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# Costus root extract improves testicular toxicity of Bisphenol A in adult male albino rats: histopathological, ultrastructural and biochemical studies

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## Abstract

**Background:** Bisphenol A (BPA) causes environmental pollution and is used as a natural antioxidant to protect against chemical side-effects. Costus is a well-known medicinal plant containing several biologically active compounds. We investigated the protective effects of costus extract against the toxic effects of BPA in the rat testes.

**Results:** Biochemical and immunohistochemical investigations revealed that bisphenol reduced the activity of antioxidant enzymes and plasma testosterone levels and significantly increased P53. Co-administration of costus root extract with BPA improved the depletion of antioxidant enzymes, returned testosterone to normal levels, and improved P53 alternations. Histological and ultrastructural examinations showed that BPA reduced body and testicular weights, and the degeneration of seminiferous tubule germ cells, and the use of costus root extract with BPA attenuated these toxic effects.

**Conclusions:** Costus protects rat testes against the toxic effects of BPA.

**Keywords:** Bisphenol A, Costus root extract, Oxidative stress, Toxicity, Testes

## 1 Background

Bisphenol A (BPA) is a chemical industrial organic compound, which is extremely famous for bisphenols. It has the chemical formula C15H16O2 and its shape is a white solid. they have been used in the production of plastics and epoxy resins since the 1960s [5].

It has been found to have negative effects on male and female reproduction by causing endocrine disruptions. BPA is used in the manufacture of polycarbonate plastics, which are usually stored in containers, such as water bottles. Some plastic containers marked with symbols (Nos. 3, 6, or 7) were made of BPA, and their use should be avoided [15].

BPA is also used in the manufacture of epoxy resins, which are used in inner metal product packagings, such as food cans, package covers, lines of water supply and lines of water supply, retainers of teeth, and their compounds. Recent research has shown that BPA seeps into food and drinks in containers manufactured with BPA [9].

This is a sign of concern because of the potential effects on the health of the brain and prostate glands in fetuses, infants, and children. In addition, it may influence children's behavior.

This was found to have a possible relationship between it and high blood pressure [38]. BPA affects rat liver cells by promoting the conversion of xanthine dehydrogenase

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to xanthine oxidase and increasing the production of Reactive Oxygen Species (ROS) [35]. Therefore, the generation of ROS and oxidative DNA are responsible for the adverse effects of BPA. on the health of humans [36]. It also affects the normal functions of the endocrine glands and reproductive system by stimulating or decreasing the activity of endogenous hormones or by modifying hormone synthesis. Studies have shown that mice are exposed to Bisphenol A (BPA) This is caused by a deficiency in mitochondrial enzymes in the testes [42].

Herbal medicines have been among the modern medicines preferred for use in recent years because they are safe, inexpensive, and readily available. In addition, the method of preparing them is free of trouble and is used to treat many different diseases because they contain antioxidant compounds that help them treat several diseases including testicular toxicity. Costus root extract is a herbal medicine that contains many flavonoids, steroids, and antioxidants, which are molecules that react with ROS to delay and neutralize their function; thus, they reduce oxidative stress and protect us from many diseases [41]. It has been found that excessive production of these types of reactive oxygen species is very harmful to sperm and leads to male infertility. Increased ROS production owing to unnatural forms of sperm, leukocyte contamination, and various types of pollution are known to cause male infertility [8]. Costus root extract has been widely used to treat many diseases caused by fungi [6], worms [27], and microbes [3], as well as in the treatment of diabetes [13], cancer, and anti-inflammatory [1]. Results of studies conducted on rats was that the costus root extract had an effect in improving and enhancing fertility in male rats, where the results indicated an increase in sperm concentration after three weeks of receiving doses ranging between 200 and 400 mg of costus root extract. On the other hand, the same study found that high doses of the CS extract harmed the liver, kidneys, and testes of experimental animals. Studies related to the effects of BPA on toxicity decrease reproductive function in mammals, and anti-anxiety effects are very limited [14].

Therefore, the present study aimed to assess the effects of BPA on the reproductive system and its functions in male rats, as well as the potential preventive and curative effects of the aqueous extract of Costus root extract.

## 2 Methods

### 2.1 Preparation of costus root extract

Costus roots were obtained from stores of herbs in Taif, Saudi Arabia, washed, crushed, prepared, and extracted by adding 100 ml of boiling water to 10 g of root powder in the dark and covering with cups for 24 h, after which the solution was filtered. The supernatant was placed in dark packages, preserved at 4 °C, and orally administered once daily through a gastric tube. The doses were selected based on domain criteria et al. 2021.

### 2.2 Chemicals

In our study, 97% pure Bisphenol A (BPA) (2,2-di(4-hydroxyphenyl) propane) was obtained from Sigma-Aldrich and diluted with olive oil to obtain a final concentration of 10 mg/kg body weight. The doses were chosen as [36].

### 2.3 Animals and experimental design

Eighty adult male albino rats of approximately the same age, weighing (250–300 g) were purchased from the Animal House of King Abdulaziz University, Jeddah. Rats were kept under standard temperature (25 animals were fed a standard commercial diet (ATMID Company. All the rats were acclimatized for at least 15 days before the beginning of the experiment. In this study, animal care was carried out by following the European Community Directive (86/609/EEC) and national rules, this by the 8th edition of the NIH Guidelines for the care and use of Laboratory Animals. Rats were equally divided into four groups containing 20 rats in each group as follows:

**The first group** control group under the same laboratory conditions received distilled water and was fed a standard commercial diet daily for eight weeks.
**The second group** was treated with Costus root extract at a dose (0.4 mg/kg) daily.
**The third group** was treated with 10 mg/kg of Bisphenol A (BPA) (body weight /day) dissolved in 5 ml/kilogram of oil of olive.
**The fourth group** received 10 mg/kg of Bisphenol A (bwt/day) dissolved in 5 ml/kg of oil of olive and Costus root extract at a dose (0.4 mg/kg) daily for eight weeks was given by gastric gavage daily.

### 2.4 Ethical considerations

Experiments occurred according to the famous acceptable means for experiments of animals. Avoid any pain during operation by injection of Ketalar to avoid discomfort or agony. Our standards of animal care and administration meet those required by applicable usual laws.

