

Effect of Both Bisphenol-A and Liquorice on Some Sexual Hormones in Male Albino Rats and The Amelioration Effect of Vitamin C on Their Actions

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ABSTRACT

Background: Xenoestrogens are defined as chemicals that mimic some structural parts of the physiological estrogen compounds, therefore may act as estrogen or could interfere with the actions of endogenous estrogens. Phytoestrogens (plant estrogens) are substances that occur naturally in plants. They have a similar chemical structure to our own body's estrogen. Vitamin C, also known as ascorbic acid, is used to prevent and treat scurvy.

Objective: The aim of the study was to clarify the effect of both bisphenol-A (BPA) and liquorice together on some sexual hormones and illustration of the effect of vitamin C on their actions.

Materials and methods: Thirty male albino rats were used and divided into three groups: Group I: control (untreated group), Group II: rats treated with BPA and liquorice, Group III: rats treated with BPA and liquorice in addition to vitamin C. Blood samples were collected for different biochemical investigations.

Results: The biochemical results showed high significant increase ($p<0.01$) in the activities of ALT, AST, urea, creatinine, FSH, prolactin, total cholesterol, triglycerides, LDL-C, VLDL, LDL/HDL and TC/HDL levels. There was high significant decrease ($p<0.01$) in HDL-C, total protein, albumin, globulin, testosterone, and LH. These results turned back to the normal values after receiving the vitamin C as compared to normal group.

Conclusion: it could be concluded that phytoestrogen and xenoestrogen had undesirable effects and it's recommended to minimize the utilizing of these compounds to protect people from its hazardous effects.

Keywords: BPA, Liquorice, Vitamin C, Hormonal assay, Biochemical parameters.

INTRODUCTION

One of the environmental contaminants and a famous estrogenic endocrine disruptor is the bisphenol-A (BPA), which is used in the creation of polycarbonate plastic ⁽¹⁾ (e.g. water bottles and baby bottles) and epoxy resins (e.g. inside coating in metallic food cans) and as a non-polymer preservative to other plastics. BPA is also found in polymers used in dental materials ⁽²⁾. However, recent evidence also points to that exposure to BPA may occur through dermal contact with thermal papers widely used in cash register receipts. Therefore, it becomes an included part of the food chain. The harmful effects of BPA are largely associated to its estrogenic activity. The exposure of animal to BPA elucidates multiple effects on the male and female reproductive system in various animal models. The herbs chosen for this study, contain phytoestrogens as shown from their chemical constituents. Liquorice (*Glycyrrhiza glabra*) a family of Leguminosae, is a plant that grows in Egypt and other countries of the world. Its roots possess some nutritional value and medicinal properties. It is widely used as a cold drink and in the preparation of some pharmaceuticals such as hematinic pills ⁽³⁾. Phytochemical analysis of liquorice (*Glycyrrhiza glabra*) root extract showed that it contains triterpenes saponin (glycyrrhizin, glycyrrhetic acid and liquiritic acid), flavonoids (liquiritin, isoflavonoids and formononetin) and other constituents such as coumarins, sugars, amino acids, tannins, starch, choline, phytosterols and bitter principles, which are most likely responsible for its

therapeutic properties. There are many phenols present in liquorice extracts such as liquiritigenin, liquiritin, isoliquiritigenin, isoliquiritin, glabridin and formononetin, which are responsible for the plant estrogen activity ⁽⁴⁾. Medicinally its root is used widely either as tincture or fluid extract or ingredient in over the counter pharmaceutical products for cough, expectorant, asthma, peptic ulcers and hepatic-protective products. This is because of its anti-inflammatory, anti-ulcerative demulcent, antimicrobial and hepatic-protective activities ⁽⁵⁾.

