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Bisphenol a Hormonal Disrupture and Preventive Effect of Rose Water and Clove Oil

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Abstract: Bisphenol A (BPA) which considered synthetic estrogen that is an essential component of many plastic industries. This research was done to see the impacts of exposure of BPA on reproductive organs and hormonal levels in male and female albino Sprague-Dawley rats. The protective effect of rose water and clove oil on BPA was investigated. Ninety rats were divided into 18 groups, 9 groups of males and they are like for females. Rats were exposed to different oral gavage route 3 times a week by doses of BPA (20 µg, 20 mg, 200 mg) /kg b.wt for 6 weeks and BPA was solubilized in corn oil. BPA induced a significant decrease in total and free testosterone in male rats, in contrast to a significant increase in thyroid-stimulating hormone (TSH), progesterone, estrogen (E2), and prolactin (PRL), while a decrease in Follicle-stimulating hormone (FSH) compared to control groups. Histopathological examination revealed that rosewater and clove oil reduced testes and ovary damages induced by BPA. Rosewater and clove oil components were scanned using GC/MS, which showed that rosewater and clove oil contains phenols, flavonoids, and these inevitably confirm that a prominent role in preventing the damage during treatment. Results indicated that the used doses of BPA disrupted the sex hormone levels in both male and female rats caused reproductive impaired. The chemical and histopathological analysis results indicated that clove oil and rose water improved the adverse effect of BPA. Rosewater and clove oil improved the changes which were stimulated by BPA.

Keywords: Bisphenol A; Clove oil; Rose water; Ovary; Testes; Sex hormones; TSH.

1. Introduction

Bisphenol A (BPA), which is considered as a synthetic environmental chemical contaminant, is utilized in the manufacture of epoxy resins, polycarbonate plastics for paper products, a reagent in polymer reactions and food containers [1-3]. BPA is a synthetic chemical compound applied in industry worldwide market almost 15 billion pounds (lbs) in 2013 and is expected to increase in 2020 by nearly 4.7% and may rise to (10.2 metric tons in 2022) [4, 5].

People exposure to BPA during many ways such as inhalation, dermal routes, but food the first route to humane exposure to BPA, particularly solid fractions, canned foods, beverage containers, drink cans, cereals, vegetables, and thermal paper [6-8] . Likewise, BPA has been found in a major scale in human Hygiene products like face cleanse, shampoos, body shower, and dental materials [9, 10].

BPA can be leached from food and other sources to interfere and accumulate in the human body. Thus BPA can appear in blood serum, skin, saliva, urine, and head hair [11-13]. The highest level of BPA found in the placenta and fetus was (1–104 ng/g of tissue).

Many *in vivo* and *in vitro* experiments have been shown that BPA is a xenoestrogen act as an endocrine disorder compound. Therefore BPA binding to estrogen receptors and contribute expression of genes in many cells, besides induce oxidative damage in many tissues such as ovary, epididymis, vagina, thyroid gland, brain, and caused obesity [11, 14].

Various studies have demonstrated that lower doses of BPA cause a cytotoxic and genotoxic toxic effect [15]. BPA has been linked with various illnesses, for example, hormone homeostasis, liver damage, miscarriage, breast, and prostate cancer [16, 17]. BPA can be bioaccumulation in lipophilic tissues and do unsafe effects on the immunological system, inhibit fatty acid β-oxidation, and neurodevelopment of child and animal [18-20].

In Egypt Osman *et al*. [21] estimated BPA levels to be range between (710.59 -5.75 ppb) in canned foods (233.65 to 933.75 ppb) in feed additives. The leaching of BPA from baby bottles to milk recorded mean levels (89.35 -123.53 ppb).

Plant medicine is utilized on an extensive scale because of the lower cost, less reaction, viability contrast with drugs, contain many phytochemicals, and antioxidants inhibition potential [22,23]. Quercetin is an effective antioxidant flavonoid that mitigate the toxic effect of BPA [24].

