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Effects of Recovery Period and Tamoxifen on Bisphenol A Treated Female Albino Rats

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ABSTRACT

Background: Bisphenol-A (BPA) is an organic synthetic polycarbonate compound [$(CH_3)_2 C(C_6H_4OH)_2$] which is widely incorporated into many plastic industries worldwide. BPA is an endocrine disruptor that exhibits hormone-like properties which may promote adverse effects in humans, triggering estrogenic signals in target tissues, which raise concern about its suitability in some consumer products and food containers. Since 2008, several governments have investigated BPA safety, which prompted some retailers to withdraw polycarbonate products. A 2010 report from the United States (US) Food and Drug Administration (FDA) identified possible hazards of BPA to fetuses, infants, and young children. However, the FDA has ended its authorization of the use of BPA in baby bottles and infant formula packaging, based on market abandonment, not safety.

Aim of the work: This study aimed to investigate the antitoxic effects of the anti-estrogen drug Tamoxifen (Nolvadex) and the recovery period on the female albino rats which received BPA.

Materials and Methods: This study was performed on forty female albino rats with an average body weight of 140-160 grams. The animals were divided into four groups (10 rats per cage); **Group I** (Control untreated for 30 days), **Group II** (BPA treated for 15 days, then sacrificed), **Group III** (BPA treated first for 15 days, then left to a recovery period of another 15 days), and **Group IV** (BPA treated first for 15 days, then treated with the anti-estrogen drug Nolvadex for another 15 days). The following analyses were done to all groups; ALT (alanine amino-transferase), AST (aspartate amino-transferase), GGT (gamma glutamyl-transferase), total proteins, albumin, globulins, A/G ratio [i.e., liver function tests], creatinine, uric acid, A/C (albumin/creatinine) ratio [i.e., renal function tests], total lipids, total cholesterol, LDL-C (low density lipoprotein cholesterol), HDL-C (high density lipoprotein cholesterol), and triglycerides [i.e., lipids profile].

Results: In the BPA treated group II the biochemical results showed highly significant increase ($P<0.01$) in the enzymatic activities of ALT, AST, and GGT with concomitant increase in globulins ($P<0.05$), creatinine, uric acid, total lipids, total cholesterol, LDL-C, and triglycerides levels when compared to the control group. On the other hand, there was highly significant decrease ($P<0.01$) in total proteins, albumin, A/G (albumin/globulin) ratio, A/C (albumin/creatinine) ratio, and HDL-C levels when compared to the control group. These results turned back to normal control values after stopping the use of BPA alone (Group III) or stopping BPA and treatment with the anti-estrogen drug Nolvadex in the recovery period, except for ALT which was elevated ($P<0.05$) with Nolvadex (Group IV). **Conclusion:** It could be concluded that BPA has dangerous toxic effects on the liver and kidney functions as well as on the lipids profile. Moreover, the recovery period (i.e., 15 days without treatment) is better than the use of the anti-estrogens (as Tamoxifen) which have no antitoxic effects to BPA, but caused hepatic toxicity instead which is noted by an increase in ALT levels. So, we recommend minimizing utilization of this compound (BPA) to protect people from its hazardous effects.

Keywords: BPA: Bisphenol-A, anti-estrogen Tamoxifen (Nolvadex), recovery period.

INTRODUCTION:

The xeno-estrogen Bisphenol-A (BPA), a food contaminant with an endocrine disruptor activity, is the monomer widely used to manufacture polycarbonate plastics including baby bottles, infant feeding containers, or tableware (plates and mugs), and epoxy resin lining food and beverage cans.[1,2] There is a global concern for human health as BPA binds to estrogen receptors (ERs)[3], and can interfere with normal sex hormone balance. BPA is thought to wield its effects through endocrine disruption,

