

## Protective Effect of Hesperidin on Kidneys and Testes of Adult Male Rats Exposed to Bisphenol A

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### ABSTRACT

**Background:** Bisphenol A, a global environmental pollutant, has been reported to induce organs toxicity. Hesperidin is a flavanone glycoside which is found in citrus fruits. It has antioxidant properties; so it can protect cells from oxidative stress. **Objectives:** The current study aimed to investigate the toxic effects of bisphenol A on the kidneys and testes after repeated oral dose and evaluate the possible protective effect of hesperidin when co-administered with it.

**Materials and Methods:** Forty-two adult male albino rats were divided into four groups: Group I (Control group), Group II (Hesperidin only group) received hesperidin orally at the dose of 200 mg /kg /day. Group III (Bisphenol only group) treated orally with bisphenol A at dose 160 mg /kg /day. Group IV (Bisphenol& hesperidin group) received bisphenol A and hesperidin. After two months, all animals were sacrificed, and blood was collected for analysis (kidney functions and hormones level). The kidneys and testes were preserved for histopathological examination.

**Results:** Repeated oral administration of bisphenol A induced statistically significant increase in the level of urea and creatinine, statistically significant decrease in serum level of FSH, LH and testosterone. Histopathological examination of kidneys and testes revealed multiple histopathological changes. These toxic effects declined markedly when hesperidin was co-administered with bisphenol A.

**Conclusion:** The present study concluded that bisphenol A has many toxic effects on kidneys and testes both structurally and functionally and hesperidin has a protective role against such harmful effects.

**Keywords:** Bisphenol A, Hesperidin, kidney, Testes toxicity.

### INTRODUCTION

Bisphenol A (BPA) is considered one of the most common industrial manufactured chemicals all over the world <sup>(1)</sup>. BPA is used in a wide range of daily used plastic products, it can contaminate the environment extensively either by leaching from plastic food and water containers, or as byproducts of industrialization. So, the food and drink are the major source of exposure <sup>(2)</sup>.

Experiments using cultured cells and laboratory animals demonstrated that BPA is able to accumulate and affect several vital organ functions, including the testis, brain, heart, liver and pancreas <sup>(3)</sup>.

Hesperidin (HSD) is a flavanone glycoside which is found in citrus fruits and is considered as anti-inflammatory and antioxidant agent. It can decrease oxidative stress and produce pro-inflammatory cytokines in mice <sup>(4)</sup>.

Due to its antioxidant and anti-inflammatory properties, HSD has several biological effects for the prevention of diseases, such as cardiovascular disease, diabetes and cancer <sup>(5)</sup>. In addition, it has been reported that HSD has beneficial effects on toxicities caused by drugs and chemicals <sup>(6)</sup>.

Majority of the population especially in the developing countries may not be aware of the harmful effects of BPA on the human body <sup>(7)</sup>. Hence, this study aimed to study toxicity of bisphenol A on the function and structure of kidneys and testes of adult male albino rats and study the efficacy of hesperidin in the protection against kidneys and testes consequences that could result from bisphenol A.

### MATERIAL AND METHODS

**Type of the study:** Experimental study.

**Experimental animals:** The study was conducted on 42 adult male healthy, albino rats having weight of 200 ± 20 g. They were housed at laboratory condition for 1 week prior to experimentation. Animals were housed 5 rats/cage in a temperature-controlled room (22 ± 2 °C) with 12–12 h dark–light cycles at a humidity of 50 ±10%. Animals were fed with standard pellet feed and water.

#### Ethics considerations:

**Ethical approval was obtained from the Medical Research Ethics Committee of Faculty of Medicine - Sohag University, according to the commitment standard operating procedure guidelines on 8/2/2022 under IRB registration number: Soh-Med-22-02-34. The experimental procedure was conducted in accordance with the guide of the care and use of laboratory animals approved by the Medical Research Ethics Committee of Faculty of Medicine, Sohag University.**

#### Drugs:

**Bisphenol A:** BPA was purchased from Sigma Aldrich corporation (manufacturing company).

**Hesperidin:** HSD was purchased from Sigma Aldrich corporation (manufacturing company).

**Study design:** The rats were divided randomly into 4 groups:

**Group I (Control group):** this group included 12 animals. They were subdivided into two subgroups (6 animals each): **Group IA** (Negative control): the rats were fed on basal diet and distilled water for two

months. **Group IB** (Positive control): the rats were treated orally with corn oil (1 ml/kg /day) for two months.

**Group II (Hesperidin only group):** This group included 10 animals. The rats were treated orally with hesperidin, dissolved in corn oil at the dose of 200 mg /kg /day for two months.

**Group III (Bisphenol only group):** This group included 10 animals. The rats were treated orally with bisphenol A, dissolved in corn oil, at dose of 160 mg /kg /day (1/20 of LD50) for two months. The LD50 of BPA for rats is 3250 mg/kg after oral administration<sup>(8)</sup>.

**Group IV (Bisphenol & hesperidin group):** This group included 10 animals. The rats were treated with bisphenol A followed by hesperidin after 1 hour, at the same mentioned doses for two months.

