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The Potential Protective Effect of Stem Cell Enhancer on Chronic Bisphenol-A Treated Female Albino Rats

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ABSTRACT

Background: The xenoestrogen, bisphenol-A (BPA), is a worldwide food contaminant with endocrine disruptor activity that is incorporated in many plastic industries. The exposure of humans to such substances starts early during the fetal life, postnatal life and extends throughout the life of the individual. Many agencies raised warnings against the excessive use of these substances. **Aim of the work:** The present study was designed to determine if the usage of stem cell enhancer (SE) moderates the physiological changes occasioned by exposure to BPA in the female albino rats. **Materials and Methods:** This study was performed on thirty female albino rats with an average 100-120 g body weight. The animals were divided into three groups; **Group I** (Control untreated-group), **Group II** (bisphenol–A treated group) and **Group III** (treated group orally supplied with bisphenol –A then treated with stem cell Enhancer (SE)). Serum was separated and used for estimation of hormonal levels [estradiol, progesterone, prolactin (PRL), follicle-stimulating hormone (FSH) and luteinizing hormone (LH)], some biochemical parameters (liver enzymes, kidney function, glucose and lipid profile) and CA-125 and CA 15-3 tumor markers. **Results**: The biochemical results showed marked significant increase ($P<0.01$) in the enzyme activities [aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase (ALP)],urea and creatinine in bisphenol–A treated group when compared to the control group. These parameters were significantly reduced in the group treated with stem cell enhancer as compared to bisphenol –A treated group . Decline in the concentration of lipid profile with increase in high density lipoprotein cholesterol (HDL- C) levels in the stem cell treated group as compared to bisphenol-A group were observed. There was a significant elevation in prolactin, FSH and tumor marker levels concomitant with a significant reduction in LH levels in bisphenol-A treated group compared to the control group. These results were changed to values very close to control by using the stem cell enhancer. **Conclusion:** It could be concluded that bisphenol-A has dangerous effects on liver and kidney functions as well as on lipid profile, female hormones and tumor markers level. So, we recommended minimizing the utililizing of this compound and the use of some protective materials as stem cell enhancer to protect people from its hazardous effects.

Keywords: BPA (bisphenol- A), stem cell enhancer, tumor markers, kidney and liver function tests.

INTRODUCTION

Xenoestrogens are nonsteroidal, man-made chemicals that can enter the body by ingestion or adsorption.[(1)] Health concerns regarding exposure to xenoestrogens stem from their potential actions as endocrine disruptors. These chemicals share no structural homology with estradiol and include substances such as pesticides and industrial by-products. Phenols are one of the most common classes of xenoestrogens found in foods and consumer products that made of polycarbonate plastics.[(2)]

Bisphenol-A (BPA), a food contaminant with endocrine disruptor activity, is the widely monomer used to manufacture polycarbonate plastics including baby bottles, infant feeding containers or tableware, and a constituent of epoxy and polystyrene resin that are extensively used in the food-packaging industry and in dentistry, lining food and beverage cans.[(3)] Bisphenol–A has adverse effects on humans because of its ubiquitous presence in the environment, resistance to degradation, and potential for accumulation in fat tissues.[(4)]

BPA binds to estrogen receptors (ERs), and can interfere with normal sex hormone balance. Bisphenol-A is thought to wield its effects

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through endocrine disruption, epigenetic modification, cytokine release and oxidative stress.[5] 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.[6] Additionally, a recent study reported that urinary BPA levels were associated with upregulated estrogen receptor and estrogen-related receptor expression among adult men.[7] Epigenetic effects of BPA have been associated with an increased risk of cancer, particularly breast and prostate malignancies. There is a relationship between urine concentration of BPA and cardiovascular disorders and type 2 diabetes.[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. In addition, absorption of large amounts of BPA through skin has been shown to cause extensive damage to liver, kidney and other vital organs in human.[9]

A stem cell is a cell that has the ability to duplicate itself endlessly and to become cells of virtually any organ or tissue in the body. Stem cells are found in human embryos, and are also found abundantly as adult stem cells in the tissues of the body that circulate and function to repair and renew aging or degenerating tissues. Stem cells are more easily released into the bloodstream in children, whereas this process slows down in adult. Stem cell enhancer (StemEnhance®) is a novel mobilizer of bone marrow adult stem cells that was shown to increase the number of circulating stem cells. One gram of StemEnhance has shown to support an increased release in the number of circulating stem cells in the body by 25% to 30% that greatly increased the potential of the body's active repair and renewal system.[10] StemEnhance® is a blend of 4 compounds: *Aphanizomenon flos-aquae* (AFA) that extracted from blue green algae, *Undaria pinnatifida, Polygonum multiflorum and Cordyceps sinensis*.