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epigenetic modification, cytokine release, and oxidative stress. When first discovered, BPA was investigated for its estrogenic properties, as it is thought to alter the synthesis of estradiol and testosterone and interfere with receptor binding.[4,5] Epigenetic effects of BPA have been associated with an increased risk of malignancies, particularly breast and prostate cancer.[6,7] There is a relationship between urine concentration of BPA and cardiovascular disorders, type 2 diabetes mellitus, and liver enzyme abnormalities in a representative sample of US population.[8] Moreover, some studies on laboratory animals have shown adverse effects of BPA on brain, reproductive system, metabolic processes, including alterations in insulin homeostasis and liver enzymes.[8,9] In addition, absorption of large amounts of BPA through the skin has been shown to cause extensive damage to the liver, kidneys, and other vital organs in human.[10] It was suggested that BPA caused tissue injury in the liver, kidneys, brain, and other organs by the formation of reactive oxygen species (ROS).[10,11]

So, this study was aimed to show the toxic effects of BPA and to examine the ameliorative effects of the recovery period or the usage of an anti-estrogen drug throughout the recovery period on the liver and kidney functions, and also on lipids profile of the female albino rats.

MATERIALS AND METHODS:

Experimental animals:

Forty female albino rats of Sprague dawley strain, weighing 140-160 grams, and aging 10-12 weeks were purchased from the Theodor Bilharz Research Institute, Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment for adaptation. The animals were fasted before sacrifices for about 12-16 hours.

Experimental design:

Experimental animals were divided into four groups (ten per each cage) as follows:

- **Group I (Control group):** Normal female rats left without any treatment for 30 days.
- **Group II (BPA treated group):** Rats were orally administered BPA 20 mg / kg b.wt / day for 15 days, and then sacrificed.
- **Group III (BPA treated + recovery period):** Rats were orally administered BPA 2 mg / 100 gram b.wt. / day for 15 days, and then stop it for another 15 days as a recovery period.
- **Group IV (BPA treated + Nolvadex):** Rats orally received BPA daily for 15 days as above, and then orally supplied with the anti-estrogen Nolvadex (0.1 mg / 100 gram b.wt. / day) for another 15 days.

Bisphenol A:

Bisphenol A (2, 2-Bis-(4-hydroxy phenyl propane) dissolved in sesame oil and orally administered to rats. The dose of BPA was calculated according to **Takahashi, and Oishi.**[12]

Nolvadex (Tamoxifen):

Tamoxifen is a non-steroidal-triphenylene-based drug that displays a complex spectrum of estrogen antagonism and estrogen-like pharmacological effects in different tissue.

Blood sample collection:

At the end of the experimental periods (30±2 days, while females in the diestrus phase) for groups I, III, and IV and 15 days only for group II, the overnight fasted animals (12-16 hours) were anesthetized under diethyl ether anesthesia. Blood samples were collected from retro-orbital veins in clean centrifuge tubes and left to incubate at 37 ^{o}C temperature for 20 minutes, then centrifuged at 3000 rpm for 10 minutes. The clear non-hemolyzed supernatant sera were quickly removed in eppendorf tubes and immediately stored at -20 °C till used for biochemical analysis for liver functions, kidneys functions, lipids, and proteins profiles.

Biochemical analysis:

Determination of serum lipids were done according to total lipids (TL)[13], triglycerides (TG)[14], total cholesterol (TC)[15], high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C).[16]

Assays of aspartate amino transferase (AST), alanine amino transferase (ALT),[17] and gamma glutamyl transferase (GGT)[18] were performed using kinetic methods. Determination of albumin, total proteins, creatinine,[19] and uric acid[20] (using the uricase-PAP enzymatic) were done by colorimetric methods. Globulins were calculated by subtraction of albumin from total proteins.

The results were expressed as Mean±SEM of the mean. The data were analyzed by one way analysis of variance (ANOVA) and were performed using the Statistical Package (SPSS) program, version 20. The Kolmogorov-Smirnov test (KS-test) was used to determine if two datasets differ significantly followed by Bonferroni test as multiple comparison method to compare significance between groups. Difference was considered significant when $P<0.05$.

RESULTS:

Liver functions: The data in ***table (1)***, showed that treatment with BPA induce highly significant increase ($P<0.01$) in ALT, AST, and GGT activities when compared to the control group. After the recovery period or Tamoxifen use no significant changes were recorded in these analyses, except for ALT activity which was ($P<0.05$) in Tamoxifen treated group, when compared to the control group.