### Methods:

#### 1- Sample collection and preparation:

- At the end of the study rats were sacrificed under light anesthesia according to guidelines of Medical Research Ethics Committee of Faculty of Medicine, Sohag University.
- Blood samples were collected from jugular vein during scarification for chemical analysis (kidney function tests, hormonal assay).
- Necropsy was done for all animals, kidneys and testes were removed for histopathological examination with light microscopy.

#### 2-Biochemical tests:

- A- **Kidney function tests:** by measuring the level of serum urea and creatinine by colorimetric measurement by spectrophotometer, by using urea/BUN liquizyme kits, (catalogue No. UREC 0104021-2 and creatinine by using creatinine -J kits, catalogue No. D 496).
- B- **Hormonal assay:** by assessment of follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone hormone using chemiluminescence immunoassay analyzer, by using VIDAS FSH Kits, (catalogue No. 1009014410, VIDAS LH Kits, catalogue No. 1009072050 and VIDAS testosterone Kits, catalogue No. 414320).

#### 3- Histopathological examination:

Kidneys and testes from all experimental rats were removed. Kidneys were fixed in 10% formaldehyde and testes in Bouin solution, formaline fixed paraffin embedded tissue blocks from both organs were obtained and slides were prepared for histopathological examination by light microscopy.

#### Statistical analysis:

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA), and calculated as mean and standard deviation. Student's t- test was used for comparison between the study groups. Quantitative data were expressed as mean ± SD (Standard deviation). P

value < 0.05 is considered significant. The results were presented in the form of tables.

## RESULTS

### I-Biochemical results:

There was no significant statistical difference in the mean values of serum urea and creatinine in control groups: group (IA&IB); so group IA was used for comparison with other study groups.

There was no significant statistical difference in the mean values of serum urea and creatinine in group II (Hesperidin only group) as compared with group IA (Negative control group) (p value > 0.05) (Tables 1 & 2).

There was a significant statistical increase in the mean values of serum urea and creatinine in group III (Bisphenol only group) as compared with control group IA (p value: 0.001 and 0.003 respectively).

There was a significant statistical increase in the mean values of serum urea in group IV (Bisphenol& hesperidin group) as compared with control group IA (p value: 0.04). While, there was no significant statistical difference in the mean values of serum creatinine in group IV as compared with control group IA.

There was a significant statistical decrease in the mean values of serum urea and creatinine in group IV (Bisphenol& hesperidin group) as compared to group III (Bisphenol only group) (p value: 0.001, 0.006 respectively) (Tables 1 & 2).

**Table (1): The mean values of serum urea and creatinine in the different study groups.**

| Groups           | Serum urea (mg/dl) | Serum creatinine (mg/dl) |
|------------------|--------------------|--------------------------|
|                  | Mean± SD*          | Mean ± SD*               |
| <b>Group IA</b>  | 41.50±3.7          | 0.57±0.04                |
| <b>Group IB</b>  | 40±3.4             | 0.56±0.03                |
| <b>Group II</b>  | 38.3±1.7           | 0.55±0.03                |
| <b>Group III</b> | 54.70±2.8          | 0.78±0.12                |
| <b>Group IV</b>  | 44.6±1.04          | 0.60±0.03                |

\* SD: standard deviation

**Table (2): Statistical comparison of serum urea and creatinine among different study groups.**

| Groups               | Serum urea (mg/dl) | Serum creatinine (mg/dl) |
|----------------------|--------------------|--------------------------|
|                      | P value by t- test | P value by t- test       |
| <b>IA versus IB</b>  | 0.13               | 0.24                     |
| <b>IA versus II</b>  | 0.14               | 0.33                     |
| <b>IA versus III</b> | 0.001*             | 0.003*                   |
| <b>IA versus IV</b>  | 0.04*              | 0.17                     |
| <b>III versus IV</b> | 0.001*             | 0.006*                   |

\* Significant difference at p value < 0.05

After comparing the hormonal level assay results of follicle stimulating hormone (FSH), luteinizing hormone (LH) and free testosterone of both control groups; group (IA) and group (IB), There was

no significant statistical difference between them ( $p > 0.05$ ); so group IA was used for comparison with other study groups. There was no significant statistical difference in the mean values of serum FSH, LH and free testosterone in group II (Hesperidin only group) as compared with group IA (Negative control group) ( $p$  value  $> 0.05$ ) (Tables 3 & 4).

There was a significant statistical decrease in the mean values of serum FSH, LH and free testosterone in group III (Bisphenol only group) as compared with control group, IA ( $p$  value 0.001, 0.002, 0.001 respectively). While there was a significant statistical increase in the mean values of serum FSH, serum LH and free testosterone in group IV (Bisphenol & hesperidin group) as compared to group III (Bisphenol only group) ( $p$  value: 0.001, 0.001, 0.01 respectively). Finally, there was no significant statistical difference in the mean values of serum FSH, serum LH and free testosterone in group IV (Bisphenol & hesperidin group) as compared with control group IA ( $p$  value  $> 0.05$ ) (Tables 3 & 4).