Ascorbic acid (AA) or vitamin C is a water soluble vitamin. Ascorbic acid is a six-carbon keto-lactone, synthesized from glucose via several intermediates. It is an anti-oxidant vitamin ⁽⁶⁾, with biological actions of particular relevance to reproduction. In humans its deficiency has been associated with a wide spectrum of clinical manifestations such as scurvy, which is a lethal condition unless appropriately treated. The antioxidant role of AA in maintaining motor abilities has been well documented. It has been shown that AA elicits its antioxidant effect through donation of electrons and protection of other compounds from oxidation. In this process, vitamin C itself is oxidized but unreactive and therefore ascorbic acid has been described as a good free radical scavenger. Several studies in human have demonstrated the effects of vitamin C on intestinal iron absorption, reduction of harmful oxidants in the stomach and vascular responsiveness. These effects have been reported to be mediated by the antioxidant actions of vitamin C, which may play a role in iron absorption, gastric cancer prevention, vascular disease and hypertension. Vitamin C appears to have beneficial

effects on erectile and testicular functions in healthy and unhealthy subjects with reproductive problems⁽⁷⁾.

AIM OF THE STUDY

This study was conducted to clarify the effect of both BPA and liquorice together on some sexual hormones and illustration of the effect of vitamin C on their actions.

MATERIALS AND METHODS

30 male albino rats of local strain weighting 110-130 g were used throughout the experiments. The animals were housed in isolated animal metal cages in a conditioned room in the animal lab of the Physiology Department, Faculty of Science, Al-Azhar University.

Ethical approval:

Approval of the Ethical Committee was obtained. This study was conducted in accordance with ethical procedures and policies approved by Animal Care and Use Committee of Faculty of science, Al-Azhar University, Cairo, Egypt. Animals were treated according to guidelines for the use and care of laboratory animal in temperature, ventilation, humidity and normal dark / light cycle with free access to water and fed on rodent chow diet all over the period of the work (4 weeks)

Experimental design:

In current study, 30 male albino rats were divided into three equal groups (10 rats in each group) as the following:

- **Group I:** (control group) comprised of normal rats and maintained on standard rodent chow diet and tap water *ad libitum* for 30 days.
- **Group II:** rats received orally liquorice (250 mg/kg body weight) and BPA (20 mg/kg body weight for 30 days).
- **Group III:** rats treated with BPA and liquorice in addition to vitamin C (7 mg/kg body weight).

Body weight measurement: Body weight was recorded before and after the experiment.

Blood sample collection:

At the end of the experimental period, the blood samples were collected from the retro-orbital sinus after anesthetizing the rat by ether. Serum was separated by centrifugation at 3000 rpm for 15 minutes at room temperature to estimate biochemical parameters.

Biochemical analysis:

Assessment of biochemical parameters:

In the present study total protein (TP), albumin concentration were estimated, then serum globulin concentrations were calculated according to the formula:

$$\text{Globulin (g/dl)} = \text{total protein (g/dl)} - \text{albumin (g/dl)}$$

The ratio of serum albumin/globulin was determined.

Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) activities, creatinine, Urea concentrations as well as lipid profile [cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C)] were also determined. All parameters were estimated using Bio Merieux SA kits, France. However, ratios of TC/HDL (risk factor 1) and LDL/HDL (risk factor 2) were also calculated after calculation of serum LDL-C (low-density lipoprotein cholesterol) and VLDL (very low density lipoprotein cholesterol) using the **Friedwald's⁽⁸⁾** and **Norbert⁽⁹⁾** formulas, respectively as following.

Friedwald's equation: $\text{LDL (mg/dl)} = \text{TC} - \{\text{HDL} + [\text{TG}/5]\}$. Norbert equation: $\text{VLDL} = \text{TG}/5$.

Hormonal assay:

Estimation of serum luteinizing hormone (LH) follicles-stimulating hormone (FSH) Prolactin (P) and testosterone (T) levels were determined by using kit manufacturer instructions. All kits used for hormone assay were obtained from (Monobind Inc. lake forest CA 92630, USA).