Blue green algae (BGA) is one of the most nutrient dense foods which is rich in substances that have useful effects on human health. This substance has a high concentration of vitamins, minerals and enzymes with a complete spectrum of essential and non-essential amino acids that are all easily absorbed by the body. Due to these properties, a large number of researchers were interested in employment of blue green algae as food supplementation. Several blue green algae, including *Aphanizomenon flos-aquae* (AFA) showed antibacterial and antioxidant properties, glucose and cholesterol-regulatory effects, enhance the phagocyte activity in macrophages as well as host immune system modulation. It has been shown that blue-green algae increases the stem cells trafficking or homing in animals through induction of a transient boosting in the population of stem cells in animal's circulatory system.[11]

Many in vitro and in vivo studies have demonstrated the anti-carcinogenic activities of *Undaria pinnatifida* seaweeds, especially against breast cancer. *Undaria* consumption was found to cause significant changes in the regulation of the menstrual cycle by increasing the length of the cycle, stimulating ovulation, and lowering the estrogen/progesterone ratio in pre-menopausal women. Such changes may be beneficial particularly with regard to women at high risk of estrogen-dependent diseases or who are experiencing fertility problems. [12]

Polygonum multiflorum (PM) was found to possess highly beneficial activities such as anti-inflammatory, anti-diabetic, anti-atherosclerotic and hypolipidemic activity with a recently documented ability to support stem cells release from the bone marrow. *Polygonum* was found to play a central role in the cell protection by enhancing of cellular superoxide dismutase (SOD), nitric oxide (NO) and reduced glutathione (GSH) activities and a decrease in malondialdehyde (MDA) production in a concentration- dependent manner.[13]

Cordyceps sinensis has been reported to have acute anti-tumor, anti-metastasis, immunomodulatory, antioxidant, and anti-inflammatory effects. Also, *Cordyceps* is clearly indicated as a therapeutic agent in treating hypolibidenism and other sexual malfunction in both sexes. [14]

There are increasing scientific and clinical evidences for StemEnhance role in controlling many chronic diseases such as arthritis and cancer.[15] However, there is not any authentic report about stem cell enhancer and BPA. Given to the dangerous of xenoestrogens effects on

health, the purpose of this study is to evaluate the safety and efficacy of StemtechTM (StemEnhance) in treatment of bisphenol-A exposed female albino rats.

MATERIALS AND METHODS

Experimental animals

Thirty female albino rats of Sprague Dawely strain, weighing around 100-120g, at the age of 6-8 weeks were purchased from Theodore Bilharz Research Institute, Giza, Egypt. Animals were kept under observation at control conditions (12 hour light/dark cycle, the temperature was 23±3 °C, and compressed food and water was available *ad-libitum*) for about 15 days before the onset of the experiment for adaptation.

Drug and dose:

(a) Bisphenol-A

Bisphenol-A (2,2-Bis-(4-hydroxy phenyl propane) dissolved in sesame oil and orally administered. BPA was purchased from Sigma Chemical Co. (St Louis, MO, USA). The dose of BPA was calculated according to **Takahashi and Oishi.** [16]

(b) Stem cell Enhancer

StemEnhance (500 mg *Aphanizomenon flos-aquae* extract per capsule) (STEM Tech Health Sciences, San Clemente, CA, USA) (StemtechTM).

Experimental design:

Experimental animals were divided into three groups (10/each)as follows:

Group I (Control group): Normal young female albino rats (without any treatment) for 105 ± 2 days. **Group II (Bisphenol-A group)**: Young female albino rats were orally administered with 20mg BPA /kg. b.wt/day for 105 ± 2 days. **Group III (Stem cell Enhancer treated group**): Young female albino rats were administered with 20mg BPA /kg. b. wt/day for 105 days with concomitant orally administered by (0.1mg /100g b. wt /day) StemEnhance at the last 15 ± 2 days.

