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Effects of Recovery Period and Stem Cell Enhancer on Bisphenol A Treated Female Albino Rats

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ABSTRACT:

Background: Bisphenol A (BPA) is a worldwide used endocrine disruptor that is incorporated in many plastic industries. Exposure of humans to such substance starts early during the fetal life, postnatal life, and extends throughout the life of the individual. Many agencies raised warnings against excessive use of such substance.

Aim of the work: This study aimed to investigate effects of the recovery period (RP) and stem cell enhancer (SCE) 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. Animals were divided into four groups (10 rats per cage); group I (control untreated for 30 days), group II (BPA treated for 15 days, and then sacrificed), group III (BPA treated first for 15 days, then left for another 15 days without any treatment "RP"), and group IV (BPA treated first for 15 days, then treated with SCE for another 15 days). The following biochemical 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, A/C (albumin/creatinine) ratio, uric acid [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 rats (group II) the biochemical results showed highly significant increases ($P<0.01$) in the enzymatic activities of ALT, AST, GGT, creatinine, uric acid, total lipids, total cholesterol, LDL-C, and triglycerides levels, with only a significant increase ($P<0.05$) in globulins levels when compared to the control group. On the other hand, there was highly significant decreases ($P<0.01$) in total proteins, albumin, A/G ratio, A/C ratio, and HDL-C levels when compared to the control group. These results turned back to about the normal control values after stopping the use of BPA and either taking a RP (group III) or receiving the SCE (group IV).

Conclusions and Recommendations: It could be concluded that BPA has dangerous toxic effects on the liver and kidney functions as well as on the lipids profile. So, we recommend minimizing utilization of this compound (BPA) as possible to protect people from these hazardous effects. Moreover, the RP (i.e., 15 days without treatment) is better than the use of SCE which has no more benefit against the antitoxic effects of BPA.

Keywords: BPA; bisphenol A, RP; recovery period, SCE; stem cell enhancer.

INTRODUCTION

Bisphenol A (BPA, 2,2-bis 4-hydroxy phenyl propane) is one of the environmental contaminants widely used in the manufacture of polycarbonate plastic (e.g., water and baby bottles), epoxy resin (e.g., inside coating in metallic food cans), and is a non-polymer additive to other plastics.[1, 2] Hence, it became an integrated part of the food chain.[3] There is a global concern for human health as BPA binds to estrogen receptors (ERs),[4] and can interfere with normal sex hormone balance. BPA is thought to wield its effects through endocrine disruption, 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.[5] There is a significant relationship between urinary concentration of BPA and cardiovascular disorders, type 2 diabetes, and liver enzyme abnormalities in a representative sample of US population.[6] Moreover, two studies on the laboratory animals have shown adverse effects of BPA on brain, reproductive system, and metabolic processes, including alterations in insulin homeostasis and liver enzymes.[7]

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In addition to that, absorption of large amounts of BPA through the skin has been shown to cause extensive damage to the liver, kidneys, and other vital organs inhuman.[8] It is highly conjugated in the liver to form bisphenol A glucuronide, a major metabolite, which is excreted in urine.[9] BPA has been shown to cause the formation of multinucleated giant cells in rat liver hepatocytes. It also causes degeneration of renal tubules in the kidney of rat and mice.[10] Laboratory studies suggest that even low levels of BPA increase oxidative stress and inflammation that promotes protein leakage into the urine, which is a biomarker for early renal impairment and future risk of developing coronary heart disease.[11] However, exogenous stem cell therapeutic strategies carry several potential risks that may limit their wider clinical application. For instance, stem cell therapy is an invasive technique that requires repeated injections often in the portal vein or hepatic artery.[12] Another limitation is that stem cells are exposed to several manipulations during their expansion in vitro before being transplanted, these manipulations lead to their contamination and/or cause deleterious changes in their intrinsic characteristics due to several intracellular and extracellular influences, with additional burden on the diseased liver.[13] Based on the fact that bone marrow-derived stem cells have the ability to migrate to sites of tissue damage and participate in tissue regeneration, stem cell enhancer may provide a promising non-invasive alternative to exogenous stem cell transplantation. Many different soluble factors have the ability to mobilize bone marrow-derived hematopoietic stem cells (BM-HSCs) from the bone marrow to the peripheral circulation and hence increase their total number.[14]