Proteins profile: In ***table (1),*** also there were highly significant decreases in total proteins, albumin, and A/G ratio, with only a significant increase in globulins levels ($P<0.05$), when compared to the control group. In the recovery period or Tamoxifen groups no significant changes were recorded in these analyses when compared to the control group.

Kidney functions: The data in ***table (2)*** demonstrated that treatment with BPA showed highly significant increases ($P<0.01$) in serum uric acid and creatinine, with a highly significant decrease ($P<0.01$) in A/C ratio. However, there were no significant changes in the other groups as compared to the control rats. These parameters were turned back to the normal values in the other groups (recovery period and anti-estrogen treated rats).

Lipids profile: table (3) showed that the female rats treated with BPA exhibited a highly significant elevation ($P<0.01$) in total lipids, total cholesterol, LDL-C, and triglycerides compared to the control group. While, there was a highly significant decrease ($P<0.01$) in HDL-C in BPA treated group compared to the control. These parameters turned back to the normal values in the other groups (recovery period and anti-estrogen treated rats).

DISCUSSION

The widespread consumption of BPA-containing products has raised concerns among scientists and regulatory agencies that human exposure to BPA may have adverse toxic effects on different vital organs. In the present study, the recorded highly significant increase ($P<0.01$) in ALT, AST, and GGT activities reflects a state of oxidative stress on the liver cells. BPA is oxidized to a reactive metabolite 4, 5-bisphenol-O-quinone and major DNA increased in rat liver DNA at the presence of peroxidase activation. BPA has been shown to decompose into many kinds of metabolites, probably including BPA radical by a reaction of radical oxygen.[21]

Reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide has been generated in liver macrophages after exposure to hepato-toxicants.[22] Accordingly, it could be concluded that the oxidative stress induced by BPA in liver of rats may be due to the formation of ROS arising from reduced mitochondrial fractions and the formation of quinine radical, one of the BPA metabolites. BPA significantly decreased the activities of antioxidant enzymes and increased lipid peroxidation in the liver, thereby increasing oxidative stress.[11]

We found that BPA highly significantly increase ALT, AST, and GGT activities over control values. ALT, AST, and GGT are the most reliable markers of hepatocellular injury or necrosis. Their levels are elevated in a variety of hepatic disorders. ALT and GGT are thought to be the most specific tests for hepatic injury because ALT is present mainly in the cytosol[23] and GGT is present in the cell membranes of the liver with low concentrations elsewhere.[24] When the hepatocytes are damaged, these enzymes are released into the blood where highly significant increase in AST and ALT activities indicates damage to the cytosol and also to mitochondria.[25] Therefore, it could be suggested that the oxidative stress induced by the high dose of BPA (2 mg/100 gram/day for 15 days) may mediate the disturbance in hepatic function which is reflected by the present increase in hepatic enzymes. The absence of any effect on hepatic function after the recovery period may support this explanation. Rats treated with the anti-estrogen Nolvadex in the recovery period

showed a significant increase ($P<0.05$) in ALT activity only. Nolvadex is known to have varied biological effects ranging from complete estrogen antagonism to pure estrogen agonism depending upon its concentrations, sex of animals, and target organ.[26]

Treatment with BPA throughout the experiment recorded a highly significant reduction ($P<0.01$) in total proteins, albumin, and A/G ratio. This may be due to liver and kidney damage induced by oxidative stress due to BPA administration where liver is the main site of the conjugation and detoxification of drugs and other foreign substances.[27] The hypoalbuminemia observed in the present results revealed the hepatotoxic nature of BPA on liver cells. The synthesis of albumin is depressed in a variety of diseases, particularly those of the liver. BPA can cause toxicity and inflammation of liver which can induce significant decrease in protein profile.[28] BPA induces significant reduction of protein in rat liver microsomes.[27] There was increased level of globulins which may be due to liver affection that produce an increase in the gamma-globulin level.[27,28] However, the increase in globulins is less than the decrease in albumin leading to both hypoproteinemia and low A/G ratio.