Blood sample collection: At the end of the experimental periods (105 ±2 days, those rats that had reached the stage of diestrus),the overnight fasted animals (18 h) were scarified. Blood samples were collected from retro-orbital vein in a clean centrifuge tubes and left to incubate at 37^{o}c temperature and centrifuged at 3000 rpm for 15 min. The clear non-haemolysed supernatant sera were quickly removed and immediately stored at -20°C till been used for further biochemical analysis.

Biochemical analysis:

Serum total lipids (TL), triglycerides (TG), total cholesterol and high density lipoprotein cholesterol (HDL-C) content were measured using enzymatic colorimetric kits (Biodiagnostic, Egypt) according to Tietz.[17] Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein (VLDL) were calculated using the **Friedwald's** formula.[18]

Friedewald's equation: **LDL (mg/dl) = TC- {HDL + [TG/5]}.**

VLDL = TG/5

Ratios of LDL/HDL (risk factors) and TC/HDL were also calculated. Glucose level was estimated according to Trinder.[19] Also, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities were assayed according to **Reitman and Frankel** [20], however serum alkaline phosphatase (ALP), urea and creatinine concentrations were measured according to Tietz.[17] In particular, serum BUN was determined by a modification of the method described by Sampson [21]. Serum estradiol, progestrone, gonadotrophic (FSH-LH) and prolactin were measured by Enzyme-linked Immune Sorbent Assay (ELISA) according to **Tsang *et al*.[22], Elder *et al*.[23], Urban *et al*.[24], Leviene *et al*.[25] and Liu and Zhous[26]** respectively**.** CA-125 and CA 15-3 levels were measured using ELISA kits (BioVision, USA). Collected serums were stored at -20C° and were analyzed simultaneously.

Statistical analysis

The results were expressed as Mean ± SE 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. Difference was considered significant at $p < 0.05$.

RESULTS

Rats treated with bisphenol-A exhibited a significant elevation ($P<0.05$) in total lipid, total cholesterol, triglycerides and LDL cholesterol as compared to those of the control

group in the experiment (Fig.1). However, HDL cholesterol was significantly reduced (P<0.05) in BPA treated group. On the other hand, the stem cell enhancer treated group (group 3) showed a statistically significant reduction in the levels of total lipid, total cholesterol, triglycerides and LDL cholesterol as compared to bisphenol A group. VLDL, LDL/HDL and TC/ HDL ratios in the stem cell enhancer treated group were approximately the same as that of the control group.

Rats received BPA also exhibited a significant elevation (P<0.05) in the glucose levels throughout the experiment compared to the normal control group. However, serum glucose of rats treated with BPA significantly reduced when treated with stem cell enhancer (group 3) (Fig.1).

Moreover, BPA treated group exhibited a significant increase (P<0.05) in the enzyme activities (ALAT, ASAT and ALP) as compared to the control group (Fig. 2). However, the stem cell enhancer treated group showed a significant decrease in the activities of these enzymes as compared to the bisphenol-A group but still higher than the values of the control group (Fig. 2).

The recorded nephrotic alterations in Fig. (3) revealed that oral administration of BPA (group 2) induced a significant elevation ($p < 0.05$) in serum urea, creatinine and BUN as compared to the control group. However, the data demonstrated a significant decrease in urea and BUN levels in the BPA treated with StemEnhance group (group 3) as compared with the values of BPA treated group. On the other hand, the stem cell enhancer treatment showed a significant decrease in the creatinine level compared to BPA group and kept its concentration with normal range compared to control group (Fig.3).

Also, a significant increase in the estrogen (Fig.4) and a significant decrease in the progesterone (Fig. 5) concentrations were observed in the bisphenol-A group as compared to the control group (P<0.05). Furthermore, the bisphenol-A treated with stem cell enhancer group (group 3) showed a statistically significant increase in the estrogen compared to the control but lesser than the values of the bisphenol-A group. However, its administration showed a significant increase in the progesterone level that was approximately equal to that of the control group.