**Table (3): The mean values of serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and free testosterone in different study groups.**

| Groups    | Serum FSH (mIU/ml) | Serum LH (mIU/ml) | Serum testosterone (pg/ml) |
|-----------|--------------------|-------------------|----------------------------|
|           | Mean ± SD*         | Mean ± SD*        | Mean ± SD*                 |
| Group IA  | 1.07 ± 0.22        | 0.44 ± 0.05       | 0.95 ± 0.14                |
| Group IB  | 0.85 ± 0.21        | 0.41 ± 0.12       | 0.95 ± 0.13                |
| Group II  | 1.04 ± 0.16        | 0.50 ± 0.05       | 0.96 ± 0.12                |
| Group III | 0.54 ± 0.13        | 0.30 ± 0.08       | 0.65 ± 0.08                |
| Group IV  | 1.01 ± 0.16        | 0.45 ± 0.05       | 0.91 ± 0.17                |

**Table (4): Statistical comparison of serum FSH, LH and free testosterone among different study groups.**

| Groups        | Serum FSH (mIU/ml)               | Serum LH (mIU/ml)                | Serum testosterone (pg/ml)       |
|---------------|----------------------------------|----------------------------------|----------------------------------|
|               | <i>P</i> value by <i>t</i> -test | <i>P</i> value by <i>t</i> -test | <i>P</i> value by <i>t</i> -test |
| IA versus IB  | 0.09                             | 0.5                              | 0.4                              |
| IA versus II  | 0.7                              | 0.07                             | 0.9                              |
| IA versus III | 0.001*                           | 0.002*                           | 0.001*                           |
| IA versus IV  | 0.5                              | 0.6                              | 0.7                              |
| III versus IV | 0.001*                           | 0.001*                           | 0.01*                            |

**II-Histopathological results:**

- **Microscopic examination of kidney tissues using H&E stain:**

**Control groups (group IA, IB):** Light microscopic examination of sections from the kidneys showed normal structure of glomeruli and tubules both proximal and distal ones (Figure 1).

**Hesperidin only group (group II):** H&E stained sections of the kidneys showed normal arrangement of glomeruli and tubules with no pathological changes (Figure 2).

**Bisphenol only group (group III):** Examination of serial sections from kidneys showed multiple histological alterations including: glomerular destruction, tubular degeneration with deposition of hyaline cast inside the lumen of some tubules, vascular congestion and lymphocytic infiltration in the interstitial tissue of the kidney (Figure 3 a, b, c, d & e).

**Bisphenol & hesperidin group (Group IV):** H&E stained sections of kidneys showed restoration of the normal appearance of glomeruli and tubules with presence of only few lymphocytic infiltration in the interstitial tissue of the kidney and absence of congestion (Figure 4).

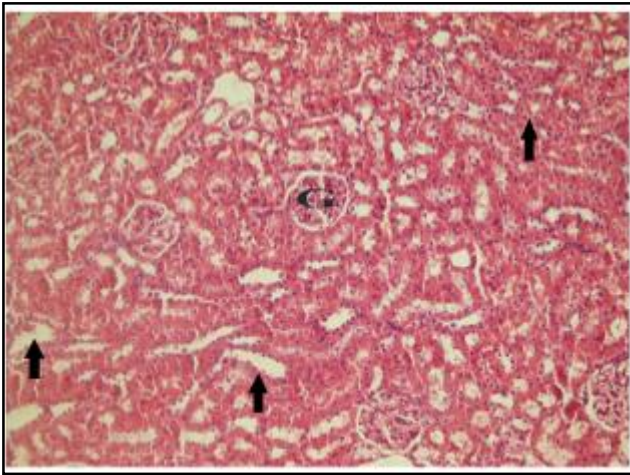
- **Microscopic examination of rat testes using H&E stain:**

**Control groups (group IA, IB):** Light microscopic examination of sections from the testes showed normal appearing seminiferous tubules with normal spermatogenic cells, spermatocytes and spermatids attached in the layers of those seminiferous tubules also with normal Sertoli cells in-between (Figure 5)

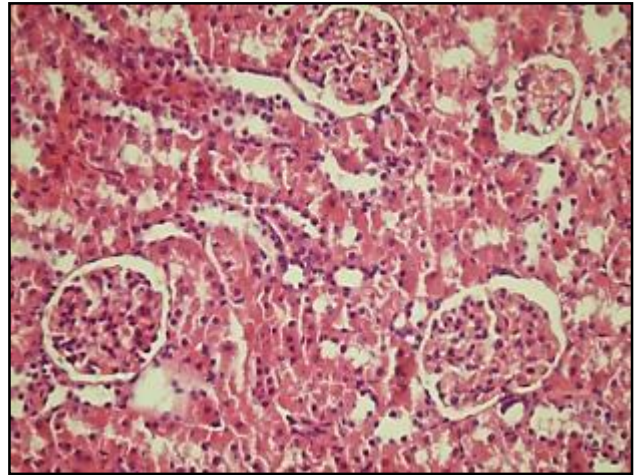
**Hesperidin only group (group II):** Microscopic examination of the testes showed normal seminiferous tubules with normal process of spermatogenesis and sperm formation (Figure 6).