Nolvadex is also a liver carcinogen in rats and has been associated with an increased risk of endometrial cancer in women.[29] **Smith *et al*.**[30] revealed tissue damage and carcinogenic change in rats by an oral route. Low doses of BPA may influence *de novo* fatty acid synthesis thereby contributing to hepatic steatosis.

Treatment of female rats with BPA for 15 days induced highly significant increase in serum uric acid, creatinine, and A/C ratio. This is because BPA induced oxidative stress on the kidney tissue of rats.[31] Furthermore, uric acid increase may be due to the effect of BPA on the heart as several studies showed an association between elevated uric acid levels and cardiovascular diseases.[32,33]

The results of this study revealed highly significant increases in serum total lipids, total cholesterol, triglycerides, and LDL-C with concomitant reduction of HDL-C in the female rat group treated with BPA. These can be induced by estrogenic activity where estrogens have a significant effect on serum lipids. The effect on cholesterol is probably due to the action of the hormone on the lipoproteins associated with cholesterol in the circulation. The higher estrogen like effect of BPA may be the reason for the incidence of myocardial infarction and other complications of arteriosclerotic vascular diseases.[34]

CONCLUSIONS AND RECOMMENDATIONS:

The use of BPA in different plasticizers and other industries should be limited and the erroneous handling of plastic containers should be avoided to reduce the health risk resulting from exposure to these endocrine disruptors including BPA. Also, not to use Tamoxifen in BPA toxicity where it is not safe to the liver. Alternatively, a recovery period, i.e., without treatment, can be more beneficial to patients exposed to BPA than the use of Nolvadex.

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Table (1): The effect of BPA, recovery period, and anti-estrogen on liver functions in female albino rats compared to the control group (M±SEM).

Groups	ALT (U/L)	AST (U/L)	GGT (U/L)	T. proteins (g/dL)	Albumin (g/dL)	Globulins (g/dL)	A/G ratio
Control	18.2±0.57	15.2±0.8	7.68±2.02	8.3±0.07	5.2±0.05	3.1±0.12	1.67±0.12
BPA	***41.9±0.601****	***33.76±0.9****	***13.39±0.5****	***7.5±0.17****	***4.0±0.07****	***3.50±0.12****	***1.14±0.1****
Recovery	19.8±0.87	15.8±0.67	8.43±2.8	8.2±.06	5.1±0.05	3.1±0.02	1.64±0.02
Nolvadex	***24.2±2.3****	16.3±1.9	9.16±2	8±0.09	5.5±0.03	2.5±.01	2.2±.03

Values were statistically significant at **P*<0.05, ***P*<0.01, *(N.S)* Non-Significant.

Table (2): The effect of BPA, recovery period, and anti-estrogen on kidney functions in female albino rats compared to the control group (M±SEM).

Groups	Uric acid (mg/dL)	Creatinine (mg/dL)	A/C ratio
Control	1.29±1.36	0.41±0.013	12.68±0.05
BPA	***2.17±1.6****	***0.57±0.18****	***7.01±0.06****
Recovery	1.73±0.98	0.39±0.011	13.08±0.04
Nolvadex	1.21±1.39	0.44±0.02	12.5±0.05

Values were statistically significant at **P*<0.05, ***P*<0.01, *(N.S)* Non-Significant.

Table (3): The effect of BPA, recovery period, and anti-estrogen on lipids profile in female albino rats compared to the control group (M±SEM).

Groups	T. lipids (mg/dL)	T. cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Control	640±5.1	190.5±1.1	168.8±0.9	101.7±2.1	55±1.4
BPA	***830±4.6****	***250.1±1.9****	***225.8±1.3****	***158.5±1.9****	***46.4±1.2****
Recovery	654±3.8	195.2±1.6	169.14±0.8	108.9±2.3	52.5±2.1
Nolvadex	692±3.2	198.2±1.3	176.7±0.10	112.6±1.5	50.3±1.02

Values were statistically significant at **P*<0.05, ***P*<0.01, *(N.S)* Non-Significant.