The data illustrated in fig. (6) showed the effect of bisphenol-A on serum PRL, FSH, and LH levels of female albino rats in all groups. BPA in dose (20 mg/kg/day) caused a significant elevation (P<0.05) in PRL and FSH levels concomitant with a significant reduction(P<0.05) in the LH levels after bisphenol administration as compared to the control group. However, the bisphenol-A treated with StemEnhance group showed insignificant increase in the levels of these hormones in female albino rats sera than the control group.

Moreover, bisphenol-A group showed a significant increase in serum concentration of CA15-3 and CA-125 (Fig.7). However, the bisphenol-A treated with stem cell enhancer (group 3) showed a significant reduction (P<0.01)in the values of these tumor markers as compared to BPA group and were kept within the normal range compared to the control (group 1).

DISCUSSION

In recent years, much attention has been paid to whether exposure to environmental BPA affects human health, development and reproduction. It has been reported that BPA increases the health concern partly because of its effect on the endocrine system and its weak estrogen receptor-binding capacity.[27]

Differences in sensitivity of animal strains to BPA and other agents that may act to disrupt estrogen signaling pathways have been much debated.[28] The animal model used in the current study, the Sprague Dawley rats, have been used previously and shown to be sensitive to estrogenic compounds. In addition, a comprehensive evaluation of the pharmacokinetics of BPA across life stages has been reported in this strain.[29]

Effects of BPA on fat deposition have been investigated in multiple experimental models with mixed results including increases, decreases, or no effect.[30] The results of this study revealed a significant increase in serum total lipid, total cholesterol, triglyceride and LDL cholesterol concomitant with reduction of

HDL cholesterol in female rats treated with BPA. Bisphenol-A can induce estrogenic activity where estrogens have a significant effect on serum cholesterol. The effect on cholesterol is probably due to an action of the hormone on the lipoproteins associated with cholesterol in the circulation as reported by Ganong[31]. This is in agreement with **Paul *et al.*[32]** who reported that the treatment of rabbits with bisphenol-A, increased serum total lipid, triglycerides and total cholesterol where the increase in cholesterol was apparent for the lipoproteins, VLDL and LDL but not for HDL.

Total lipids, triglyceride, total and LDL cholesterol in the BPA treated with stem cell enhancer group were significantly decreased with concomitant increase in HDL cholesterol levels from that of BPA treated group. Similar data were obtained by **Sanaei *et al.*[11]** who stated that blue green algae inhibits intestinal cholesterol absorption, decreases the hepatic lipids and leads to attenuation of plasma total cholesterol and triglyceride concentrations. Additionally, *Polygonum multiflorum* (PM) was found to possess an anti-atherosclerotic and hypolipidemic activity**.**[13]

The significant reduction in the TC/ HDL cholesterol level in the stem cell treated group from the BPA group, indicating that the protective effects of stem cell enhancer treatment were more pronounced on the atherogenic LDL cholesterol levels than on the HDL cholesterol levels in the treated rats. This protective effect may be related to cellular protection effect of *Polygonum multiflorum* (constituent in StemEnhance) which attenuate lipid peroxidation by up-regulating of cellular antioxidants and decrease MDA concentration**.**[13]

BPA administration induced a highly significant hyperglycemia while the treatment with StemEnhance greatly reduced the glucose concentration. This could possibly be due to the high fiber content of blue-green algae that interferes with the glucose absorption or probable action of producing polypeptides after digestion of blue green algae [33]. The results of this study are in agreement with the result of **Sanaei *et al.*[11]** and **Anwer *et al.*[34]** who recommend the use of StemEnhance as a functional food in management of diabetes. They attributed the antihyperglycemic effects of StemEnhance to increase the insulin secretion from β-cells of the pancreatic islet or due to enhancement of transport of blood glucose to the peripheral tissue.