Numerous products are obtained from rose damask like rosewater and rose oil [25]. Rosewater is applied in perfumes, cosmetics, a preventing agent of various diseases and food flavors [26]. Rosa species are rated antimicrobial, antioxidant, and hypolipidemic properties [27]. Rose damask flower contents phenolic compounds like anthocyanin, cyanidin 3, 5 diglycoside, kaempferol, quercetin, vitamin C, terpenoids, and essential oil as citronellal, linalool, geraniol [28].

Pharmacological screening has stated that clove essential oil has antioxidant activity, superoxide anion radical scavenging, and decreases lipid oxidation in food, antifungal, and antimicrobial properties [29].

The current study planned to assess the hormonal disturbance impacts of various concentrations of BPA on thyroid-stimulating hormone, Follicle-stimulating hormone, estrogen, progesterone, and prolactin in female rats, total and free testosterone in male rats and histopathology examination. It was then estimating the preventives effect of rose water and clove oil as of the adverse effects of BPA.

2. Materials and Methods

2.1. Materials.

Bisphenol A (BPA, Purity 99%) was purchased from Sigma-Aldrich Chemical Co. Appropriate amounts of BPA were mixed with corn oil to achieve the desired concentrations. Rosewater from the local market, Clove oil from Al - Jabri Herbal Medical Company, Tween 20 (Sigma-Aldrich) for solubilizing clove oil in water.

2.2. Experimental animals.

Mature male (45) and mature female (45) Albino Sprague-Dawley rats with a mean weight 120 ± 0.53 g were purchased from the animal house of the Egyptian Organization of Biological products and vaccines, Cairo, Egypt. The rats were breeding in the animal home of

Biology Laboratory, Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt. The rats were split to 18 groups, 9 groups for males and 9 groups for females (five rats each group) and lived in cages made from stainless steel and preserved at (25 ± 2 °C), light /dark cycle (twelve/twelve h) and relative humidity of (50-60%) pending the experiment for a ten-day before the beginning of the experiment. BPA was administered by oral gavage route 3 times/week for six weeks.

This experiment was confirmed by the Committee of Ethics in Animal Experiments of the Institutional Animal Care and Use Committee (CU- IACUC), Cairo University. The approval number CU-II- F- 45- 18.

2.3. Animal diet.

The basal diet was designed, according to Reeves *et al*. [30] A.I.N. 93G. Basal diet consist of (g/ kg): casein, corn starch and dextrin (200, 400,132) respectively; sucrose 100; corn oil 70; cellulose 50; mineral mixture, vitamin mixture (35: 10); l. DL-methionine 3; choline chloride 2.5 and tert-butyl hydroxy quinone 0.008. Rosewater and clove oil treatment were given in drinking water. Clove oil dissolved (0.1 ml/ 1 L of water) in Tween 20 and solubilized in water. The rosewater was prepared by adding (0.1 ml from rose oil / 1 L of water) in Tween 20 and solubilized in water.

2.4. Design of the biological experiment.

2.4.1. Male rats groups.

G1: (control) rats were fed on basal diet + drank normal water. G2: (rose water control) rats were fed on basal diet and drank rose water. G3: (clove oil control) rats were fed on basal diet and drank clove oil dissolved in water. G4: (tween 20 control) rats were fed on basal diet and drank tween 20 dissolved in water. G5: (20 µg/kg b.wt. from BPA) rats were fed on a basal diet, drank normal water and ingested orally with BPA (20 µg/kg b.wt.). G6: (20 mg/kg b.wt. from BPA) rats were fed on a basal diet, drank normal water and ingested orally with BPA (20 mg/kg b.wt.). G7: (200 mg/kg b.wt. from BPA) rats were fed on a basal diet, drank normal water and ingested orally with BPA (200 mg/kg b.wt.). G8: (200 mg/kg b.wt. BPA+ Rosewater) rats were fed on a basal diet, drank rose water and ingested orally with BPA (200 mg/kg b.wt.). G9: (200 mg/kg b.wt. BPA+ clove oil) rats were fed on a basal diet, drank clove oil dissolved in water and ingested orally with BPA (200 mg/kg b.wt.).