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean \pm standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

□ □ Independent-samples t-test of significance was used when comparing between two means.

□ □ Chi-square (χ^2) test of significance was used in order to compare proportions between two qualitative parameters.

□ □ The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:

□ □ Probability (P-value)

- P-value < 0.05 was considered significant.

- P-value < 0.001 was considered as highly significant.

- P-value > 0.05 was considered insignificant.

RESULT

Results of the present study regarding the body weight are in Table 1.

Table (1): Change of body weight in the control and treated groups

Parameter	Control (mean ± SEM) N=10	Mixture (mean ± SEM) N=10	Mixture and vitamin C (mean ± SEM) N=10
% of body weight change	13.2 ± 0.04	21.4 ± 2.9* 8.1%	118 ± 1.08 4.0 %

P* < 0.005 as compared to control group.

Protein profile: (Table 2)**Table (2):** Change in protein profile in the control and treated groups

Groups Parameters	Control (mean ± SEM) N=10	Mixture (mean ± SEM) N=10	Mixture and vitamin C (mean ± SEM) N=10
Total protein (g/dl) % change	6.18 ± 0.09	4 ± 0.10** - 37.3 %	4.94 ± 0.1 - 17.8%
Albumin (g/dl) % change	3.44 ± 0.02	1.95 ± 0.07** - 46.9 %	2.48 ± 0.06 -27.9 %
Globulin(g/dl) % change	2.78 ± 0.24	2.1 ± 0.1* - 24.5 %	2.46 ± 0.15 -11.5%
Albumin/ Globulin % change	1.30 ± 0.21	0.90 ± 0.03 -32.8 %	1.034 ± 0.07 -20.5%

P* < 0.005, p** < 0.001 as compared to control group.

Liver functions are shown in Table 3.**Table (3):** Change in liver function test in the control and treated groups

Groups Parameters	Control (mean ± SEM) N=10	Mixture (mean ± SEM) N=10	Mixture and vitamin C (mean ± SEM) N=10
ALT (U/dl) % change	19.8 ± 2.3	2.7 ± 0.75** 40.3%	29.1 ± 0.005 15.9%
AST (U/dl) % change	23.4 ± 3.1	0.0 ± 0.52** 69.5%	33.0 ± 0.06 41.5%

p**<0.001 as compared to control group

Lipid profile: (Table 4).**Table (4):** Changes in lipid profile in the control and treated groups

Groups Parameters	Control (mean ± SEM) N=10	Mixture (mean ± SEM) N=10	Mixture and vitamin C (mean ± SEM) N=10
T-C (mg/dl) % change	142±2.2	172.6 ± 0.06** 21.8%	149 ± 0.71 4.93%
T-G (mg/dl) % change	82 ± 2.6	100.2 ± 0.17** 22.6%	88 ± 0.7 7.32%
HDL-C (mg/dl) % change	56 ± 1.7	38.31 ± 0.9** -31.0%	43 ± 0.005 -23.2%
LDL-C % change	70.6 ± 1.8	113.4 ± 0.13** 61.2%	88.4 ± 0.14 25.21%
VDL-C % change	16.4 ± 0.5	20.02 ± 0.04** 25.7%	17.6 ± 0.31 7.32%
LDL/HDL % change	1.27 ± 0.04	3.0 ± 0.06** 136.6%	2.05 ± 0.04 61.42%
TC/HDL % change	2.56 ± 2.4	4.62 ± 0.07** 76.94%	3.47 ± 0.03 35.6%

P** < 0.001 as compared to control group

Kidney functions: The data are in table (5).

Table (5): change in kidney function tests in the control and treated groups

Groups Parameters	Control (mean ± SEM) N=10	Mixture (mean ± SEM) N=10	Mixture and vitamin C (mean ± SEM) N=10
Urea (mg/dl) % change	40.2 ± 0.9	66.7 ± 1.3** 62.9%	53.7 ± 0.7 34.4%
Creatinine(mg/dl) % change	0.5 ± 0.2	0.72 ± 0.07** 46%	0.58 ± 0.04 16%

P** < 0.001 as compared to control group

Hormones: (Table 6).