ASAT and ALAT are two enzymes of the most reliable markers of hepatocellular injury or necrosis. Their levels are elevated in a variety of hepatic disorders. Our data revealed an increase in ALAT, ASAT and ALP activities in female rats after exposure to bisphenol-A. This may be due to accumulation of toxic substances generated from BPA in liver that can induce liver toxicity. The present results are in agreement with those of **Korkmaz *et al.*[35]** who reported a significant increase in ALAT and ASAT activities in rats treated with 25 mg/kg BPA for 50 days. Meanwhile, stem cell enhancer treatment recorded a significant decrease in the activities of these enzymes than that of the bisphenol-A group. This action may be due to the antioxidant activity of some StemEnhance constituents that exhibited a hepatoprotective effect against BPA liver damage.[36]

This study also demonstrated that treatment of female rats with BPA induced a highly significant increase in serum urea, BUN and creatinine that may be due to the oxidative stress induced by BPA on the rats kidney tissues as investigated by **Korkmaz *et al.*[35]**. However, the treatment with StemEnhance was significantly improved urea, creatinine and BUN levels. This may be due to its antioxidant properties that improved renal functions via attenuating oxidative stress-mediated decline in GFR and renal hemodynamics.[37]

Estrogen has a pervasive effect on body function in both males and females through a variety of mechanisms. The action of BPA at estrogen related receptor (ERR) and the estrogen membrane receptor (mER) has been documented and shown some ligand cross reactivity.[38] The present data recorded a highly significant increase in the estrogen with a significant decrease in the progesterone levels after treatment with BPA. This is in agreement with the results of **Lü and Zhan[39]** who showed that the serum hormone levels of parental female rats were increased by BPA exposure. The treatment with StemEnhance was significantly decreased

estrogen and increased progesterone values as compared to BPA group, however, it still higher than that of control group. This may be attributed to the ability of StemEnhance to modulate serum hormone levels and urinary excretion of estrogen metabolites and phytoestrogens. [12]

As revealed from the data obtained, BPA administration caused hyperprolactinemia which is significantly decreased with stem cell enhancer treatment despite of its value remain higher than control value. Similar data were obtained by **Rosmary *et al.*** [40] who stated that BPA has estrogen-like effect mimicked estradiol in inducing hyperprolactinemia in rats. They revealed that BPA may be alter estrogen receptor and/or estrogen-responsive genes that affect the lactotrophs in rats. However, **Funabashi *et al*.** [41] suggested that BPA could have hypothalamic actions and can alter levels of progesterone receptor expression. This induced changes in neural systems that could impact upon gonadotrophin secretion.

Similarly, the present study reported that BPA induced a significant elevation in FSH with concomitant reduction in LH levels. The decreases in LH observed in this study are consistent with estrogen induced alterations observed by others.[42] This is also in agreement with the results of **Lü and Zhan** [39] that reported a differences in the response of FSH and LH to BPA that could be due to differential sensitivity of the systems regulating FSH and LH secretion to BPA at the level of the pituitary or the hypothalamus**.**

To evaluate that the use of stem cell enhancer has not a pre-carcinogenic potential, measurement of CA15-3 and CA-125 expressions were done. The CA15-3 and CA-125 are regarded as the most reliable tumor markers used in diagnosis and monitoring of breast[43] and ovarian cancer[44] respectively. The observed significant reduction in serum levels of CA15-3 and CA-125 in stem cell enhancer treated rats as compared with the bisphenol-A treated group could be an indication that the stem cell enhancer might have an anti-carcinogenic potentials as reported in previous studies.[17]

In the light of the present results, it could be concluded that high doses of BPA have serious effects on the liver, kidney, hormonal levels and have a pre-carcinogenic potential indicated from the elevated tumor marker levels. These effects are mediated by the oxidative stress induced by BPA. Although treatment with natural product substances as StemtechTM (StemEnhance) suggesting to be safe and efficient in treatment of bisphenol exposed female albino rats.

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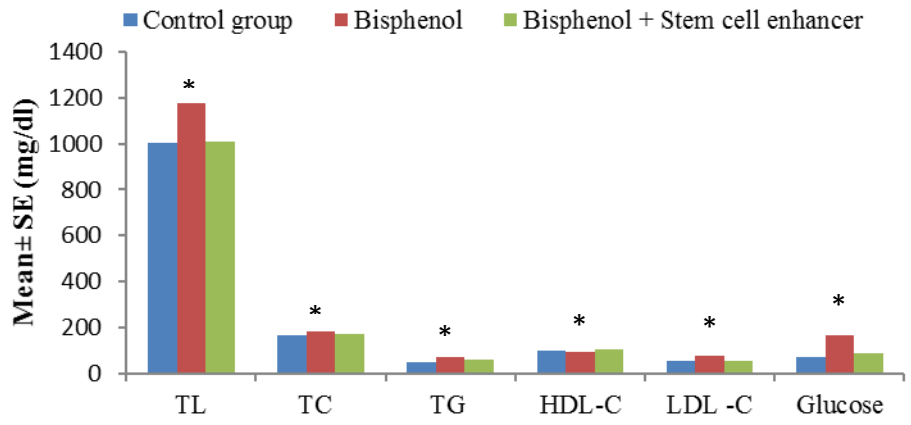