2.4.2. Female rats groups.

G10: (control) rats were fed on basal diet + drank normal water. G11: (rose water control) rats were fed on basal diet and drank rose water. G12: (clove oil control) rats were fed on basal diet and drank clove oil dissolved in water. G13: (tween 20 control) rats were fed on basal diet and drank tween 20 dissolved in water. G14: (20 µg/kg b.wt. from BPA) rats were fed on a basal diet, drank normal water and ingested orally with BPA (20 µg/kg b.wt.). G15: (20 mg/kg b.wt. from BPA) rats were fed on a basal diet, drank normal water and ingested orally with BPA (20 mg/kg b.wt.). G16: (200 mg/kg b.wt. from BPA) rats were fed on a basal diet, drank normal water and ingested orally with BPA (200 mg/kg b.wt.). G17: (200 mg/kg b.wt. BPA +Rosewater) rats were fed on a basal diet, drank rosewater, and ingested orally with

BPA (200 mg/kg b.wt.). G18: (200 mg/kg b.wt. BPA + clove oil) rats were fed on a basal diet, drank clove oil dissolved in water and ingested orally with (200 mg/kg b.wt. of BPA).

Each group of rats has collected 5 blood sample rats of each group from eye plexus after six weeks in clean dry sterile and labeled centrifuge tubes. Rats were slightly anesthetized by sodium pentobarbital 40 – 50 mg/kg I.P. Separating serum was done by centrifugation at 1500 r.p.m. for 5 min. Testes of rats were weighed and immersed in a 10% formalin solution for the histopathological examination.

2.5. Biochemical analysis.

2.5.1. Hormonal analysis.

Estimation of hormones in the serum of male and/or female rats was carried out using different methods. Total and free Testosterone [31], Serum thyroid-stimulating hormone (TSH) [32], follicles stimulating hormone (FSH) [33], estrogen (E2) [34], progesterone [35] and prolactin [36], were determined by Siemens ADVIA Centaur CP Immunoassay System. All kits used for hormone assay were (Sigma-Aldrich).

2.5.2. Determination of total antioxidant activity test (TAA).

The phosphomolybdenum method was used for determining TAA of rose water and clove oil, according to Prieto *et al*. [37]. Each sample solution (0.1 mL, 0.5 mg/mL) was combined with 0.3 mL of reagent solution sulfuric acid, sodium phosphate and ammonium molybdate (0.6 mol/L, 28 mmol/L, and 4 mmol/L respectively). The mixture was incubated at (95℃, 90 min). Next, the mixture had cooled down to room temperature; the absorbance of the mixture was measured at (695 nm) versus a blank utilizing a spectrophotometer (UVD-3500). The antioxidant activity was expressed as ascorbic acid (l mol/g). Ascorbic acid (0.5 mg/mL) was used as reference compounds. All tests were repeated 3 times, and means were calculated. TAA of the tested samples was determined utilizing the calibration equation (Absorbance = 0.0049 x concentration + 0.0409; R^2 = 0.998). TAA of rosewater and clove oil (l mol of ascorbic acid/g extract) were taken.

2.6. Rosewater and clove oil preparation for GC MS.

Rosewater and clove oil 0.1 mL were taken from each solution, diluted with 1.5 mL of ethanol, and with the assistance of magnetic stirrer 5 min. The extraction was filtered through a 0.45 μm membrane filter. 1 μL of the subsequent filtrate was injected into GC/ MS for analysis.

2.6.1. GC/MS analysis program.

The analysis of rose water and clove oil was carried utilizing GC/MS. The helium was a transporter gas, and the linear velocity was (1ml/min). The temperature of the oven was started at (55 ^{o}C for 3 min) and next programmed pending 280 ^{o}C at a rate of (11 ^{o}C/min). The injector was (220 ^{o}C), and detector temperatures were (220 ^{o}C). The injection mode (splitless) and volume injected (1 µl). The Mass operating parameters were as follows: ionization potential and interface temperature (70 eV, 280 ^{o}C), respectively. Selected ion monitoring (Scan mode) was applied used (m/z) at start mass and end mass (35 to 600). The identification of components depended on a comparison of their mass spectra and those of the authentic

compounds by computer matching with (NIST and WILEY) library as well as by comparing the fragmentation pattern of the mass spectral data with those reported in the literature review [38].