Table (6): changes in hormonal profile in the control and treated groups.

Groups Parameters	Control (mean ± SEM) N=10	Mixture (mean ± SEM) N=10	Mixture and vitamin C (mean ± SEM) N=10
Prolactin(ng/ml)% change	4.3 ± 0.07	6.71 ± 0.07 ** 55%	4.91 ± 0.06 15.1%
Testosterone(ng/ml) % change	16.2 ± 0.6	9.4 ± 0.16** - 39.5%	13.7 ± 0.08 -14.7%
FSH (mlu/ml) % change	2.7 ± 0.07	5.22 ± 0.95** 92.6%	3.77 ± 0.02 45.1%
LH(mlu/ml) % change	10.7±0.5	7.51 ± 0.07** -25.5%	9.0 ± 0.1 -15.1%

P* < 0.005, P** < 0.001 as compared to control group.

DISCUSSION

Body weight:

The findings in this study showed an increase in body weight in mixture experimental group. This effect may be attributed to the *Glycyrrhizaglabra*. Increase in body weight by *Glycyrrhizaglabra* root extract is in accordance with that reported by Miller ⁽¹⁰⁾ who demonstrated that *Glycyrrhizaglabra* inhibits 11 β-hydroxysteroid dehydrogenase and induces excess release of mineralocorticoids, which cause retention of sodium and water that leads to edema and increase in body weight. However, the increased body weight is explained by the improvement in feeding efficiency.

Liver function:

In the present study there was an increase in activities of ALT and AST in mixture group. BPA has adverse effects on the liver, as indicated by increased activities of ALT and AST enzymes. 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. 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. BPA has adverse effects on the liver, as indicated by increased activities of ALT and AST enzymes ⁽¹¹⁾. Bisphenol A is reported to increase the hepatic oxidative stress and mitochondrial dysfunction ⁽¹²⁾. Also liquorice has toxic effect on the liver after *G.*

glabra administration. It causes changes in hepatocyte tissue, congestion and necrosis of hepatocytes in the liver of extract-treated animals which caused by metabolism of plant extract in the liver. Also, glycyrrhizin and coumarins, are two active gradients of liquorice, which have toxic effect in the liver causing hepatic conjugation or hemorrhage ⁽¹³⁾. When the animals were treated by vitamin C, elevation of ASAT and ALAT was reduced to a value close to the normal. This may be due to the antioxidant activity of vitamin C. This protective effect of vitamin C against toxicity of the mixture groups occurred because of its antioxidant action ⁽¹⁴⁾. A previous study suggested that the ameliorating effects of vitamin C may be likely mediated via inhibition of free radicals generation and/or increasing free radical scavenging activity.

Protein profile:

The present work results showed changes in protein profile in the mixture-treated rats. The physiological and biochemical activities in the albino rats were completely disturbed after the oral administration of mixture. In the present study, mixture-treated group showed, decreased protein content when compared to the control. Decreased levels of serum protein might be due to deactivation of protein disulfide isomerase, a multifunctional protein critically involved in the folding and shedding of cellular proteins ⁽¹⁵⁾. The decreased protein levels could be related to damage of cells caused by BPA. The decrease in protein content