Fig.1:Total lipid (TL), total cholesterol (TC), triglycerides (TG), HDL cholesterol (HDL-C), LDL-cholesterol (LDL-C) and glucose parameters in the control and treated groups (*p< 0.05)

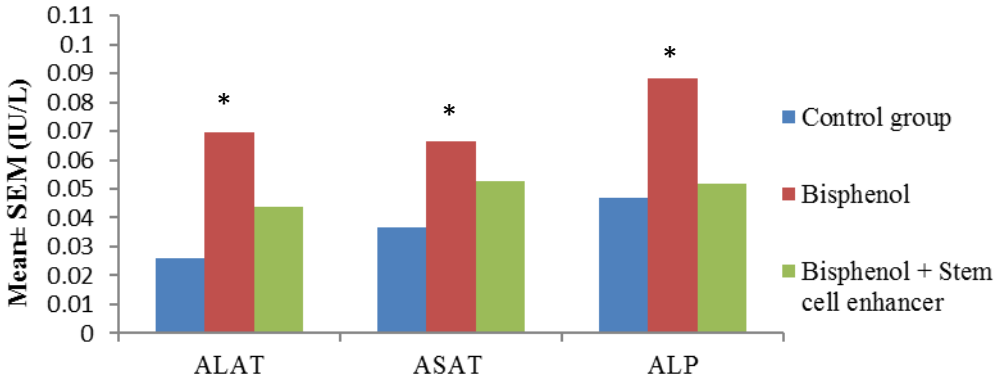

Fig. 2: Changes in the ALAT, ASAT and ALP activities in the control and treated groups (*p< 0.05)

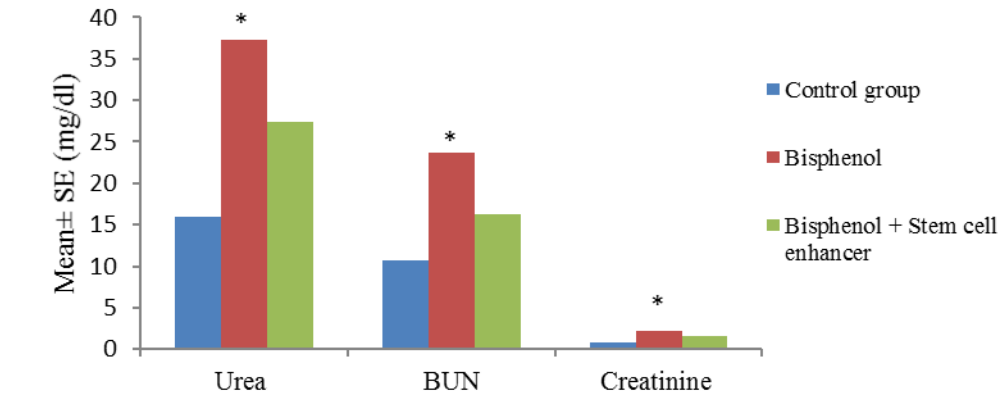

Fig. 3: Changes in urea, BUN and creatinine levels in the control and treated groups (*p< 0.05)

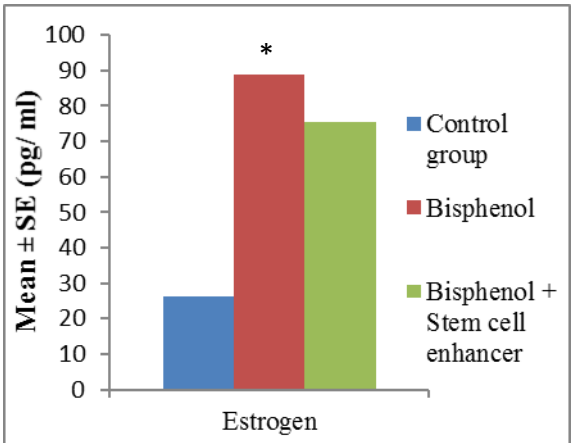

Fig. 4: Levels of Estrogen in the control and treated groups (*p< 0.05)