### 2.5 Biochemical evaluation

#### *2.5.1 Tissue homogenization preparation*

Crush 0.5 g of tissue testes in nitrogen by blender. Homogeneity directly in phosphate buffer (pH 7.8) containing 1 ml EDTA. Then by centrifugation 20,000xg for 25 min in a cold environment, and storing of supernatant in a deep freezer till usage of measurements of (GPx), (CAT), (SOD) enzymes, and for measuring (GSH) and (MDA).

The enzyme activity assays for CAT, SOD, and GPX were done according to [4, 18, 26], respectively). MDA accumulation was measured in the tissue according to the method of Ohkawa et al. [33], while total GSH levels were determined according to [34].

#### 2.5.2 Enzyme activity assays

Testicular samples were dissected and put in Petri dishes, then washed with normal saline (0.9% NaCl). Part of these samples was taken for histopathological examination. The remaining part was rapidly washed in ice-cold 0.9% NaCl, and homogenized in nine volumes of buffer (0.1 mol/L phosphate buffer; pH 7.4), ground with liquid nitrogen in a mortar for determining the activities of enzyme activity assays.

#### 2.5.3 ELISA serum hormonal assays

Pre-stored frozen serum samples were used until thawed at room temperature. Then, it was centrifuged at 1000 rpm for 5 min. Then an ELISA reader (Rayto microplate RT 2100C) with wavelengths of 450 and 630 nm was used, the concentrations of testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) were measured [36].

#### 2.5.4 Histopathological examination

At the end of the experiment, the testicles of rats were dissected under general anesthetic, then cut into three samples, the first for histopathological examination, the second for transmission electron microscope examination, and the third for enzyme activity assays.

The first sample was fixed immediately in formalin 10%, then placed in paraffin blocks, then cut the blocks by microtome to small slices thickness 5 mm and placed in glass slides. Sections were stained with Hematoxylin and Eosin. Examination of sections was done with a light microscope [7].

#### 2.5.5 Immunohistochemistry staining, detection of P53 apoptotic markers

The samples were placed in paraffin blocks. The block was cut by microtome to small slices of thickness 5 mm and placed in glass slides. Dewax Sections by putting in xylol for 20 min, then hydrate in descending degrees of alcohol to distilled water. Immerse sections in citrate buffer (pH 6) at 95–99 °C then in a water bath for 40 min, then cool at room temperature. Rinse sections in phosphate-buffered saline (PBS), then stained with an expression of P53, to detect sections of the testis by using avidin Biotin Complex (ABC) method as mentioned by El-Masry et al. [19].

Transmission Electron Microscope (TEM) examination: Cut into small pieces 1 mm thick then fixe in phosphate buffer solution (pH 7.2) for 3 h at 4 °C, then fixed in buffered 2% osmium tetroxide for one hour at 4 °C, rinsed and dehydrated in a graded degree of ethanol, embedding in Epon, cutting in ultrathin sections (50 nm thick), then stained with uranyl acetate [16].

### 2.6 Analysis of statistics

It was done by the usage of SPSS version 16. The results were evaluated as mean + SD. The importance of variation between results was planned using one-way analysis of difference (ANOVA) test and followed by Tukey test.

## 3 Results

### 3.1 Biochemical findings

The findings of Table 1 show effects of the taking Bisphenol A itself or together with costus root extract on the weight of the body and testes. Mean ± SD indicated that the administration of Bisphenol A (third group) showed a significant decrease in the weight of the body and testes compared with the first and second groups. Administration of costus root extract with Bisphenol A (fourth group) prevented the reduction of body weight and testes weight when compared with the third group.

**Table 1** Showed the effect of the treatment with Bisphenol A itself or together with costus root extract on the weight of body and testis

| | First (control) group | Second group costus root extract seeds | Third group Bisphenol A | Fourth group Bisphenol A with costus root extract |
|---|---|---|---|---|
| Body weight | 287 ± 2.4 | 264 ± 1.9 | 183 ± 1.8* | 265 ± 3.1** |
| Testis weight | 2.72 ± 0.2 | 2.31 ± 0.3 | 1.89 ± 0.6* | 2.9 ± 0.2** |

The first group received distilled water daily for eight weeks

The second group was treated with Costus root extract at a dose (0.4 mg/kg) daily

The third group was treated with 10 mg/kg of Bisphenol A (BPA) (body weight /day) dissolved in 5 ml/kilogram of oil of olive

The fourth group received 10 mg/kg of Bisphenol A (bwt/day) Costus root extract at a dose (0.4 mg/kg) daily for eight weeks was given by gastric gavage daily

* $p < 0.05$ (significant difference in comparison with control group and second groups)

** $p < 0.05$ (significant difference in comparison with third group)

Data in Table 2 showed that administration of Bisphenol A in the third group caused a significant reduction in the antioxidant enzymes of activity of (CAT), (GPx), (SOD) and (GSH) while significantly increasing (MDA) and (NO) in tissues of testis in comparison with the first group. The treatment of costus root extract with Bisphenol A in the rat of the fourth group prevented the exhaustion of antioxidant enzymes; CAT, GPx, SOD, and GSH, and inhibition of rising of MDA and NO when compared with the third group.

### 3.2 Hormonal assay

The findings of Table 3 showed the effect of the administration with Bisphenol A itself or together with costus root extract on serum levels of testosterone, FSH, PRL, and LH. The mean ± SD data indicated that the administration of Bisphenol A (third group) showed a significant decrease in levels of testosterone in comparison with the control and second groups but no significant changes in FSH, PRL, or LH. as compared with the control and second groups, administration of costus root extract with Bisphenol A (fourth group) prevented the reduction of observed alterations in the serum levels of testosterone as compared with the third group.