In the case of significant injury or degeneration, the numbers of new tissue cells found in healing tissue far exceed the capacity of local stem cells to duplicate and differentiate, suggesting that stem cells coming from other sites must be involved in the process of repair. Tissue stem cells are traditionally believed to be limited in their ability and differentiate into other tissue; however bone marrow stem cells were recently shown to have significant capacity to become cells of other tissue. Bone marrow stem cells, including marrow stromal cells (MSCs) are released from tissue of origin, and circulate in a subject's circulatory or immune system to migrate into various organs and tissue to become mature, terminally differentiated cells. Therefore, enhancement of stem cell trafficking (i.e., release, circulation, homing, and/or migration) can amplify these physiological processes and provide potential therapies for various pathologies.[15]

Stem cell enhancer is a natural stem cell mobilizer that can trigger the release of millions of adult stem cells from bone marrow into the circulation, and its considerable safety allows for foe a sustained oral daily intake over long periods of time. The product we carry is a blend of many compounds extracted from the cyanophyta Aphanizomenon flos-aquae (AFA), Undaria pinnatifida, Polygonum multiflorum, and Cordyceps sinensis to support the release of additional stem cells. They may have individual physiological effect or synergistic effects with one another, such as serving as both a releasing agent and migration agent.[16]

Stem cell therapeutic strategies are being evaluated as an attractive promising approach for liver repair. Several studies have reported the ability of various types of stem cells to improve the pathological outcome of liver cirrhosis and to attenuate the clinical symptom of the disease.[17]

Aim of the work:

This work was aimed to investigate effects of bisphenol A on the female albino rats, the recovery period (RP) and stem cell enhancer (SCE) on the female albino rats, which received BPA.

MATERIALS AND METHODS:

Experimental animals:

Forty female albino rats of Sprague dawley strain, weighing 140-160 grams, at the age of 10-12 weeks were included in the study. They 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/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 20 mg BPA/kg b.wt./day for 15 days, then sacrificed.
- **Group III (BPA treated + recovery period):** Rats were orally administered 20 mg BPA/kg b.wt./day for 15 days, then stopped it for another 15 days as a recovery period.
- **Group IV (BPA treated + stem cell enhancer):** Rats orally received BPA daily for 15 days as above, then orally supplied with the stem cell enhancer (0.1 mg/100 g/day) only 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.**[18]

Stem Cell Enhancer:

Is a blend which is formed of;

- Cyanophyta Aphanizomenon flos-aquae concentrate.
- Cordyceps sinensis.
- Undaria pinnatifida extract.
- Polygounum multiflorum extract.

Blood sample collection:

At the end of the experimental periods (30±2 days, while females are in the diestrus phase) for groups I, III, and VI) and 15 days only for group II, the overnight fasted animals (12-16 hours) were anesthetized with 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 analyses for liver functions, kidneys functions, and lipids profile.

Biochemical analysis:

Determination of serum lipids were done according to; total lipids (TL),[19] triglycerides (TG),[20] total cholesterol,[21] high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C).[22]

Assays of aspartate amino transferase (AST), alanine amino transferase (ALT),[23] and gamma glutamyl-transferase (GGT)[24] were performed.

Determination of albumin, total proteins, creatinine,[25, 26] and uric acid[27] (using the uricase-PAP enzymatic) was done by colorimetric methods. Globulins were calculated by subtraction of albumin from total proteins.

Statistical analysis:

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 data sets differ significantly, followed by Bonferroni test as a multiple comparison method to compare significance between groups. Difference was considered significant when $p<0.05$ and highly significant when $P<0.01$.

RESULTS

Liver functions: The data in **table (1)** showed that treatment with BPA induced highly significant increases ($P<0.01$) in ALT, AST, and GGT activities, with only a significant increase in globulins levels ($P<0.05$) accompanied with a highly significant decreases in total proteins, albumin, and A/G ratio when compared to the control group. After both the recovery period and stem cell enhancer treatment all these parameters improved (no significant changes) 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. These parameters also turned back to normal values in the other two groups (recovery period and stem cell enhancer treated rats) when compared to the controls.