2.7. Histopathological examination.

Necropsy samples were taken after careful anatomy examination from the testes and ovaries of rats of each group and set in buffered formalin (10%, 24 h) and treated for paraffin wax set with the automatic tissue processor (SAKURA FINE TECH from the Netherlands) by dehydrating through (70%, 90%, and 95%) and changes of absolute ethanol two for 90 min each. The clearing was carried out during changes of xylene (twice, for 2 h) each, infiltrating through two changes of paraffin wax at (70°C) and placed in paraffin wax. Sections were cut at 4 µm with the rotary microtome (SAKURA FINE TECH, from the Netherlands) and installed on glass slides and dried at (65°C,45 min), and following stained by (hematoxylin, eosin stain), and then examined by the light microscope [39].

2.8. Statistical analysis.

Standard deviation (SD) and standard error (SE) were calculated according to Fisher [40]. Least significant difference (LSD) test was applied to compare the significant variation between means of treatment [41]. The Costat program was applied for full analysis.

3. Results and Discussion

Human care is daily exposed to many environmental xenobiotics, which cause a rise in oxidative stress in the body. One of these wade range of contaminates is BPA which stimulated oxidative damage in many tissues [42]. Vandenberg *et al*., [43] suggested that lower dose BPA exposure can cause endocrine-disrupting effects.

3.1. Biological and biochemical effects of Bisphenol A.

3.1.1. Behavior.

It was notes aggression, and hypertension in male rats received BPA at 3 doses only while females appeared signs of weakness, and uncomforted may due to BPA caused oxidative stress. Adesanoye *et al*., [44] and Musachio *et al*., [45] noticed that BPA caused the behavioral deficit and Parkinsonian in flies. The results agree with Jiang *et al*. [46], who stated that there was a relationship between BPA exposure and increased hypertension risk.

3.1.2. Body and testes weight.

Result in Table (1,2) indicated that body weights were significantly decreased in both female and male rats groups exposed to BPA (G 6, 7) as compared to the control group (G1) and showing linear relation between dose and final body weight. Treated groups with either rosewater or clove oil in drinking water (G8, G9) showed a significant increase in body and testes weight compared to treated rats groups by BPA only and were closed to control groups (Table 1, 2 and Figure 1). However, no significant changes in b.wt. were observed in rosewater or clove oil alone treated groups (G2, G3) when compared to the control group (G1). The testes weight compared to control were significantly decreased in male rats exposed to all BPA dose.

Table 1. Body and testes weight of male rats as affected by BPA for 6 weeks.

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Daily body weight gain (g)	Testes weight (g)
G1 (Control)	119.25 ± 1.65^{a}	282.55 ± 1.72^{a}	163.30^{a}	3.89^{a}	1.89 ± 0.02^{a}
G2 (Control rose water)	122.88 ± 1.09^{a}	282.20 ± 1.74^{a}	159.33^{ab}	3.79^{ab}	1.91 ± 0.08^{a}
G3 (Control clove oil)	120.00 ± 1.08^{a}	281.13 ± 0.69^{a}	161.13^{ab}	3.84^{ab}	1.88 ± 0.01^{a}
G4 (Control Tween 20)	122.38 ± 1.91^{a}	285.80 ± 1.37^{a}	163.43^{a}	3.89^{a}	1.90 ± 0.06^{a}
G5 (Rats treated 20 µg BPA /kg b.wt.)	120.75 ± 1.38^{a}	277.00 ± 1.43^{ab}	156.25^{ab}	3.72^{ab}	1.79 ± 0.01^{ab}
G6 (Rats treated 20 mg BPA / kg b.wt.)	117.25 ± 1.31^{a}	271.85 ± 0.93^{bc}	154.60^{ab}	3.68^{ab}	1.75 ± 0.02^{ab}
G7 (Rats treated 200 mg BPA / kg b.wt.)	118.00 ± 1.47^{a}	269.35 ± 1.03^{c}	151.35^{b}	3.60^{b}	1.70 ± 0.02^{b}
G8 (Rats treated 200 mg BPA / kg b.wt. + rose water)	117.50 ± 1.89^{a}	282.24 ± 4.65^{a}	164.74^{a}	3.92^{a}	1.92 ± 0.03^{a}
G9 (Rats treated 200 mg BPA / kg b.wt. + clove oil)	118.75 ± 0.75^{a}	280.40 ± 0.98^{a}	161.65^{ab}	3.85^{ab}	1.91 ± 0.06^{a}
L.S.D 0.05	4.1778	5.7159	6.9793	0.1661	0.1161