Findings of Table 4 showed the effect of the administration with Bisphenol A itself or together with costus root extract on immunohistochemical staining for P53 indicated that the administration of Bisphenol A (third group) showed strong positive of immunohistochemical staining for P53 in comparison with the control and

**Table 2** Effect of the administration of Bisphenol A itself or together with costus root extract on antioxidant enzymes of testis CAT, GPx, SOD, GSH, MDA, and NO

| | First (control) group | Second group costus root extract | Third group Bisphenol A | Fourth group Bisphenol A with costus root extract |
|---|---|---|---|---|
| CAT | 08.01 ± 0.31 | 09.03 ± 0.83 | 03.8 ± 0.05* | 07.9 ± 0.02** |
| SOD | 08.03 ± 0.21 | 08.9 ± 0.11 | 04.5 ± 0.02* | 07.89 ± 0.23** |
| GPx | 71.18 ± 0.48 | 64.08 ± 0.03 | 52.81 ± 0.5* | 61.91 ± 0.4** |
| GSH | 23.03 ± 0.14 | 22.01 ± 0.01 | 10.19 ± 0.2* | 022.9 ± 0.1** |
| MDA | 14. 03 ± 0.39 | 13.9 ± 0.12 | 017.01 ± 0.92* | 12.1 ± 0.01** |
| NO | 99.09 ± 0.98 | 92.34 ± 0.43 | 110.2 ± 0.13* | 94.01 ± 0.02** |

The first group received distilled water daily for eight weeks

The second group was treated with Costus root extract at a dose (0.4 mg/kg) daily

The third group was treated with 10 mg/kg of Bisphenol A (BPA) (body weight /day) dissolved in 5 ml/kilogram of oil of olive

The fourth group received 10 mg/kg of Bisphenol A (bwt/day) Costus root extract at a dose (0.4 mg/kg) daily for eight weeks was given by gastric gavage daily

* $p < 0.05$ (significant difference in comparison with control group and second groups)

** $p < 0.05$ (significant difference in comparison with third group)

**Table 3** Effect of the treatment with Bisphenol A alone or together with costus root extract on levels of testosterone, FSH, PRL, and LH

| | First (control) group | Second group costus root extract | Third group Bisphenol A | Fourth group Bisphenol A with costus root extract |
|---|---|---|---|---|
| Testosterone | 4.01 ± 0.3 (nmol/L) | 4.13 ± 0.03 (nmol/L) | 2.2 ± 0.02* (nmol/L) | 3.9 ± 0.01** (nmol/L) |
| FSH | 018.03 ± 0.21 (IU/L) | 018.9 ± 0.11 (IU/L) | 017.1 ± 0.01 (IU/L) | 16.11 ± 0.01** (IU/L) |
| PRL | 16.18 ± 0.48 (mIU/L) | 16.08 ± 0.03 (mIU/L) | 15.91 ± 0.2 (mIU/L) | 14.91 ± 0.4** (mIU/L) |
| LH | 21.9 ± 0.14 (IU/L) | 22.01 ± 0.01 (IU/L) | 20.19 ± 0. 2 (IU/L) | 019.9 ± 0.1** (IU/L) |

The first group received distilled water daily for eight weeks

The second group was treated with Costus root extract at a dose (0.4 mg/kg) daily

The third group was treated with 10 mg/kg of Bisphenol A (BPA) (body weight /day) dissolved in 5 ml/kilogram of oil of olive

The fourth group received 10 mg/kg of Bisphenol A (bwt/day) Costus root extract at a dose (0.4 mg/kg) daily for eight weeks was given by gastric gavage daily

* $p < 0.05$ (significant difference in comparison with control group and second groups)

** $p < 0.05$ (significant difference in comparison with third group)

**Table 4** Effect of the treatment with Bisphenol A alone or together with costus root extract on immunohistochemical staining for P53

| | First (control) group | Second group costus root extract | Third group Bisphenol A | Fourth group Bisphenol A with costus root extract |
|---|---|---|---|---|
| P53 | + | + | +++++ | ++ |

The first group received distilled water daily for eight weeks

The second group was treated with Costus root extract at a dose (0.4 mg/kg) daily

The third group was treated with 10 mg/kg of Bisphenol A (BPA) (body weight / day) dissolved in 5 ml/kilogram of oil of olive

The fourth group received 10 mg/kg of Bisphenol A (bwt/day) Costus root extract at a dose (0.4 mg/kg) daily for eight weeks was given by gastric gavage daily

(+ staining) Faint staining of immunohistochemical staining for P53 was observed with the control and second groups

(+ staining) Moderate reduction of expression of immunohistochemical staining for P53 was observed with the fourth group compared to the third group

(+++++ staining) Strong positive of immunohistochemical staining for P53 in comparison with the control and second groups

second groups, while little or no staining was observed with the control and second groups of immunohistochemical staining for P53. Administration of costus root extract with Bisphenol A (fourth group) showed a reduction in expression of immunohistochemical staining for P53 (++ staining). There was no difference in immunohistochemical staining for P53. In the costus root extract group compared to the control group.

### 3.3 Histopathological results findings by light microscope

The light microscopic observations of the present study of first (control) and second groups showed seminiferous tubule were normal structure, covered by basement membrane containing myoid cells, the normal structure of spermatogonia appeared as small rounded cells with rounded nuclei, successive layers of spermatogenesis as both primary spermatocytes appeared as large cell with large rounded nuclei and secondary spermatocytes, normal spermatids appeared as small, rounded cells with pale nuclei, normal sperms in the lumen and normal Sertoli cells had triangular cell and triangular nuclei inside acidophilic cytoplasm and attached to the basement membrane in between the layers of spermatogonia and spermatocytes, normal blood vessels and interstitial Interstitial cells present between the tubules (Fig. 1a, b).

*Light microscopic observations third group (BPA treated group)*: The testes showed degeneration and disorganization of the basement membrane of seminiferous tubules surrounded by degenerated myoid cells and separated by vacuolated interstitium and congestion of dilating blood vessels. Also, wide spaces among the deteriorating germinal epithelium, replaced by wide spaces within the seminiferous tubules, damaged sperm with vacuoles, degeneration of both primary and secondary spermatocytes as well as a few debris of sperm in the centers of the seminiferous tubules and degenerated Sertoli cells with pyknotic nuclei. Multi-forms of spermatids, abnormal blood vessels, and degenerated interstitial cells between the tubules (Fig. 1c).

*Light microscopic observations of the fourth group (BPA and costus-treated group)*: The testes showed marked improvement in the seminiferous tubules; its basement membrane is contained myoid cells, nearly normal stages of spermatogonia as primary, secondary spermatocytes, spermatids, and sustentacular cells (Fig. 1d).

### 3.4 Immunohistochemical staining for P53

As shown in Fig. 2a and b, testicular tissues from control and costus-treated group rats stained, little or no staining was observed for P53. In contrast, in Fig. 2c testicular tissues from rats treated with Bisphenol A (BPA) alone stained strongly with P53. This was reversed in the BPA and costus-treated group where there was reduced expression of P53 as in Fig. 2d. There was no difference in the expression of P53 in the costus-treated group compared to the control group.