**Bisphenol only group (Group III):** H&E stained sections of the testes showed multiple histopathological changes including: reduced number of spermatogenic cells with few number of spermatids in the lumen of some tubules, maturation arrest. There were severe edema and congestion in the interstitial tissue of the testes. Also, there was detachment and disruption of the germinal epithelium with destruction of some spermatogonic cells (Figure 7 a, b, c).

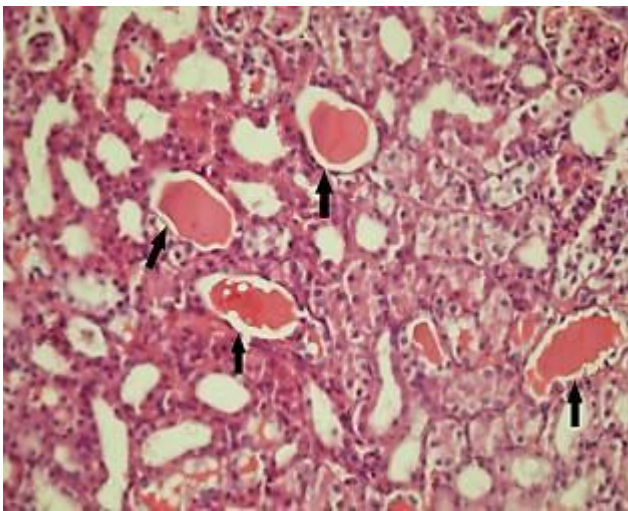
**Bisphenol & hesperidin group (Group IV):** Light microscopic examination of sections from the testes showed mild decrease in the number of spermatogenic cells with partial improvement in the process of spermatogenesis. Also, there was few areas of congestion compared to group III (Figure 8).



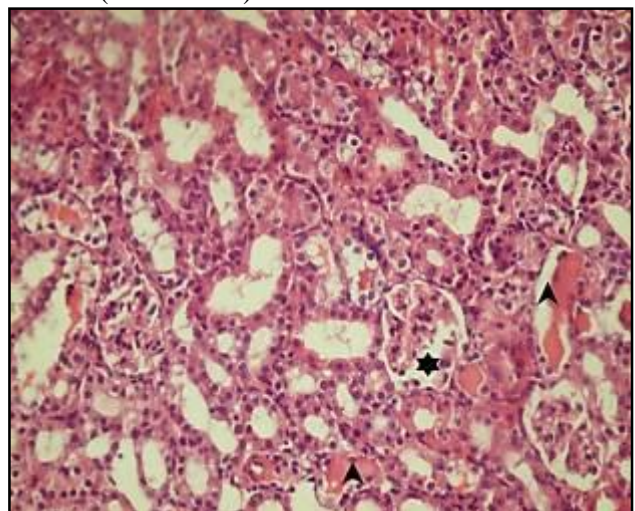
**Figure (1):** A photomicrograph of rat kidney in group I showing normal glomeruli (G) and normal proximal and distal tubules (thick arrows) (H&E X200).



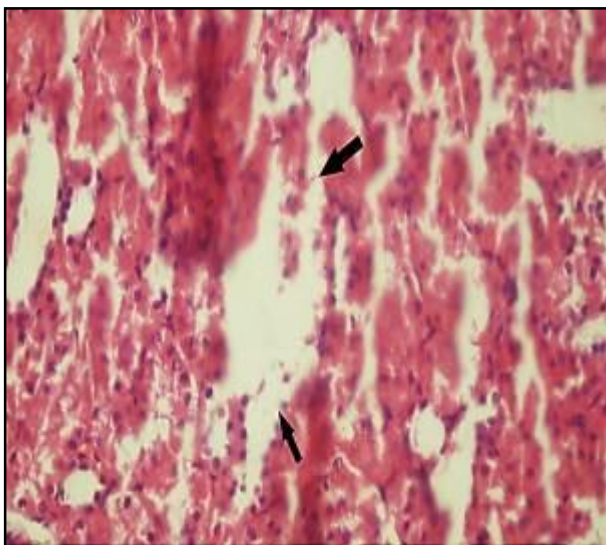
**Figure (2):** A photomicrograph of rat kidney from group II showing normally appearing glomeruli and tubules (H&E X400).