- Each value represents the mean ± S.E (Standard Error) and mean of five replicates.
- Values in the same column with the same letter are not significant at p≤0.05.

Table 2. Bodyweight of female rats as affected by BPA for 6 weeks.

Groups	Initial body weight (g)	Final body weight (g)	Bodyweight gain (g)	Daily body weight gain (g)
G1 (Control)	120.88 ± 0.43^{a}	202.15 ± 2.75^{ab}	81.28^{ab}	1.94^{ab}
G2 (Control rose water)	120.88 ± 4.26^{a}	206.34 ± 2.9^{a}	85.46^{a}	2.03^{a}
G3 (Control clove oil)	121.25 ± 2.14^{a}	200.06 ± 0.72^{ab}	78.81^{ab}	1.88^{ab}
G4 (Control Tween 20)	121.75 ± 0.85^{a}	203.47 ± 2.67^{ab}	81.72^{ab}	1.95^{ab}
G5 (Rats treated 20 µg BPA / kg b.wt.)	120.00 ± 2.80^{a}	195.63 ± 1.63^{bc}	75.63^{ab}	1.80^{ab}
G6 (Rats treated 20 mg BPA /kg b.wt.)	121.00 ± 4.24^{a}	190.93 ± 2.03^{c}	69.93^{ab}	1.66^{ab}
G7 (Rats treated 200 mg BPA / kg b.wt.)	114.00 ± 3.74^{a}	181.38 ± 2.02^{d}	67.38^{b}	1.60^{b}
G8 (Rats treated 200 mg BPA /kg b.wt. + rose water)	119.75 ± 4.03^{a}	200.20 ± 0.96^{ab}	80.45^{ab}	1.92^{ab}
G9 (Rats treated 200 mg BPA / kg b.wt. + clove oil)	119.50 ± 2.33^{a}	201.33 ± 2.09^{ab}	81.83^{ab}	1.95^{ab}
L.S.D 0.05	8.9418	5.8512	9.9013	0.2354

- Each value represents the mean ± S.E (Standard Error) and mean of five replicates.
- Values in the same column with the same letter are not significant at p≤0.05.