### 3.5 Ultrastructural study

Testes of the first (control) and second groups showed the basement membrane formed of sustentacular cells and normal stages of spermatogonia. Spermatogonia has oval heterochromatin nuclei. Primary spermatocytes revealed rounded to oval nuclei and spermatids were rounded shapes that had acrosomal caps (Fig. 3c, d). The sertoli cell appeared as a large pyramidal cell having a large serrated nucleus with a prominent nucleolus (Fig. 3a–c).

The third group (the BPA-treated group) showed damaging effects of Bisphenol A BPA on the cells. The cytoplasm of stages of spermatogonia cells and Sertoli cells have degenerated with intracellular vacuoles and electron-dense bodies; cell debris, swollen mitochondria; and other spermatogonia cells were shrunken and separated by spaces due to degenerated neighboring cells. Some vacuolated cytoplasm of degenerated primary spermatocytes and spermatids (Fig. 3d). The cytoplasm primary spermatocytes and spermatids showed vacuoles, excessive lipid droplets, irregular nuclear membranes, and shrunken pyknotic nuclei (Fig. 4a).

The testis of rats of the fourth group (BPA and costus treated group, showed marked improvement of spermatogonia, normal spermatids also primary spermatocytes and sperms appeared nearly normal (Fig. 4b,c).

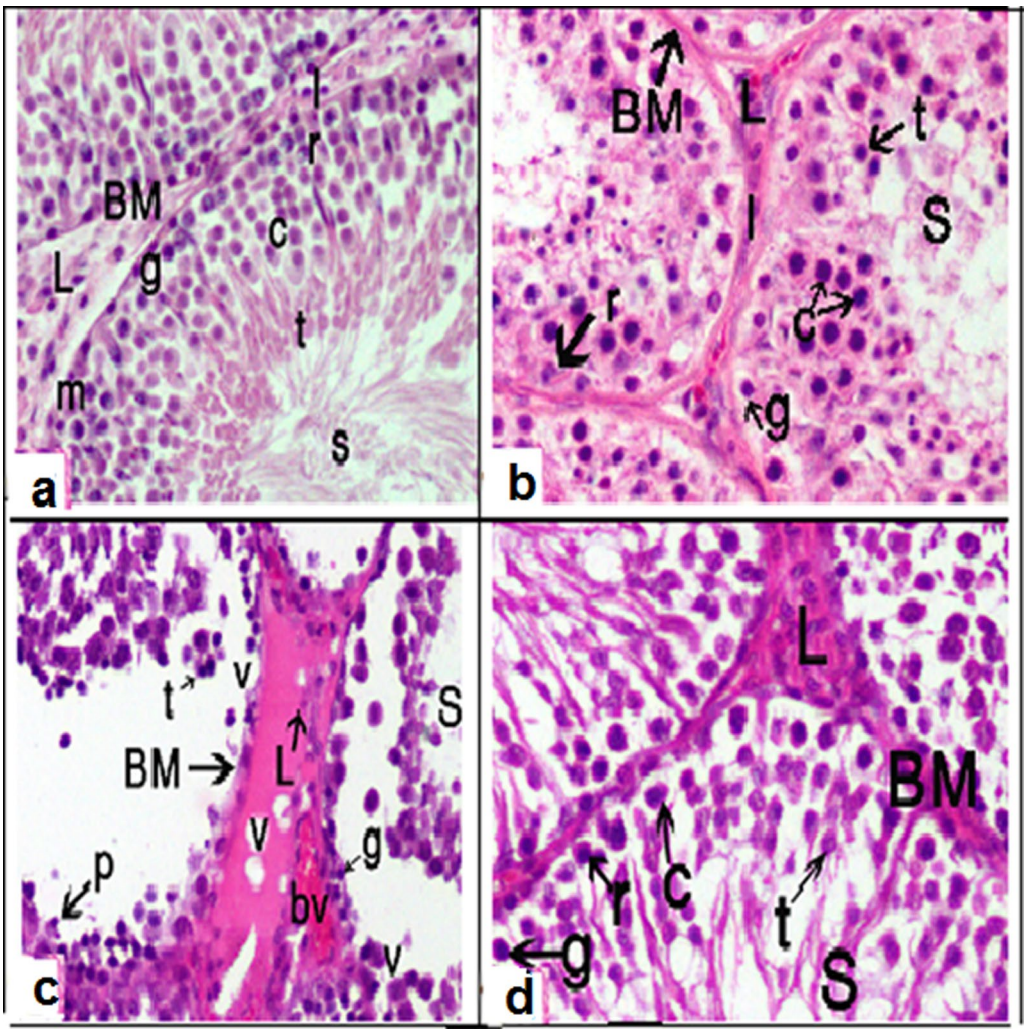


**Fig. 1** **a** Light micrographs (LM) a transverse section of a control group of rats of testis showing seminiferous tubules lined with basement membrane (BM) had myoid cells (m), interstitial tissue (l) among the tubules, spermatogonia appeared as small, rounded cells (g), primary spermatocyte as large, rounded nuclei (c). Spermatids as small, rounded cells with pale nuclei (t); Sertoli cells have triangular nuclei attached to the basement membrane (r), sperms (S) in the center, and Leydig cells (L). (Hematoxylin and Eosin × 400). **b** Light micrographs (LM) a transverse section of second groups of rats of testis showing seminiferous tubules lined with basement membrane (BM) had myoid cells (m), interstitial tissue (l) among the tubules, spermatogonia appeared as small, rounded cells (g), primary spermatocyte as large, rounded nuclei (c). Spermatids as small, rounded cells with pale nuclei (t). Sertoli cells had triangular nuclei attached to the basement membrane (r), sperms (S) in the center, and Leydig cells (L). (Hematoxylin and Eosin × 400). **c** Light micrographs (LM) of a transverse section testis of rat third group showed well-defined disorganization with necrosis, vacuoles (v) of germ cells (g), degenerated Leydig cells (L), and degenerated basement membrane (BM), congested blood vessels (bv). (Hematoxylin and Eosin × 400). **d** Light micrographs (LM) of the transverse section of testis of the fourth group showed the normal structure of spermatogonia cells (g) with normal basement membrane (BM); Sertoli cells (r) and Leydig cells (L) normal spermatocytes c), spermatid (t) and sperms (S). (Hematoxylin and Eosin × 400)

## 4 Discussion

Bisphenol A is a chemical product as epoxy and polycarbonate resins that has a marked endocrine disrupting effect and environment due to its wide use in many fields and has an anti-androgen effect [28] and is prevalent in plastic used in food and beverage packaging such as bottles and water pipes [32]. Our work is the first work that revealed the improved effects of costus versus, the toxicity of Bisphenol A on the testis of the rat.