Lipids profile: table (3) showed that female rats treated with BPA exhibited highly significant elevations ($P<0.01$) in total lipids, total cholesterol, LDL-C, and triglycerides and 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 stem cell enhancer treated rats) when compared to the control group.

DISCUSSION

The impact of endocrine disrupting chemical exposure on human health is receiving increasingly focused attention especially bisphenol A (BPA). In the last decade, several studies demonstrated the toxicity of BPA even at low doses. Studies have shown that BPA can cause injury in the liver, kidney, brain, epididymal sperm in rodents, and other organs by forming reactive oxygen species (ROS).[28] The adverse effects of BPA are largely related to its estrogenic activity,[29] and result in disturbances to the reproductive function,[30] steroidogenesis,[31] and adipogenesis.[32] However, BPA has other effects such as inflammatory cytokine[33] and an increase of oxidative stress,[34] which is independent of estrogenic activity.

The liver is the major organ for the metabolism and detoxification of xenobiotics, including BPA.[35] Therefore, the liver could be largely exposed to BPA and could be susceptible to lower doses than other organs. In humans, the urinary concentration of BPA was associated with abnormal liver function.[36] There are some reports that high doses of BPA cause altered liver weights in mice or rats[37] and decreased the viability of rat hepatocytes.[38] Recently, the oxidative stress was proposed as another adverse cellular effect of BPA in the liver.[39] BPA increased the generation of ROS and induced cellular apoptosis in hepatocytes.[40] Oxidative stress can induce mitochondrial damage, and damaged mitochondria can generate more ROS. Mitochondria are vulnerable to ROS due to impairment of the antioxidant and DNA repair enzyme systems.[36] Accumulation of oxidative damage in the mitochondria induces mitochondrial dysfunction, mitochondrial DNA depletion, and cell apoptosis.[39] Previous studies reported that increase in the levels of IL-6 and TNF-α was observed following BPA treatment, also BPA reduced catalase and glutathione peroxidase 3 (GPX3) expression in the liver and kidney of male ICR mice.[41, 42] Although ROS can increase proinflammatory cytokines,[43] proinflammatory cytokines themselves can induce oxidative stress.[44]

In our study, there were adverse effects on the liver of rats i.e., hepatotoxicity due to BPA ingestion in large doses that manifests itself in the form of a highly significant increase of hepatic enzymes ALT, AST, and GGT when compared to the controls. Globulins were also significantly increased compared to the first group. On the contrary, liver affection was manifested by a highly significant decrease in the production of albumin and consequently of total proteins compared to the controls. A/G ratio was also highly significantly lowered due to both highly significant decrease in albumin and significant increase in globulins.

The liver is a target tissue for endocrine-disrupting chemicals. Specific estrogen receptors exist in the liver and cellular responses involving estrogen interactions have been identified.[45] The current study demonstrates that BPA has adverse effects on the liver, as indicated by increased activities of ALT, AST and GGT enzymes. They are released in the blood stream when the liver is damaged.[46] Bisphenol A is reported to increase the hepatic oxidative stress and mitochondrial dysfunction.[47] Elevated levels of serum enzymes ALT and AST are indicators of cellular leakage and loss of functional integrity of the cell membrane in the liver.[48] These are of major importance in assessing and monitoring functional status of liver. Thus, their increased presence in serum may give information on organ dysfunction.[49] It has been reported by **Gao et al.**[50] that ALT activity is an important index to measure the degree of cell membrane damage, while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme.

Decreased serum protein biosynthesis in the liver of rats treated with BPA may be attributed to the formation of BPA adducts. **De Flora et al.**[51] had reported the ability of BPA to form DNA adducts in *vitro* in an acellular system and in *vivo* in rodent liver. **Atkinson and Roy**[52] had found that BPA is converted to bisphenol o-quinone, which might bind to DNA. When this occur the transcription of DNA to

mRNA will be impaired resulting ultimately in the inhibition of protein synthesis.