under stress induced by BPA may be attributed to the utilization of amino acids in various catabolic reactions. BPA may either act by activating or inhibiting enzyme activities in the cell or destruction of the cell organelles with liberation of particular enzymes is one of the reasons to alter the expression of total proteins. Another reason is oxidative stress influenced by excess reactive oxygen species (ROS) produced in serum that are known to damage proteins⁽¹⁶⁾. The depletion of total protein content observed in this investigation can be correlated to this fact. BPA-induced oxidative stress in serum are known to damage proteins⁽¹⁷⁾. This can lead to various diseases, including cancer, infertility and neurodegenerative diseases. There is much concern that exposure to BPA causes endocrine toxicity in man and wild life. Also, *liquorice* has toxic effect. It is possible that the chemical constituents of the plant parts of liquorice, majorly the alkaloids, are responsible for protein depletion as a result of increased protein catabolism and increase excretion in urine⁽¹⁸⁾. Liquorice is an extract from the *Glycyrrhizaglabra* plant, which contains glycyrrhetic acid, or GZA. GZA is made of one molecule of glycyrrhetic acid and two molecules of glucuronic acid. When administered orally, the product of glycyrrhetic acid is found in urine.

Additionally, vitamin C resulted in a significant improvement in the serum total protein level. Vitamin has the ability to prevent oxidative stress, which affects the liver that is the main site for protein synthesis in the body⁽¹⁹⁾. This may be due to the antioxidant activity of vitamin C that exhibited a hepato-protective effect against liver damage.

Lipid profile:

In the present study, there was a significant increase in all the parameters of lipid profile, except HDL which recorded a significant decrease in mixture group and nonsignificant change in vitamin C group when compared to control group. Exposure to low doses of the mixture increases the insulin expression and production by the pancreas⁽²⁰⁾. Insulin is known to increase genesis by both post-translational protein modifications and transcriptional mechanisms. It was 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, this 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 treated mice⁽²¹⁾.

BPA caused hyperlipidemia that is an important agent for cardiovascular, atherosclerosis disease, myocardial infarction and cerebrovascular disease. Mechanistically, liquorice or environmental BPA acts

on estrogen receptors and do estrogen action. Both BPA (xenoestrogen) and liquorice (phytoestrogen) are a well-known endocrine-disrupting chemical that bind to the estrogen receptors (ER alpha and ER beta) and disrupting the lipid profile⁽²²⁾.

Meanwhile, after treating by vitamin C, a water-soluble antioxidant, which is an important factor in lipid regulation, there was an increase in HDL levels and protection against LDL oxidation⁽²³⁾.

Kidney function:

This study also demonstrated that treatment of rats in the mixture group induced a highly significant increase in serum urea and creatinine. BPA can cause injury in the kidney, and other organs by forming reactive oxygen species (ROS) that may be due to the oxidative stress induced by BPA on the rats kidney tissues as investigated by **Korkmaz et al.**⁽²⁴⁾. Oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases. The toxicity of liquorice extract showed toxic changes in the kidney after *G. glabra* administration. An increase in intake of liquorice can cause many toxic effects. Hyper-mineralocorticosteroid syndrome can occur when the body retains sodium, loses potassium altering biochemical and hormonal activities. Many adverse effects of liquorice poisoning can be seen and most are attributed to the mineralocorticoid effects of GZA. People with previously existing kidney problems may be more susceptible to GZA and liquorice poisoning⁽²⁵⁾. However, treatment with vitamin C resulted in a significant improvement in serum levels of biochemical parameters of the kidneys. **Tawfik and Al-Badr**⁽²⁶⁾ recorded that dietary antioxidants such as vitamin C had a modulator effects on mixture group-induced serum urea due to oxidative damage of the kidneys of rats.

Hormone:

In the present study, the mixture of bisphenol A and liquorice caused hormonal disturbances, where it showed a significant increase in FSH and prolactin and significant decrease in LH and testosterone hormone levels. As revealed from the data obtained, BPA administration caused hyperprolactinemia. Similar data were obtained by **Rosmary et al.**⁽²⁷⁾ who stated that BPA has estrogen-like effect mimicked estradiol in inducing hyperprolactinemia in rats. They revealed that BPA may alter estrogen receptor and/or estrogen-responsive genes that affect the lactotrophs in rats. However, **Funabashi et al.**⁽²⁸⁾ suggested that BPA could have hypothalamic actions and induce 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 is consistent with estrogen-induced alterations observed by others. This is in