The present study showed that Bisphenol-A- reduced body weight and testes weight these reports parallel with reports by Samuel et al. [36] who postulate that treatment with BPA significantly reduction in the testes and body weights of the rats in comparison to the first group Anthonet and Orish [10]. He added that the cause

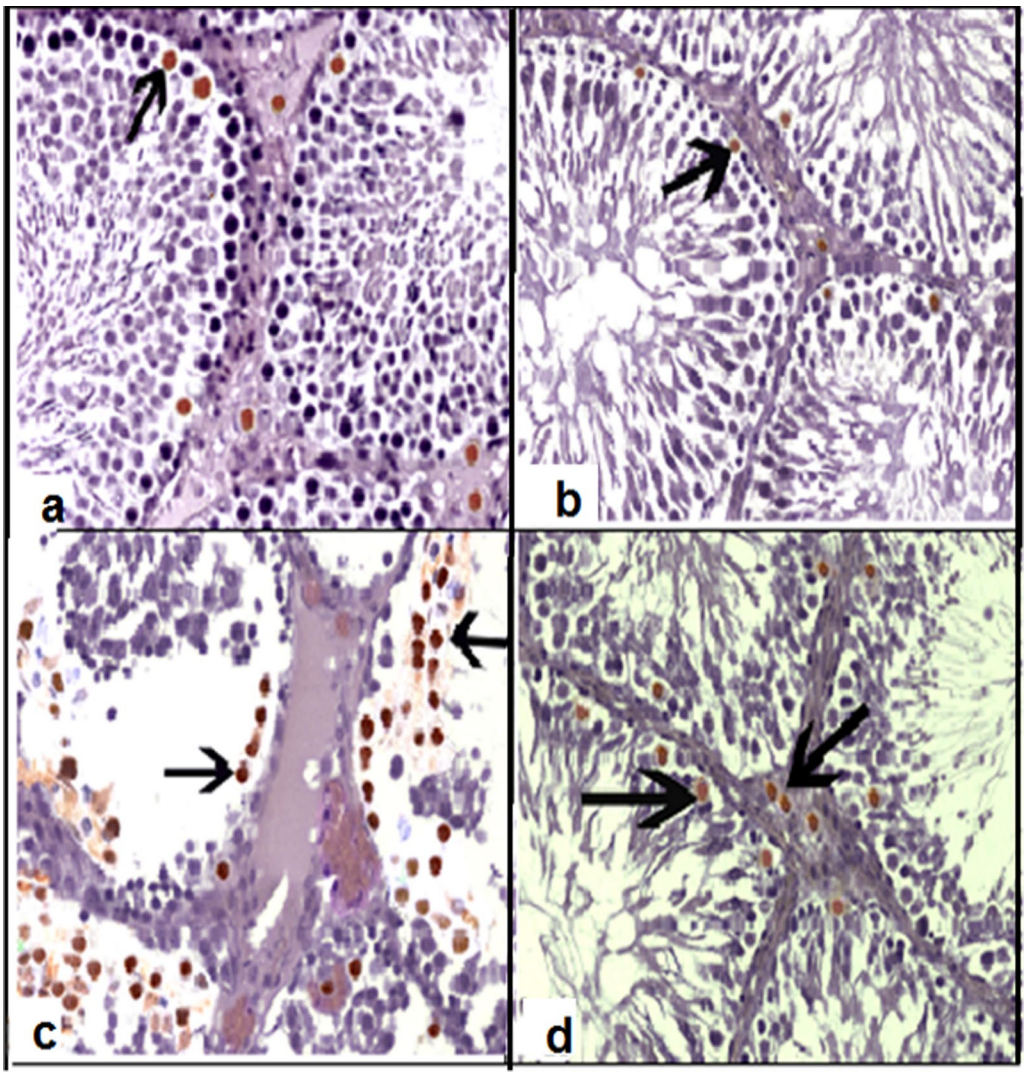


**Fig. 2** **a** Light micrographs (LM) of a transverse section of the control group stained with P53-ir showed a faint positive affinity for P53 (arrows) (P53 X400). **b** Light micrographs (LM) of a transverse section of the costus second group stained with P53-it showed a faint positive affinity for P53 (arrows) (P53 X400). **c** Light micrographs (LM) of a transverse section of testis of rat (third group) stained with P53-ir showed a strongly positive affinity for P53

of reduction in male albino Wistar rats may be due to reduced tubule size, a decrease in the number of germ cells, and degenerated spermatids after reproductive toxicity of lead. In addition to that Abdel-Halim et al. [2] showed that reduction may be due to a decrease in serum testosterone levels or may be due to decreased number of germ cells; suppression of spermatogonia cells also counts activity of the steroidogenic enzyme; these results were parallel with our results. Also, our present study showed that Costus had a remarkable ability to ameliorate the Bisphenol-A-induced decrease in body and testis weights, so Costus protects against the toxicity of Bisphenol A on the testis. This is consistent with reports by Khattab and Mansoury [30] who postulated that Costus afer leaf extract had a protective effect against testicular toxicity associated with cyclosporine, also our results were in coincidence with Abd El-Rahman et al. [1] who stated that administration S. lappa extracts significantly opposite TA-weight loss effects., they also added that this refinement may be due to improved function immunity and activities of antioxidant enzymes in rats. Improvement could be the consequence of the improvement of immune functions and antioxidant activities in rats.