In this study the kidneys of rats were also intoxicated by BPA which resulted in a highly significant increase in creatinine and uric acid concentrations compared to the controls. Decreased albumin/creatinine ratio was due to both decreased albumin synthesis in the liver with decreased creatinine excretion from the kidneys. BPA is an estrogenic endocrine disruptor molecule of phenolic structure, which has renal elimination, and builds up when the glomerular filtration rate decreases.[53]

In the present study there were highly significant increases in total lipids, total cholesterol, LDL-cholesterol, and triglycerides in BPA treated group when compared to the controls. On the other hand, there was a highly significant decrease in HDL-cholesterol in BPA group when compared to the control group. Exposure to low doses of BPA increases the insulin expression and production by the pancreas.[54, 55] Insulin is known to increase genesis by both post-translational protein modifications and transcriptional mechanisms.[56] **Xie et al.**[57] found that activity of sterol regulatory element binding protein 1c (SREBP-1c), which regulates cholesterol metabolism, were activated by increased insulin level. So, insulin is likely to contribute to hypercholesterolemia following BPA treatment. However, **Marmugi et al.**[55] did not rule out the contribution of other mechanisms, independent of insulin and possibly involving direct effects of BPA on the liver, to the hepatic transcriptional impacts detected in BPA-treated mice.

In our study, SCE administration improved liver function with no significance when compared to the controls. Stem cell therapeutic strategies are being evaluated as an attractive promising approach for liver repair. The ability of SCE aphanizomenon flos-aquae (AFA) to mobilize BMSCs has been reported to help reverse hair color and support overall healing and regeneration.[15] Polygonum multiflorum possess important properties for tapping into regenerative and restorative potential of the body. Many scientific studies have confirmed that extracts of Polygonum multiflorum are indeed of promoting hair follicle growth, through increased expression of sonic hedgehog (Shh) and I-catenin expression, two important pathways involved in both early embryogenesis and maintaining stem cell identity.[58] Another study found that polygonum multiflorum extracts promotes proliferation of stem cells and progenitors, as shown by increase in the number of bone marrow stem cells and lymphoid progenitors following administration of Polygonum multiflorum extract in mice.[59] A previous study reported that Undaria pinnatifida has a significant elevation in the number of circulating CD34+ HSCs (HSC express a surface antigen known as stem cell antigen or $CD34^{+}$).[60]

Cordyceps sinensis protect both hematopoietic progenitor cells directly and the bone marrow stem cell through its effects on osteoblast differentiation. Cordyceps sinensis enhanced the colony forming ability of both granulocyte macrophage colony forming unit (GM-CFU) and osteogenic cells from bone marrow preparations and promoted the differentiation of bone marrow mesenchymal stromal cells into adipocytes, alkaline phosphatase-positive osteoblasts, and bone tissue, this action attributed to enhanced expression of CBFa1 (core binding factor alpha 1) and BMP-2 (bone morphogenetic protein-2) with concurrent suppression of ODF (osteoclast differentiation factor/RANK [receptor activator of nuclear factor-kB]) ligand.[61] Furthermore, SCE- mobilized BM-HSCs increased the number of insulin- producing cells in islets of Langerhans and reduced blood glucose levels in diabetic rats.[62]

Combination of Cyanophyta Aphanizomenon flos-aqua (AFA), Undaria pinnatifida, Polygonum multiflorum, and Cordyceps sinensis may have individual physiological effects, additive effects, and/or synergistic effects with one another, such as serving as both a releasing agent and migration agent.[15]

Stem cells circulating in the peripheral blood stream are recruited to sites of tissue in need of repair and regeneration through homing and extravasation. This mobilization of stem cells into the bloodstream and subsequent migration to the site of tissue injury result from a

combination of mechanical and chemoattractant signals.[63] Mechanical force or other factors may activate L-selectin on the surface of stem cells. Activation of L-selectin, in turn, may promote elevated expression of the receptor, CXCR4 (an alpha-chemokine receptor specific for stromal-derived-factor-1 "SDF-1 also called CXCL12"). Cells at the site of tissue injury may also secrete SDF-1 ligand, thereby attracting stem cells expressing receptor CXCR4 to the injury site. The interaction of SDF-1 and CXCR4 promote sufficient adhesion to halt circulation of a stem cell in the peripheral blood stream.[15] SCE treatment not only mobilized HSCs but also other types of bone marrow stem cells, such as mesenchymal stem cells, were mobilized and homed to the injured liver.[64]