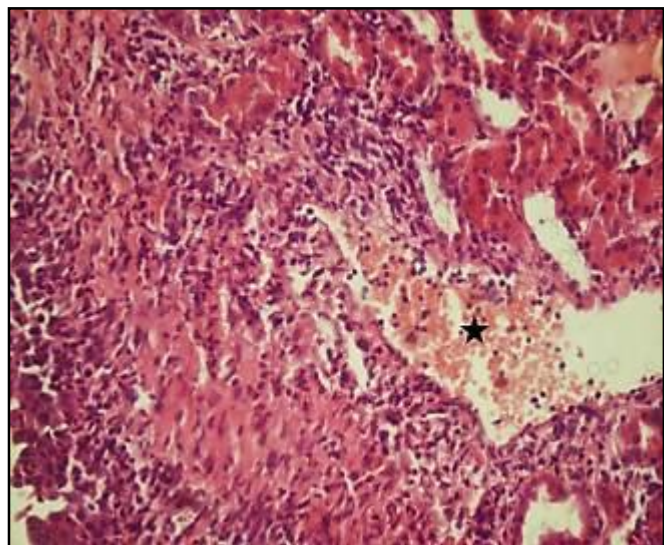
**Figure (3a):** A photomicrograph of rat kidney in group III showing hyaline cast deposition inside the lumen of renal tubules (black arrows) (H&E X400).



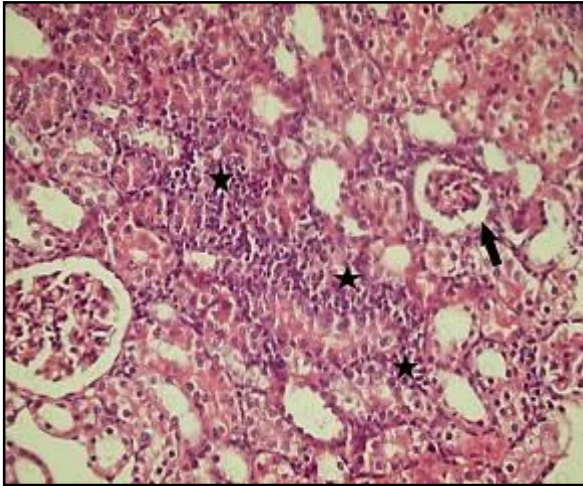
**Figure (3b):** A photomicrograph of rat kidney in group III showing glomerular destruction (star) and hyaline cast inside tubules (arrow head) (H&E X400).



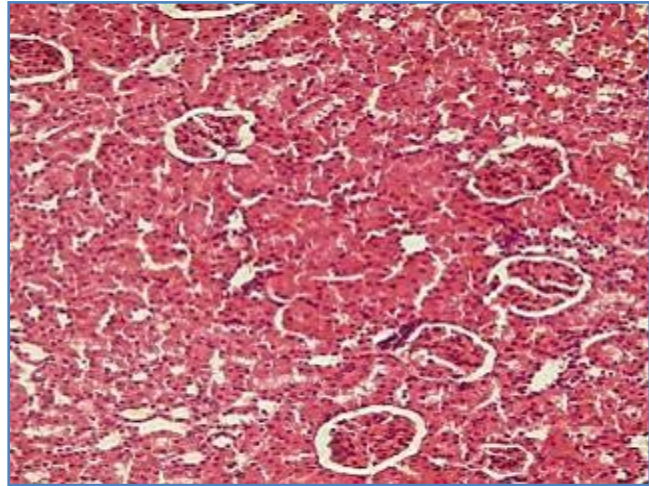
**Figure (3c):** A photomicrograph of rat kidney in group III showing disruption of the epithelial lining of some renal tubules (arrows) (H&E X400).



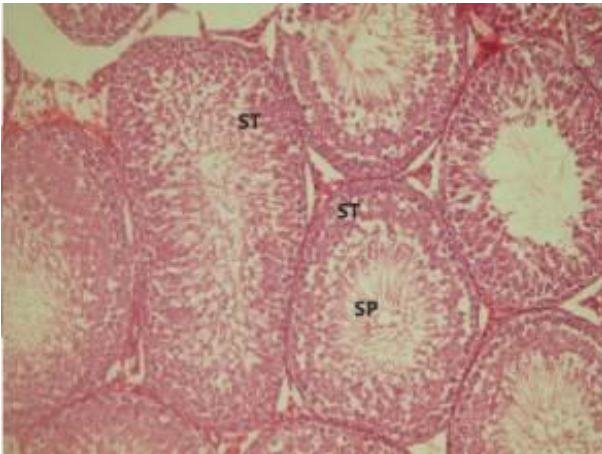
**Figure (3d):** A photomicrograph of rat kidney in group III showing vascular congestion (star) along with lymphocytic infiltration (H&E X400).