Also, the present study, showed that Bisphenol A induced significant decreases of activity of antioxidant enzymes: (CAT), (GPx), (SOD) and (GSH), in the third

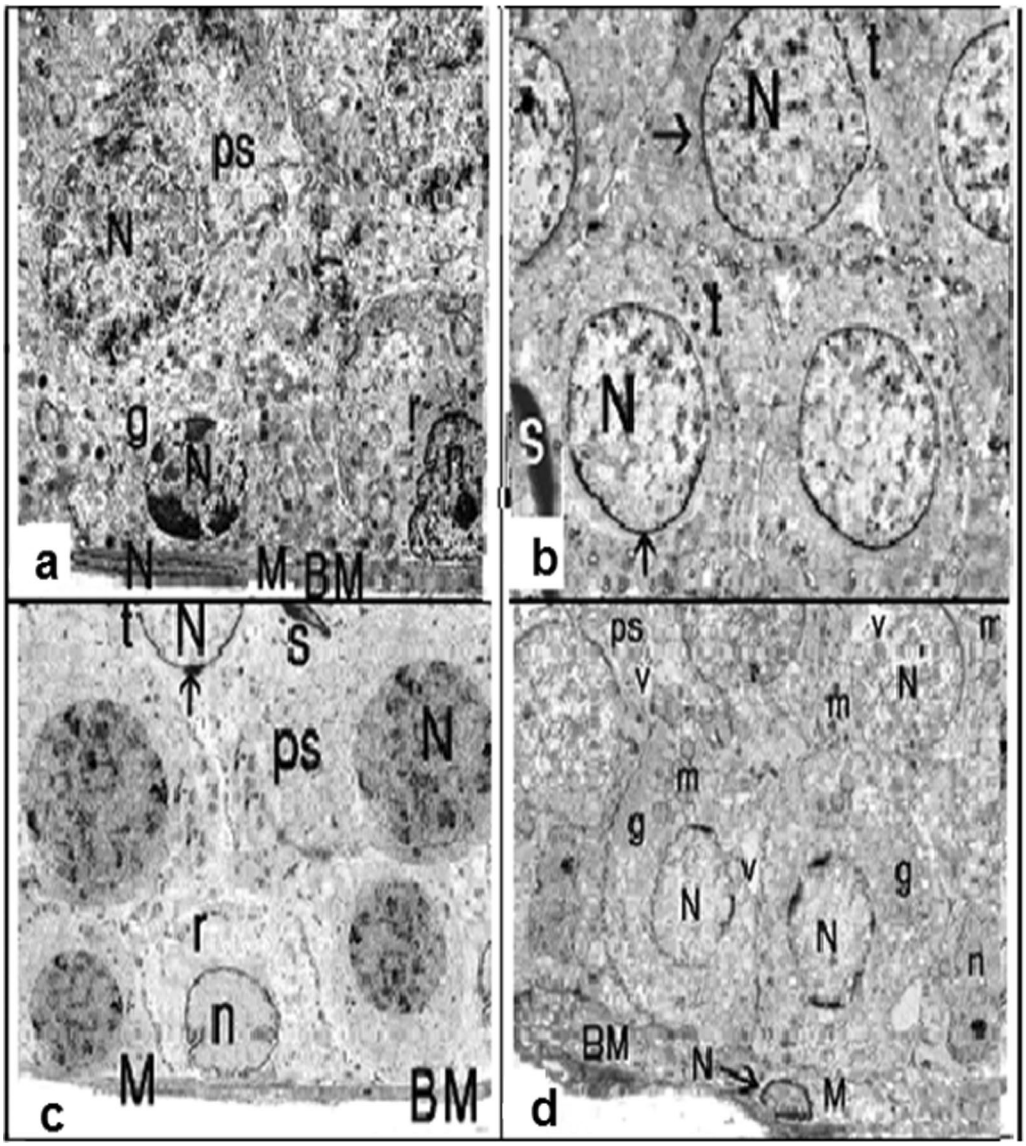


**Fig. 3** **a** Transmission electron micrograph of sections testis of rats of a control group. showing: spermatogonia (g) with its small nucleus (N), primary spermatocyte cell (ps) with its large nucleus (N); Sertoli cell (r) with its dentate nucleus (n), rest on regular basement membrane (BM) with flat myoid cell (M) nucleus (N) (X 5000). **b** Transmission electron micrograph of sections testis of rats of a control group. showing: a group of spermatids (t) with a rounded nucleus (N) and acrosomal cap (arrow) and sperm (s) X15000. **c** Transmission electron micrograph of sections testis of rats of second groups. showing: spermatogonia (g) with its small nucleus (N), primary spermatocyte cell (ps) with its large nucleus (N), Sertoli cell (r) with its dentate nucleus (n), rest on regular basement membrane (BM) with flat myoid cell (M) nucleus (N), spermatids (t) with rounded nucleus (N) and acrosomal cap (arrow) and sperm (s) (X 5000). **d** Transmission electron micrographs of the third group rat testis showed irregular thick basement membrane (BM) with nuclear atrophy (n) of a myoid cell (M), vacuoles of cytoplasm (v) of degenerated spermatogonia (g) with its degenerated small nucleus (N), also damaged primary spermatocytes (ps), also vacuoles of cytoplasm (V), degenerated mitochondria (m), and endoplasmic reticulum. Also, damage of nuclear membrane (N), atrophy of Sertoli cell (r) with its atrophy nucleus (n) (X 5000)

group while significantly increased in the levels of Reactive Oxygen Species (ROS) as (MDA) and (NO) in tissues of testis of rat when compared with the first group. These results were parallel to the results of Samuel et al. [36]. Who stated that Bisphenol A caused a noticeable decrease of antioxidant enzymes with an increase in the levels of reactive oxygen species (ROS) they also added that these reductions of antioxidant enzymes may be due to enhanced utilization of GSH for detoxification of Bisphenol A induced free radicals or may be due to SOD converted the superoxide anion radicals into $H_2O_2$, which thereafter accumulated in the testis which inhibits CAT