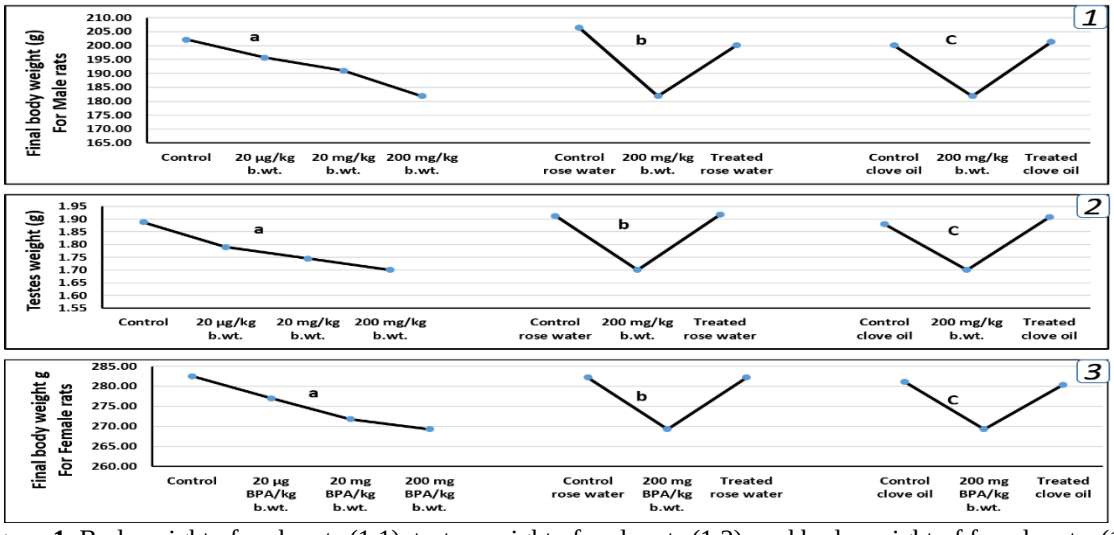

Figure 1. Bodyweight of male rats (1.1), testes weight of male rats (1.2), and body weight of female rats (1.3) as affected by BPA for 6 weeks.

In this study, the body weights (b.wt.) of male and female rats were decreased due to BPA treatments inhibited the increase in body weight, which may be due to BPA instigated a significant decrease in fat stores, energy metabolism, and feeding of the effectiveness of

rodents [47, 48]. On the other hand, BPA caused weaker peroxisome proliferator-activated receptor gamma (PPAR-γ) function, which impaired adipocyte development and decreased body weight [49].

The results agree with Vom Saal *et al*. [50] who found a significant reduction in body weight in the mice exposed to 2 µg/kg of BPA and conform by Honma *et al*. [51] and Lü and Zhan [52].

In the current study, it was noted that BPA decreased the weights of testes in male rats due to the inhibition of (spermatogenesis, steroid biosynthesis of Leydig cells), decreased (elongated spermatids, tubule size) and steroidogenic enzyme activity [53, 54]. Furthermore, the reduction of testes weight depends on the dose of BPA has been reported by Samova *et al*. [55].

This study reduction of testes weight and this agree with Takahashi and Oishi [53] who stated that dose (200 mg/kg/day) of BPA for 4 weeks caused significantly decreased seminal vesicle and testes weights in rats. Similarly, a study on adult male *Holtzman* rats (W13) administered with BPA 20 µg/kg b. wt. showed a reduction in the testis weight, a decrease in the epididymal weight, and a reduction in sperm production in rodents during pubertal development [56].

Table 1 and Figure 1.1, 1.2, generally, the relation between body or testes weight and BPA dose was linear (Figure 1.1a, 1.2a). The high dose of BPA (200 mg/kg b.wt) significantly decreased body weight and testes weight in male rats; this reduction came to the control values by the administration of rosewater or clove oil (Figure 1.1 b and c, 1.2 b and c). The final body weight of female rats decreased significantly at 20 and 200 mg of BPA only (Table 2 and Figure 1.3).

3.1.3. Effect of bisphenol A on TSH and sexual hormones for male and female rats

3.1.3.1. Male hormone.

Table 3 and Figure 2 summarized the mean values ± SD of total and free testosterone levels. Bisphenol A (20 µg, 20 mg, and 200 mg /Kg body weight) tended to significantly decrease in testosterone levels after 6 weeks of treatment contrast with the control group (G1). Furthermore, group (G 2), which received rose water only significantly increased testosterone levels compared to the control group (G1), while groups (3 and 4) which received clove oil or tween 20 respectively showed no significant change compared with the control group (1). While total and free testosterone levels significantly increased when rose water or clove oil were administrated to rats (G 8 and 9) compared to the height dose of BPA (G 7) and reached the normal control value.

The present study indicates that exposure of male rats to BPA decreases the total and free testosterone levels, which may be due to BPA has been considered a weak estrogen [56]. Furthermore, BPA may cause male infertility, and inhibition of steroidogenesis was estrogen-mediated and was associated with the inhibition of enzyme activity [58].

The low dose (20 µg/kg b.wt) of BPA reduced total and free testosterone as the high dose (200 mg/kg b.wt), which may indicate the hazard of low doses of BPA which might be ingested as a pollutant. Rosewater treatment only showed a significant increase in total testosterone in male rats.

Results showed that the very low dose of BPA (20 µg), gave an adverse effect on both male and female sex hormones as well as TSH (Table 3, 4).

Table 3. Effect of BPA on the male hormone for 6 weeks in albino rats.