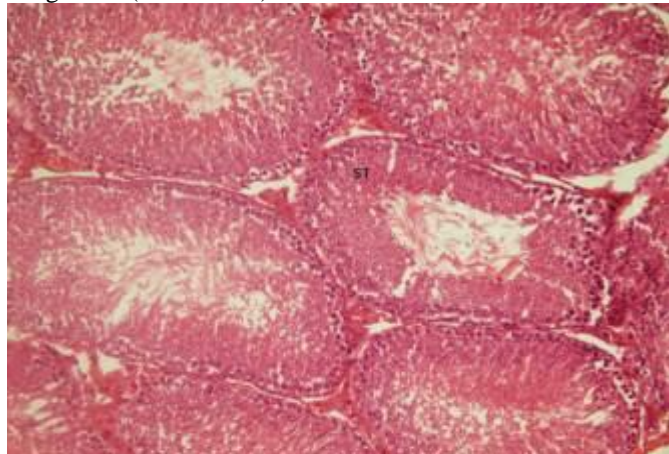
**Figure (3e):** A photomicrograph of rat kidney in group III showing lymphocytic infiltration in the interstitial tissue of the kidney (stars) and glomerular destruction and atrophy (arrow) (H&E X400)



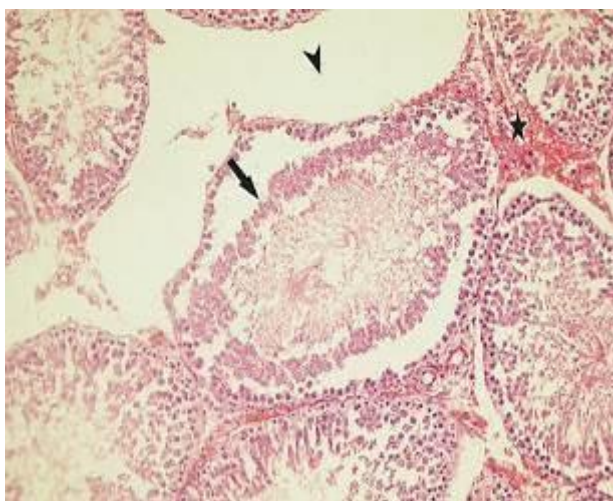
**Figure (4):** A photomicrograph of rat kidney from group IV showing more or less normal glomeruli and tubules with very few lymphocytic cells in the interstitial tissue and absence of congestion (H&E X200)



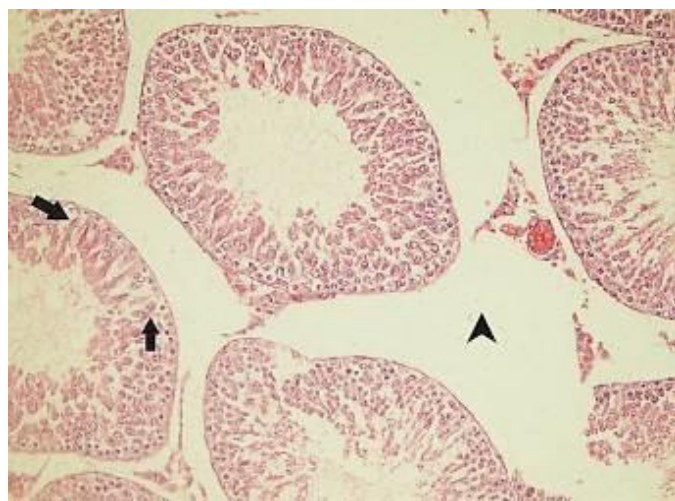
**Figure (5):** A photomicrograph of rat testis in group I showing normal structure of seminiferous tubules (ST) and normal process of spermatogenesis up to sperm formation (SP) (H&E X200).



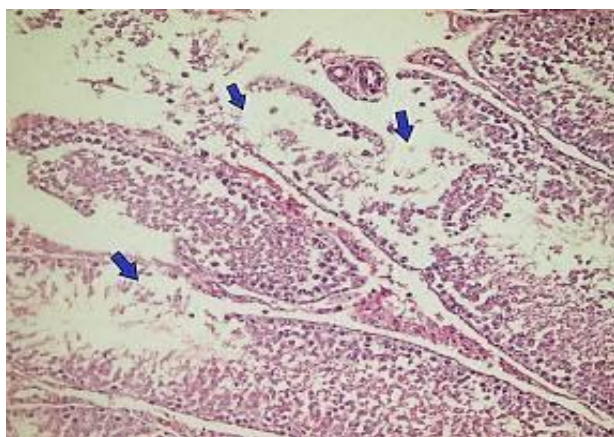
**Figure (6):** A photomicrograph of rat testis from group II showing normal seminiferous tubules (ST) and normal process of sperm formation (H&E X200).



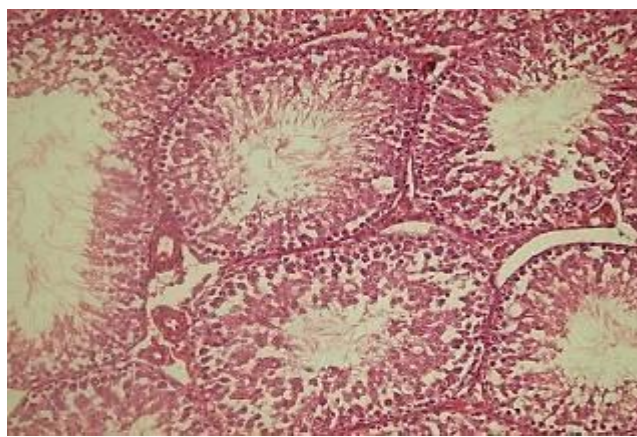
**Figure (7a):** A photomicrograph of rat testis in group III showing disruption of germinal epithelium and detachment (black arrow), severe oedema in-between seminiferous tubules (arrowhead) and marked congestion in the interstitial tissue of the testis (star) (H&E X200).