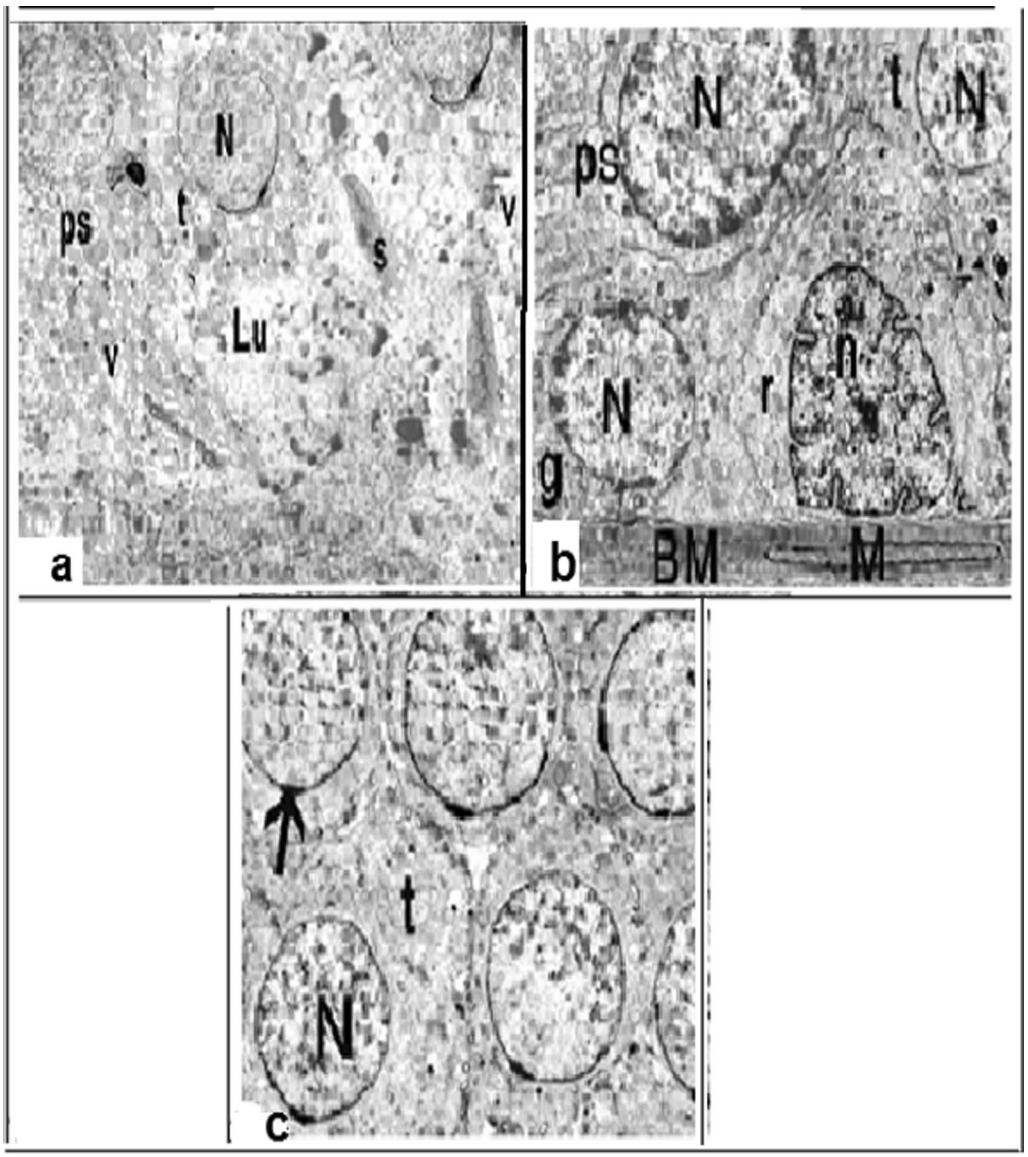


**Fig. 4** **a** Transmission electron micrographs of the third group rat testis showed damaged primary spermatocytes (ps) with cytoplasmic vacuolation, degenerated mitochondria, dilation endoplasmic reticulum, vacuoles of cytoplasm (v) of spermatid with irregular acrosomal cap (t), and irregular nuclear membrane (N) close to the lumen (Lu) (X4000). **b** Transmission electron micrograph of sections testis of rats of the fourth group: showing: spermatogonia (g) with its small nucleus (N), primary spermatocyte cell (ps) with its large nucleus (N); Sertoli cell (r) with its dentate nucleus (n), rest on regular basement membrane (BM) with flat myoid cell (M) nucleus (N) (X5000). **c** Transmission electron micrograph of sections testis of rats of the fourth group showing: a group of spermatids (t) with rounded nucleus (N) and acrosomal cap (arrow) and sperm (s) X15000

activity. Also, antioxidant enzymes can become inhibited with an elevation of lipid peroxidation as MDA Increase in reactive oxygen species (ROS) as MDA levels of the testis may cause a decrease in motility of sperm and destruction of the membrane of spermatozoa [24]. Apaydin et al. [12] added that **e**nzymatic antioxidants (SOD, CAT, GPx, and GST) are useful in defending against the injury of cells and protecting cells and organs from the unfavorable effects of ROS by catalyzing the toxic $H_2O_2$ to water and oxygen. Also, these may cause the failure of the formation of sperm and steroid hormones, and hence, male sterility with infertility of males.

The present study deduced that Bisphenol-A caused toxicity on the male reproductive system in adult rats; these results were parallel to Kazemi et al. [29]. Who stated that Bisphenol A caused toxicity on the male reproductive system in adult rats due to elevation of ROS levels leads to oxidative stress, which also increases

disorder caused by free radicals of oxygen and antioxidant enzymes of the cell [23].

Co-administration of costus in the present study is associated with a deficiency of MDA and H2O2 and a rise in the action of CAT, SOD, and GPx. These results were parallel with findings from Anthonet and Orish [10], who reports that these results were due to an inhibition of GPx1 gene code, and GPx4, associated with disturbance in the oxidation of protein; they also added that costus root extract showed obvious changes in oxidative stress of testis, hormonal, sperm analysis and histopathological changes caused by lead.

The present study showed that administration of Bisphenol A (third group) showed a significant reduction in the serum levels of testosterone, but no significant changes in FSH, PRL, and LH. as compared with the control and second groups, these findings are in coincidence with the results of Gonçalves et al. [20], who stated that the Bisphenol A is harm to TM3 cells of Leydig and damage their steroid action Also, our results were in coincidence with the results of Sohrab et al. [37], who reported that the decrease in the level of testosterone was most probably due to a deterioration in the activity of the Leydig and Sertoli cells; these results correspond with our ultrastructural results.

Kamel et al. [28] postulated that reduction of serum levels of testosterone is an indicator of chemical toxicity on the reproductive system,see also Samuel et al. [36] added that decreased levels of testosterone caused the failure of spermatogenesis and destruction of seminiferous epithelium, Leydig cell, and Sertoli cells, which is correlated with the histological and ultrastructural results of the present study.

Administration of costus root extract with Bisphenol A (fourth group) prevented the reduction of observed alterations in the serum levels of testosterone when compared with the third group; these findings were coincidence with the results of Anthonet and Orish [10], who postulated that costus Afer had a protective effect by elevating levels of plasma testosterone, in the treated groups to near normal.