Groups	Testosterone Total (ng/ml)	Testosterone Free (pg/ml)
G 1 (Control)	3.06 ± 0.14 ^{bc}	8.66 ± 0.87^{ab}
G 2 (Control Rose water)	3.95 ± 0.19^{a}	9.89 ± 0.20^{a}
G 3 (Control Clove oil)	2.95 ± 0.03 ^{bc}	8.01 ± 0.34^{ab}
G 4 (Control Tween 20)	3.08 ± 0.14 ^{bc}	8.53 ± 0.29^{ab}
G 5 (20 µg/kg b.wt. from BPA)	2.16 ± 0.33^{d}	5.74 ± 0.90^{c}
G 6 (20 mg/kg b.wt. from BPA)	2.78 ± 0.15^{cd}	7.01 ± 0.37^{bc}
G 7 (200 mg/kg b.wt. from BPA)	2.04 ± 0.02^{d}	5.66 ± 0.08^{c}
G 8 (200 mg/kg b.wt. from BPA + Rose water)	3.51 ± 0.22^{ab}	8.80 ± 0.15^{ab}
G 9 (200 mg/kg b.wt. from BPA + clove oil)	3.40 ± 0.14^{ab}	8.73 ± 0.45^{ab}
L.S.D 0.05	0.5189	1.4603

- Each value represents the mean ± S.E. (Standard Error) and the mean of five replicates.
- Values in the same column with the same letter are not significant at p≤0.05.

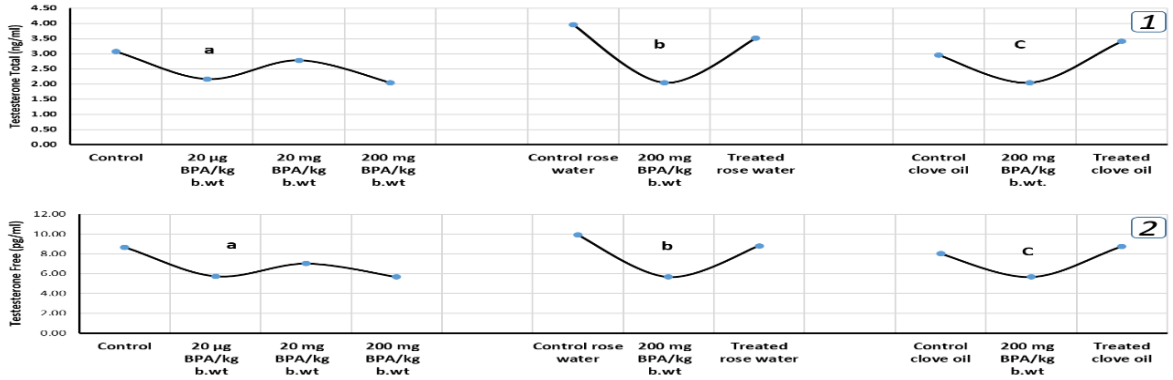

Figure 2. Effect of BPA on testosterone total (1) and testosterone free (2) hormones for 6 weeks in male albino rats.

The obtained result for dose-response was similar to hypothetical nonmonotonic response recorded by Xu *et al*., [59], who presented that there are adverse responses are relative to the exposure of BPA, which means that higher concentration of BPA causes major effects than low concentration. Notwithstanding, a few non-linear dose-response curves intervened in which there is no- linear relationship over the range from low to the high concentration of BPA.

Rosewater improves testosterone levels in the group treated with BPA (200 mg/kg b. wt.); this may be due to direct effects of the compounds contained in rosewater. Rose is rich in flavonoids and polyphenols, especially quercetin [27]. Flavonoids increase testosterone levels. They also competitively bind to an enzyme called aromatase, decrease enzymatic expression, inhibit the conversion of testosterone to estrogen and consequently increase testosterone level [60]. Thus, *damask rose* extract may have affected lote trop cells in the anterior pituitary extract, which would increase LH and consequently increase testosterone reported by Farnia *et al.* [61].

Administration of rose water only to normal rats significantly increased total testosterone in male rats compared to control (Table 3).