agreement with the results of **Akingbemi et al.** (29) where they reported that BPA has adverse effects on testicular function by decreasing pituitary LH secretion and reducing Leydig cells steroidogenesis. Decreased Leydig cells was compared with the decreased serum LH and testosterone (T) levels as well as Leydig cell androgen biosynthetic capacity. So, BPA suppressed T production via decreased LH secretion. There is also evidence that BPA interferes with LH receptor-ligand binding. Different doses of BPA caused inhibition in spermatogenesis and seminiferous tubule in male. BPA caused a decrease in sperm count and motility and also affected sperm morphology of adult male rats. BPA has been associated with declined semen quality and increased sperm DNA damage. This also supports the toxicity of BPA on germ cell. **Nakamura et al.** (30) stated that BPA, dose dependently, decreased the expressions of steroidogenic acute regulatory (StAR) protein and steroidogenic enzymes like cholesterol side chain cleaving enzyme P450scc (CYP11A1), P45017, and 17-HSD mRNA. Therefore, the decreased expression of steroidogenic enzymes and StAR protein concerned in testosterone synthesis might be primarily related to the decreased testosterone levels as a result of BPA treatment. **Gurmeet et al.** (31) supposed that the decreased testosterone level may be the cause of alteration of spermatogenesis and disturbance of the seminiferous epithelium. Additionally, the disturbances in hypothalamic-pituitary-gonadal axis and decrease in Sertoli cell phagocytic function might be induced by BPA administration. The cytotoxic agents can affect the spermatogenic cells due to their high mitotic activity. The results are in accordance with **Vandenberg et al.** (32). They showed morphological damages in testis after administration of BPA and provided the evidence for their toxicity on the male reproductive system. It has been reported that liquorice reduced serum testosterone level and affected androgen metabolism by inhibiting the enzyme β -6 HSD, and 17β HSD hydroxysteroid dehydrogenase or by stimulating aromatase. Therefore, it was proposed that liquorice could cause the deficiency of serum testosterone, leading to sexual dysfunction or decline of libido in men (33).

Tamer et al. (34) and **Helal et al.** (35) demonstrated that liquorice affects the adrenal cortex by stimulating glucocorticoids production, which affects the reproductive hormones. Small amounts of testosterone are secreted by the adrenal glands and estradiol is also secreted to a lesser degree by the adrenal glands. The effect of liquorice on serum testosterone among healthy women was found to decrease testosterone because of the inhibition of 17-hydroxysteroid dehydrogenase. This finding indicates that liquorice may be beneficial in treating women with hirsutism and polycystic ovary syndrome. Liquorice also supports the adrenal gland in stimulating excretion of hormones from the adrenal cortex and in promoting

estrogenic activity. Therefore, it was proposed that liquorice causes the deficiency in serum testosterone. Glabridin, the major isoflavone in liquorice and many other phenols such as liquiritigenin, liquiritin, isoliquiritigenin, isoliquiritin, glabridin and formononetin are responsible for phytoestrogen activity and estrogenic properties, which cause hormonal disturbance and decrease in sperm count (36). Meanwhile, vitamin C recorded a significant improvement in the value of testosterone hormone and elevated its level as compared to the mixture-treated animals. **Biswas et al.** (37) reported that ascorbic acid stimulate testicular steroid dehydrogenase activity and increase plasma testosterone level. **Karanth et al.** (38) reported that ascorbic acid is a vitaminergic transmitter that activates the release of both FSH and LH from the anterior pituitary gland by autocrine action by means of nitric oxide. Then, LH causes the release of testosterone from Leydig cells.

CONCLUSION

It could be concluded that phytoestrogen and xenoeostrogen have undesirable effects and it is recommended to minimize the utilizing of these compounds to protect people from its hazardous effects.

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