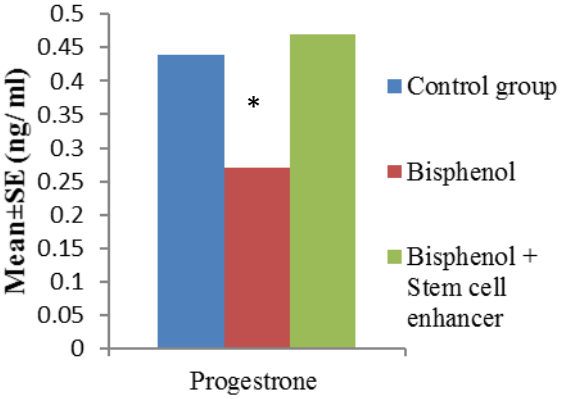

Fig. 5: Levels of progestrone in the control and treated groups (*p< 0.05)

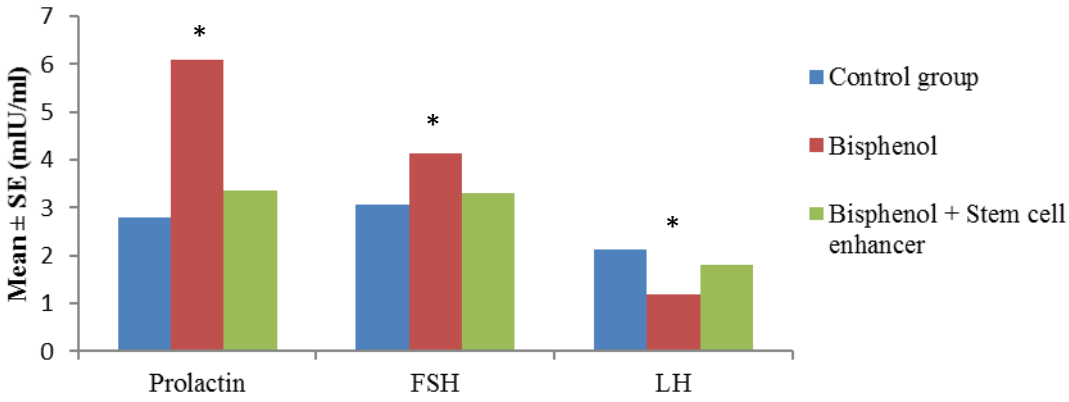

Fig. 6: Changes in the prolactin, FSH and LH levels in the control and treated groups (*p< 0.05)

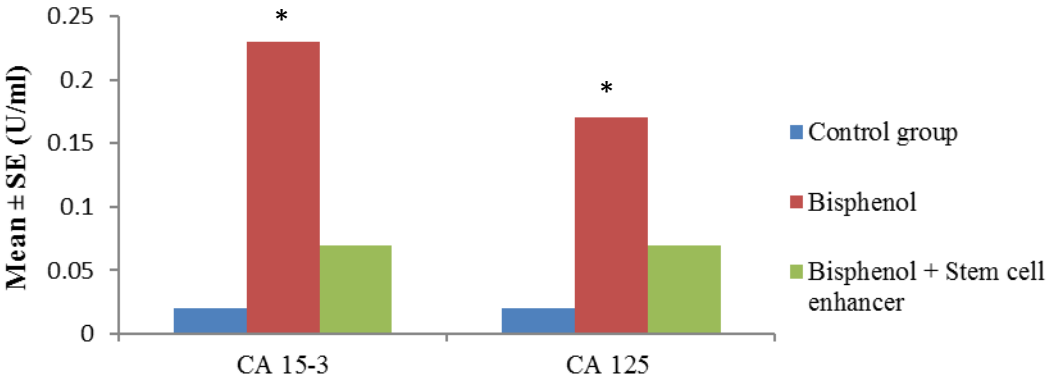

Fig.7 : CA 15-3 and CA-125 levels in the control and treated groups (*p< 0.05)