**Figure (7b):** A photomicrograph of rat testis in group III showing severe oedema (arrow head) and diminished number of spermatogenic cells (arrow) (H&E X200).



**Figure (7c):** A photomicrograph of rat testis in group III showing severe destruction of spermatogenic cells (blue arrows) (H&EX200).



**Figure (8):** A photomicrograph of rat testis from group IV showing restoration of the process of spermatogenesis with few areas of congestion, no oedema in between tubules and no disruption of germinal epithelium (H&EX200).

## DISCUSSION

Bisphenol A (BPA) is a universal synthetic material which is used mainly as a monomer in the production of polycarbonate plastics and as a precursor of epoxy resins. Polycarbonate plastics are used in food and drink containers, the resins are used as lacquers to coat metal products such as food cans, bottle tops and water supply pipes<sup>(9)</sup>. Also, BPA can be transmitted directly through the skin from some types of thermal printing paper, for example: cashier's receipts<sup>(2)</sup>.

Human exposure to BPA is inevitable due to the widespread use of these chemicals. Subsequently, BPA is found nearly in all examined serum samples taken from people in developed countries. It can be found in human serum, urine, placental tissue samples, amniotic fluid and blood taken from umbilical cord<sup>(4)</sup>.

In the present study, there was a significant statistical increase in the mean values of serum urea and creatinine in group III (Bisphenol only group) as compared to control group. While, co-administration of hesperidin (group IV) resulted in a significant statistical decrease in the mean values of serum urea and creatinine in comparison to group III (Bisphenol only group).

The nephrotoxic effect of BPA can be explained by accumulation of BPA toxic metabolites and inability of the kidney to eliminate them<sup>(10)</sup>. Also, exposure to BPA can induce oxidative damage in the tissues by enhancing free radical production owing to disrupting the redox status of BPA<sup>(11)</sup>.

The present results agree with the results of **Poormoosavi et al.**<sup>(12)</sup> who reported that urea and creatinine levels exhibited a significant increase in rats treated with BPA (10 mg/kg/day for 8 weeks). Also, BPA supplementation with *Asparagus officinalis* extract (herbal medicine with antioxidant properties) significantly normalized these markers' levels.

Additionally, **Moustafa et al.**<sup>(13)</sup> reported that HSD (200 mg/kg/day, orally, for 1 week) improved the significant rise in serum urea and creatinine levels following acute aluminum phosphide toxicity in adult albino rats; as it is a potent antioxidant.

Furthermore, it was reported that HSD is a powerful antioxidant agent as it improved acetaminophen induced nephrotoxicity and was superior to other antioxidants agents<sup>(14)</sup>.

Opposite results were reported by **Haroun et al.**<sup>(15)</sup> who noticed that co-administration of Vit C with BPA (25 mg/kg/day, orally for six weeks) could not improve the rise in creatinine level.

In the present study, the nephrotoxic effect of BPA was confirmed by the renal histopathological alterations: including glomerular destruction, tubular degeneration with deposition of hyaline cast inside the lumen of some tubules, vascular congestion and lymphocytic infiltration in the interstitial tissue of the kidney. Also, the ameliorative effect of hesperidin was confirmed by restoration of the normal appearance of glomeruli and tubules.

The histologic alterations might be explained by the generation of reactive oxygen species and free radical by bisphenol metabolism. These radicals disrupt the permeability of cell and organelle membranes<sup>(16)</sup>.

This coincides with **Aslanturk and Uzunhisarcikli** who reported histopathological changes in the kidney of rats received BPA (130 mg/kg/day for 28 days) in the form of tubular and glomerular degeneration. These changes were slighter when curcumin was co-administered with BPA<sup>(17)</sup>.

The results of the current research agrees with the results of **Küçükler et al.**<sup>(18)</sup> who reported that treatment of rats with hesperidin (50 mg/kg & 100 mg/kg) reduced the chlorpyrifos (insecticide) induced renal congestion, inter-tubular hemorrhage,

degeneration and necrosis changes. The authors explained the nephron-protective effect of hesperidin by its antioxidant properties.

Opposite results were observed by **Korkmaz et al.** (19) who noticed that necrotic lesions, congestion areas and mononuclear cell infiltration in the kidney of rats received BPA with vitamin C were similar to those observed in BPA treated group.

Over the last decades, the adverse effects of human exposure to the so-called "endocrine disruptors" have been a subject of concern by the scientific community. Special attention has been paid to their toxicity on the reproductive function. Bisphenol A is among the most well-known endocrine disruptors (20).

Reproductive function depends on suitable organization of the hypothalamic-pituitary-gonadal axis, proper harmony of the neurological and endocrinal systems including the hypothalamus, the pituitary gland and the gonads (9).

