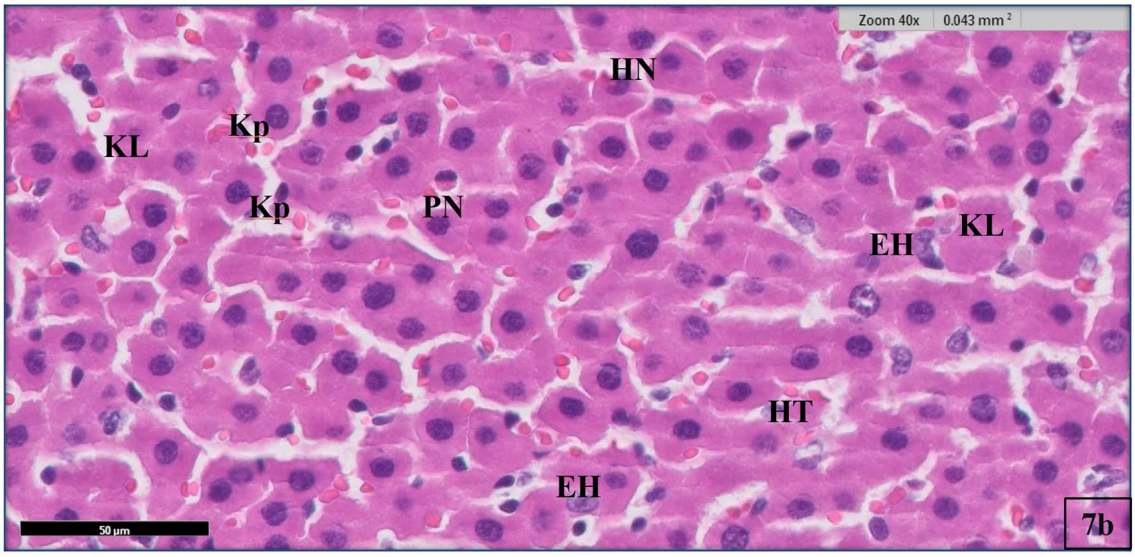


***Photomicrograph (6b)*. light micrograph of rats exposed to 25 mg/Kg (bw) of mixed concentrations of Bisphenol A & Nonylphenol showing:** Hepatocytes (H) in areas surrounding the central vein showing few cytoplasmic vacuolation (V), deformed and necrotic nuclei (N). Notice karyolysis nuclei (KN). H&E (×50µm).

Moreover, liver of rats treated with high dose mixture (BH&NH) group showed severe damage represented in the form of disarrangement of hepatic plates, distended, ruptured and congested blood vessels, surrounded by highly fibrous tissue and hepatocellular necrosis in the portal area. Additionally, hepatocellular necrosis was evident with karyolytic and pyknotic nuclei, and the proliferation of Kupffer cells in the dilated sinusoid and endothelial hypertrophy as shown in *photomicrograph (7a and b)*.



***Photomicrograph (7a)*. light micrograph of rats exposed to 100 mg/Kg (bw) of mixed concentrations of Bisphenol A & Nonylphenol showing:** Disarrangement of hepatic plates (HP) with distended (D), congested and ruptured blood vessels (BV). Notice: congestion of portal vein (PV) surrounded by a highly fibrous tissue (FT) and hepatocellular necrosis (HN) in the portal area. H&E (×100µm)**.**



***Photomicrograph (7b)*. light micrograph of rats exposed to 100 mg/Kg (bw) of mixed concentrations of Bisphenol A & Nonylphenol showing:** dissociation of hepatic tissue (HT) and hepatocellular necrosis (HN). Notice: karyolysis (KL) and pyknotic nuclei (PN) marked dilation of blood sinusoid with increased number of necrotic Kupffer cells (Kp) and endothelial hypertrophy (EH). H&E (×50µm).

DISCUSSION

Environmental concerns associated with plastic use are not only because of the amount of waste, but also the leaching of substances out of the plastic. Concerns are growing regarding potential health effects from widespread human exposure to plastic components (Verma et al., 2016). Bisphenol A (BPA) and nonylphenol (NP) are known phenolic compounds in the plastic industry that leak in the environment through industrial and municipal waste. They disrupt the inner system of animals due to its negative effect on the developmental and physiological properties through interfering with the normal endocrine functions of the living organism (Jayakanthan et al., 2015). This study explored the potentiality of subchronic exposure on the liver function and structure of albino rats. Results showed dose dependent elevation on some oxidative stress biomarkers lipid peroxidation biomarker (MDA) oxidized protein (PC) and oxidized DNA biomarker (8- OHG) pronounced in high doses of BH and NH groups as well