### 4.1 Histopathology

The present work showed that the histological structure of control and second groups of rat testes are like normal testes of other mammals; these results were parallel to the results of Anthony [11].

The light microscope of the present study of the third group treated with Bisphenol A showed degeneration of seminiferous tubule, spermatogonia with vacuoles of sustentacular cells, degeneration in spermatocytes, spermatids, and degenerated interstitial cells, these were in correspondence with other studies reported by Samuel et al. [36]. Who reported that Bisphenol A caused degeneration of seminiferous tubules, and spermatocytes, also parallel with the results of Anthonet and Orish [10]. Who reported that Bisphenol A caused degeneration of spermatogonia with vacuoles of sustentacular cells [25]. Added that Bisphenol-A inhibits the growth of spermatogonia cells, developing Leydig cells, and steroidogenesis.

Co-administration of costus with Bisphenol A in the fourth group showed marked improvement in the testis of rats; these findings were parallel with the results of Domiaty et al. [17]. Who stated that the treatment with Costus extract and risperidone together showed an effective role in the improvement of damage caused improvement the pathological and anatomical changes they also added improvement of costus may be due to presence of flavonoids in the roots of Costus extract which has beneficial effects in certain diseases such as cancer and cardiovascular, neurological disorders.

Our results were parallel with the results of Anthonet and Orish [10]. Who reported that treatment with Costus afer showed marked improvement of histopathological changes induced by lead, they also added that extract of leaves of Costus had conservative effect versus damage of testis caused by lead.

Our data in Table 4 also showed that Bisphenol A caused a marked increase in the levels of the proapoptotic protein P53. Immunohistochemical staining has also revealed intense staining of P53; these results were in coincidence with the results of Yuan et al. [31]. Who reported that Bisphenol-A treatment caused an increase in p53 apoptotic cells in testicular tissues of the rat.

So, the rise of p53 in the present work detects the prospect of the occurrence of apoptosis after Bisphenol A. treatment.

Co-administration of costus with Bisphenol A in the fourth group caused marked improvement and decrease in the levels of proapoptotic proteins P53 these findings were parallel with the results of Gules et al. [21]. Who stated that **c**ostus extract contains flavonoids that improve the reduction of oxidative stress, which has emerged as a cell protective agent when exposed to activity scavenging of free radicals that cause damage to cell structures, leading to inhibiting DNA damage. So costus appeared to have anti-apoptotic properties.

### 4.2 Ultrastructural study

The ultrastructural examination of the present study showed that it showed enlarged vacuoles, swollen mitochondria, cell debris, and cytoplasmic vacuoles of Sertoli cells; these findings were parallel with the results of Gurmeet et al. [22]. The current study showed swollen degeneration of spermatogonia cells, primary spermatocytes with excessive lipid droplets, irregular nuclear

membrane, and pyknotic nuclei; these results were parallel with the findings of Toyama et al. [39]. Who stated that Bisphenol A caused several degenerative effects on spermatogonia like the disappearance of the nuclear envelope and numerous apoptotic changes. Our ultrastructural results also revealed Bisphenol A caused damages of spermatogonia cells and spermatocytes as condensation of chromatin material, the disappearance of the nuclear envelope, and numerous apoptotic changes; these results were parallel with the findings of Tushara et al. [40]. Who postulated that Bisphenol-A caused ultrastructural several degenerative effects in the form of the presence of vacuoles in mitochondria of spermatids, Sertoli, Leydig, and peritubular myoid cells as compared to control.

In addition to that Zaki et al. [42]. Reported that an increase of vacuoles in the cytoplasm of the spermatogonia and sustentacular cells might be caused by swelling of the smooth endoplasmic reticulum that demonstrates changes in the permeability of cells in addition to that the ultrastructural changes may be due to bisphenol A had apoptosis in various spermatogonia and destruction of basement membrane which has the main function in the integrity of testicular tissues.

The testis of rats of the fourth group Bisphenol A and costus-treated group, showed marked improvement in spermatogonia. Normal spermatids, also primary spermatocytes and sperms appeared nearly normal as condensation of chromatin material. These results were parallel with the findings of Domiaty et al. [17]. Who postulated that ultrastructural changes of testis of treated rats with risperidone and costus fourth group identified that developmental stages of spermatogenic epithelium including spermatogonia, normal spermatids, primary spermatocytes, and sperms were detected also Sertoli cells appeared as elongated cells with its giant nuclei, prominent nucleoli, ER and mitochondria. The intertubular space was full of a moderate number of Leydig cells; they also added that treatment with Costus inhibited the histopathological alterations induced by risperidone within the testis.

## 5 Conclusions

From the present study, we could conclude that bisphenol A on the rat testis has been demonstrated induced reproductive toxicity in an adult male rat in the form of histological, biochemical, and ultrastructural alterations of testis observed in testicular morphology may be indicative of disturbance of sperm production and functioning. These effects might be the result of direct toxic effects on related components and/or indirect effects mediated by dysregulation of bioassay parameters. The oral use of costus has a beneficial protective effect against the histopathological, biochemical, and ultrastructural alterations in testis induced by bisphenol-A. Given the genetic and spermatogenesis similarity between rats and humans, this study will be useful in correlating adverse effects in humans.

#### Abbreviations

BPA: Bisphenol A; TST: Testosterone; ROS: Reactive oxygen species; EEC: European community directive; NIH: National Institutes of Health; EDTA: Ethylenediaminetetraacetic acid; GPx: Glutathione peroxidase; GSH: Glutathione; CAT: Catalase; SOD: Superoxide dismutase; NO: Nitric oxide; MDA: Malondialdehyde; NaCl: Sodium chloride; PH: Potential of hydrogen; ELISA: Enzyme-linked immunosorbent assay; T: Testosterone; LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; PRL: Prolactin; ABC: Avidin biotin complex; SPSS: Statistical package for the social sciences; SD: Standard deviation; BM: Basement membrane; LM: Light micrographs.

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#### Authors' contributions

All authors have participated equally in this research, and took the responsibility for the decision to submit for publication. All authors read and approved the final manuscript.

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#### Availability of data and materials

Not applicable.

### Declarations

#### Ethics approval and consent to participate

In this study, animal care was carried out by following the European Community Directive (86/609/EEC) and national rules, this by the 8th edition of the NIH Guidelines for the care and use of Laboratory Animals.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing of interests.

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