Leydig cells stimulated by LH provide the local production of testosterone, and Sertoli cells stimulated by FSH provide the local production of estradiol. In addition, Sertoli cells maintain the spermatogonial stem cells responsible for the continuity of spermatogenesis (21).

In the present study, there was a significant statistical decrease in the mean values of serum FSH, LH and testosterone in bisphenol only group as compared with control group. However, hesperidin co-administration with bisphenol normalized the serum levels of FSH, LH and testosterone.

Several mechanisms of BPA toxicity have been described. BPA exhibits weak estrogenic and antiandrogenic properties. It binds to both estrogen receptors (ERs): ER $\alpha$  and ER $\beta$ , and at high concentrations, BPA binds to the androgen receptor on which it acts as an antagonist (22).

Moreover, BPA decreases the activity of aromatase. Aromatase usually appears in the brain, Leydig cells and adipose tissue and is important in the synthesis of steroid hormones. It can catalyze the irreversible alteration of androgens into estrogens (2).

The decreased serum testosterone level could be due to the diminished expression of the steroidogenic enzymes and cholesterol carrier protein (steroidogenic acute regulatory protein "StAR") involving the testosterone production (23).

Additionally, BPA can impair oxidative homeostasis via direct or indirect mechanisms: including the increase of oxidative mediators and reduction of antioxidant enzymes, leading to mitochondrial dysfunction, alteration in cell signaling pathways and induction of apoptosis (24).

Antioxidants have an important role in protecting against BPA induced oxidative stress (25). The antioxidant activity of HSD is due to its direct radical scavenging activity, also it augments the antioxidant

cellular defenses via the ERK/Nrf2 signaling pathway as well (26).

These findings agree with the results of **Rashad et al.** (27) who noticed improvement of serum testosterone level in animal groups receiving antioxidants (Vit E, melatonin) together with BPA, 50 mg/kg, 3 days a week for 3 weeks by intraperitoneal injection.

Similar results were observed by **Olukole et al.** (28) who reported that BPA administration (10 mg/kg/day, orally for 45 days) caused significant reduction in the serum levels of testosterone compared to the control. Co-treatment with gallic acid (20 mg/kg/day) protected against such effect.

This is in line with the study of **Zahra et al.** (29) which revealed that rats treated with BPA (25 mg/kg i.p. for 30 days) showed decreased serum concentrations of FSH, LH and testosterone as compared to control group. The co-administration of vincetoxicum arnotianum, (flavonoids) (300mg/kg) to BPA-intoxicated rats restored levels of FSH, LH and testosterone.

Additionally, potent anti-oxidative effect of oral HSD has also been demonstrated in testicular tissue following cadmium toxicity in rats (30).

The results of the present study didn't agree with the study of **Gamez et al.** (31) who studied the effect of low dose of BPA (3  $\mu$ g/kg/day, orally) on prepuberal male Wistar rats. An increase in serum FSH and LH levels was observed. The different results may be due to different dose of BPA or maturation of rats.

On contrast to the results of the present study, **Sánchez et al.** (32) reported that BPA treated Wistar rats (25  $\mu$ g/Kg/d, 300  $\mu$ g/Kg/d, s.c. daily for four days) did not exhibit a decrease of testosterone levels. This variation may be due to differences in dose of BPA, method and duration of exposure.

In the present study, the testes of BPA only group showed multiple histopathological changes. There were reduced number of spermatogenic cells, severe edema and congestion in the interstitial tissue of the testes, with destruction of some spermatogenic cells. These changes were less severe when hesperidin was co-administered with BPA.

It was reported that the endocrine environment of the testes has a major impact on the antioxidant status of this organ. Treatment with any chemicals that diminish the concentration of testosterone inhibits the testicular expression of antioxidant enzymes (33).

Also, it is believed that BPA generates high concentrations of reactive oxygen species and results in the decline of the testicular antioxidant enzymes, which could increase the oxidative damage of testicular cellular membranes (34).

These findings agree with the results of **Rashad et al.** (27) who reported that testicular sections of BPA treated rats showed widened lumen of seminiferous tubules, vacuolated epithelium, separated epithelial

lining from the basement membrane and congested blood vessels. Significant improvement occurred after administration of vitamin E and melatonin antioxidants compared to the BPA group.

This is in line with the study of **Olukole *et al.*** <sup>(28)</sup> which revealed disruption of testicular architecture induced by BPA. Rats that co-treated with gallic acid had better testicular architecture: including normal pattern of spermatogenic cells, compact interstitium and mild congestion.

Also, **Wei *et al.*** <sup>(35)</sup> reported that rats exposed to BPA (5 mg/kg) exhibited deformed seminiferous tubules, decreased number of Sertoli cells and Leydig cells, decreased spermatogonia and spermatozoa. However, *Cuscuta chinensis* flavonoids treatment significantly alleviated these injuries caused by BPA.

## CONCLUSION

The findings that arose from this study confirmed the impact of bisphenol A on the kidneys and testes; structure and function. Also, the study provided a new insight regarding the potential beneficial role of hesperidin in reducing the deleterious outcomes following bisphenol A exposure.

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