Amelioration of hepatic inflammatory and fibrotic injury via bone marrow stem cell therapy can promote the proliferation of residual hepatocytes. Down-regulation of pro-inflammatory cytokines, such as TNF-α, has been described in kidney, lung injury, and fulminant hepatic failure models after bone marrow stem cells transplantation.[65] Moreover, controlling the production of cytokines, such as TGF-β and TNF-α via mesenchymal stem cell infusion improved liver fibrosis.[66]

Bone marrow stem cell mobilization may enhance the intrinsic capability of hepatocytes to proliferate by releasing proliferative cytokines and/or reducing fibrosis, thereby removing the block in the way to hepatocyte proliferation.[67] BMSCs and HSCs were shown to have the ability to become muscle cells,[68] heart cells,[62] endothelium capillary cells,[69] liver cells,[70] lung cells, gut cells,[71] skin cells,[72] and brain cells.[73] As a further illustration, a previous study performed an experiment which not only demonstrated the ability of HSCs to become liver cells upon contact with specific liver derived molecules, but also shows that this process took place within hours in injured liver cells, but the conversion was minimal and delayed when HSCs were exposed to undamaged liver.[70]

The results in our study showed that there areinsignificant differences between the two groups (i.e., III"BPA treated first for 15 days, then left another 15 days for without any treatment" and IV"BPA treated first for 15 days, then treated with SCE for another 15 days") when compared to the control group I. So, there is no advantage or need to take SCE in such situations (i.e., hepatotoxicity, nephrotoxicity, or dyslipidemia due to BPA toxic exposure). Up till now, we found no other researches done in this area to compare with.

CONCLUSIONS AND RECOMMEND-ATIONS

In this study bisphenol A (BPA) administration actually disturbed liver and renal biochemical parameters in addition to deterioration of the lipids profile, these effects refer to as the bad effects of BPA on the rats. On the reverse, RP or intake of the SCE improved these effects by returning analyses to around the normal control values. So, we recommended stopping usage of BPA and replacing it with other safe materials as the use of BPA-free materials. Also, not to use SCE in BPA toxicity because it had no more advantage over RP in this situation.

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Table (1): The effect of BPA, recovery period, and stem cell enhancer 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 Period:	19.8±0.87 (N.S.)	15.8±0.67 (N.S.)	8.43±2.8 (N.S.)	8.2±.06 (N.S.)	5.1±0.05 (N.S.)	3.1±0.02 (N.S.)	1.64±0.02 (N.S.)
Stem Cell Enhancer:	21.5±1.3 (N.S.)	14.5±1.02 (N.S.)	7.46±1.5 (N.S.)	8.4±0.05 (N.S.)	5.5±0.06 (N.S.)	2.9±.03 (N.S.)	1.8±0.05 (N.S.)

Values were either; statistically highly significant (**P<0.01), significant (*P<0.05), or non-significant (N.S.) compared to the control.

Table (2): The effect of BPA, recovery period, and stem cell enhancer 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 Period:	1.73±0.98 (N.S.)	0.39±0.011 (N.S.)	13.08±0.04 (N.S.)
Stem Cell Enhancer:	1.64±1.9 (N.S.)	0.42±0.05 (N.S.)	13.1±0.06 (N.S.)

Values were either; statistically highly significant (**P<0.01), significant (*P<0.05), or non-significant (N.S.) compared to the control.

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

Groups	T. lipids (mg/dL)	T. cholesterol (mg/dL)	LDL-C (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)
Control:	640±5.1	190.5±1.1	101.7±2.1	168.8±0.9	55±1.4
BPA:	830±4.6**	250.1±1.9**	158.5±1.9**	225.8±1.3**	46.4±1.2**
Recovery Period:	654±3.8 (N.S.)	195.2±1.6 (N.S.)	108.9±2.3 (N.S.)	169.14±0.8 (N.S.)	52.5±2.1 (N.S.)
Stem Cell Enhancer:	632±3.6 (N.S.)	185.2±1.7 (N.S.)	97.06±2.6 (N.S.)	156.7±0.09 (N.S.)	56.8±1.7 (N.S.)

Values were either; statistically highly significant (**P<0.01), significant (*P<0.05), or non-significant (N.S.) compared to the control.