as high and low dose mixture groups Mix (BH & NH) and Mix (BL & NL). Oxidative stress is a state of imbalance between oxidants and antioxidants in favor of oxidants causing significant cellular damage. Free radicals play an important role in cellular damage resulting from the use of toxic chemicals, which can lead to cell death, and the development of many diseases (Sabour, 2019). Membrane phospholipids of aerobic organisms are continually subjected to oxidant challenges from exogenous and endogenous sources. For this reason, the MDA concentrations can indicate the rate and intensity of lipid peroxidation within the organism (Khene et al., 2017). In humans, several studies also have reported associations between urinary bisphenol concentrations and markers of oxidative stress, including 8hydroxydeoxyguanosine (8-OHdG), isoprostane, and malondialdehyde (MDA) (Wang et al., 2019). Meanwhile, administration of NP at a dose level of 50 µg/kg body weight/day for 30 days has been shown to increase oxidative stress parameters in blood of adult male rats (Karafakioglu and Aslan, 2010). As the two phenolic compounds exert the same actions, the pronounced elevation in all oxidative stress markers in mixture groups can be referred to the synergistic effect of the two compounds, which was more pronounced in high doses mixture groups. Concurrent to the above descriptive mechanism, reduction in the measured serum antioxidants markers superoxide dismutase (SOD), catalase (CAT) in addition to the total antioxidant capacity (TAC) was recorded in the present study in all treated groups pronounced in mixture groups. These findings were in consistence with previous studies treatment with nonylphenol (15,150 and 1500 lg/kg body weight per day for 45 days) induced dose dependent increase in the level of H_2O_2 and decreased activities of antioxidant enzymes in the liver of rats (Jubendradass et al., 2012). Production of MDA is a significant sign of oxidative stress, which together with a reduction in anti-oxidative ability of the cell can destroy the membrane integrity. These are the recorded results produced from rats treated with different doses of nonylphenol (Abnosi, and Masoomi, 2019). Likewise in animal studies, a range of BPA doses from µg/kg/bw to mg/kg/bw per day were shown to significantly reduce the TAC of a number of tissues and organs, including liver, pancreas, and testes (Kalb et al., 2016; Moghaddam et al., 2015), and a decrease in the activities of SOD, CAT, and/or GPx were also reported in brain, epididymal sperm, liver, kidney, pancreas, testes, and germ cells. While it appears that the observed reduction in antioxidant activities correlates well with the induction of ROS by BPA over a variety of doses, as noted for ROS induction, alterations in the enzymatic and non-enzymatic antioxidant schemes appear to be highly cell, tissue, and organ specific (Gassman, 2017). The liver is the largest internal organ in the human body, and it is the main organ for the metabolism and detoxification of drugs and environmental chemicals (Klaassen, 2007). Measured serum liver biomarkers showed dose dependent response to the treatment with Bisphenol A and Nonylphenol as well as their mixtures. High doses groups induced significant elevation in each of ALT, AST, TP and albumin pronounced than low doses. These findings were partially included in a previous study by Helal et al. (2018) that recorded high significant increase of hepatic enzymes ALT, AST, and GGT when compared to the controls in rats treated with bisphenol A for 28 days. Gao et al. (2015) reported that Bisphenol A increased the hepatic oxidative stress and mitochondrial dysfunction. Elevated levels of serum enzymes ALT and AST as indicators of cellular leakage and loss of functional integrity of the cell membrane in the liver; their increased presence in serum may give information on organ dysfunction. Similarly, Ola-Davies et al. (2018) reported elevation in serum proteins and albumin in rats which can be based on amount of dosage as well as the period of exposure. This increase is an indicative of the accumulation of BPA metabolites with an impaired ability of the liver to excrete them. Meanwhile, Jubendradass et al. (2012) recorded that Levels of AST and ALT were increased in rats treated with 15,150, 1500 µg/kg nonyl phenol for 45 days. Yu et al., (2017) also reported that male rats exposed to NP for 3 months increased liver mass, increased adipose tissue mass, liver dysfunction and increase in the levels of ALT and AST in blood correspond with liver damage.

The above findings were confirmed by the histopathological changes, varying in severity and damage with increasing doses gavaged to rats, especially in high and mixture groups. The results of the present study were in accordance with those of Kamel et al. (2018), where the hepatic histopathological sections of the rats exposed to low dose BPA (20 mg/kg (bw)), and high dose BPA (100 mg/kg (bw) revealed vacuolar degeneration, necrosis, widening of blood sinusoids, vacuolization swelling in hepatocytes, and pyknosis in nuclei with increased number of Kupffer cells. These findings are in congruence with the results of previous studies (Hassan, Ismail, and Khudir 2013; Eid, Eissa, and EL-Ghor 2015; Poormoosavi et al., 2018). Verma and Sangai (2009) reported that BPA treatment has led to cell and membrane damage of human erythrocytes which was due to oxidative stress. Notably, the number of Kupffer cells and degree of cellular infiltration gradually increases with higher doses of BPA. Zimmermann and Tacke (2011) mentioned that hepatic macrophages and Kupffer cells are considered vital players in the propagation of acute liver injury. These cells demonstrated their essentiality in chronic liver inflammation due to their dual pro- and antifibrotic qualities. Li et al. (2012) revealed that cell death due to BPA has turned from apoptosis to necrosis. Thompson and Patel (2010) stated that chronic liver

injury is one of the major causes behind progressive liver fibrosis, leading to cirrhosis, liver failure and carcinoma.

Regarding the effects of High NP on the hepatic tissue of rats, where the histoarchitecture appeared abnormal with degenerated and necrotic hepatocytes, with lipid droplets of different sizes which matched with previous studies (Bin- Dohaish, 2012; Bernabò et al., 2014). A study has shown that fatty infiltration, vacuolar degeneration, acute inflammatory edema and activation of kuppfer cells occur in cases of poisoning due to different contaminants (Bin- Dohaish, 2012). Kourouma et al. (2015) stated that through hepatic histopathological examination micro-vesicular steatosis was observed in 4-NP-treated group. The hepatocytes ballooning is the most characteristic feature of steatosis-hepatitis. The associated effects and major hepatic damage (synergistic degeneration) was noticed in mixture treated groups bigger than in groups with individual effects of single matter.

CONCLUSION

Bisphenol A and nonylphenol exposure induced elevation in studied oxidative stress parameters MDA, PC and 8OHG, concomitant with reduction in antioxidant and defense system that has great hazards on biological systems, especially on liver function and liver histoarchitecture. It is clear that these components have damaging effects and more hazardous than expected in its consequences even at low concentrations and continuous exposure.

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