On the other hand, Selvage *et al.* [62] and Całka *et al.* [63] found that *damask rose* increase in LH, FSH may be due to the release of norepinephrine by *damask rose* extract. Norepinephrine increases the synthesis of nitric oxide, which increases the release of Gonadotropin-releasing hormone (GnRH) from the hypothalamus as well as FSH and LH gonadotropins from the anterior pituitary gland.

The results indicated that clove oil recover testosterone levels in the group treated with BPA (200 mg/kg b. wt.) this may be due to phytochemical compounds contained phenols and flavonoids in clove oil. Clove extract increase in the sexual activity of normal male rats [64].

The present study showed that rose water contented apigenin 6-C-glucoside and apigenin effect on the recovery of a testis and increase in testosterone level by apigenin inhibiting oxidative injuries reported by Liu *et al*. [65]. Quercetin is a more effective antioxidant compound than else antioxidant nutrients, for example, vitamins (C, E) and β-carotene, and it can chelate transition metal particles, inclusive iron, in this way prohibition the iron-catalyzed Fenton reaction [66]. Treatment with clove oil and rose water, which contend quercetin improved the testosterone level, might be flavonoid compound assistance to normalize the hormonal axis and the influenced testicular capacities [67]. The treatment with quercetin caused a significant increase in gene expression for (LHr and LH subunit gene), testosterone and LH, and FSH. [68, 69].

The result agrees with Castro *et al*. [70] and Salian *et al*. [71], who stated that rats exposed to BPA, decreases testosterone levels.

3.1.3.2. Female hormone.

Table 4 and Figure 3a, no significant difference in groups (G11, G12, and G13) compared to the control group (G10) were noted, FSH values were significantly decreased while prolactin, estrogen, progesterone, and TSH levels were a significant increase in BPA treated groups compared with control groups. In contrast of, Groups received rose water or clove oil (G 17, G18) showed a significant increase in FSH level in (Figure 3.2 b, c) and significantly decreased in prolactin in (Figure 3.5 b, c), estrogen in Figure 3.3 b, c), progesterone in (Figure 3.4 b, c) and TSH levels in (Figure 3.1 b, c) compared with BPA treated group (G16) and returned to the control group level (G10).

Table 4. Effect of BPA on TSH and female hormone for 6 weeks in albino rats.

Groups	TSH (uIU/ml)	FSH (mIU/ml)	E2 (pg/ml)	Progesterone (ng/ml)	PRL (ng/ml)
G 1 (Control)	0.91 ± 0.01^{cd}	1.60 ± 0.01^{a}	35.20 ± 1.70^{c}	5.43 ± 0.76^{b}	2.35 ± 0.03^{b}
G 2 (Control rose water)	0.90 ± 0.01^{cd}	1.63 ± 0.01^{a}	31.83 ± 0.58^{c}	6.71 ± 0.57^{b}	2.33 ± 0.03^{b}
G 3 (Control clove oil)	0.91 ± 0.01^{cd}	1.58 ± 0.01^{ab}	37.10 ± 1.51^{c}	6.85 ± 0.12^{b}	2.31 ± 0.06^{b}
G 4 (Control tween 20)	0.95 ± 0.02^{c}	1.57 ± 0.01^{ab}	31.77 ± 1.46^{c}	5.96 ± 0.50^{b}	2.32 ± 0.04^{b}
G 5 (20 µg/kg b.wt. from BPA)	1.36 ± 0.02^{b}	1.43 ± 0.03^{c}	46.05 ± 1.14^{ab}	9.36 ± 0.49^{a}	3.43 ± 0.02^{a}
G 6 (20 mg/kg b.wt. from BPA)	1.40 ± 0.01^{a}	1.49 ± 0.02^{bc}	42.77 ± 1.22^{b}	9.50 ± 0.64^{a}	3.40 ± 0.01^{a}
G 7 (200 mg/kg b.wt. from BPA)	1.44 ± 0.0^{a}	1.45 ± 0.01^{c}	49.10 ± 2.40^{a}	10.01 ± 0.68^{a}	3.45 ± 0.03^{a}
G 8 (200 mg/kg b.wt. from BPA + rose water)	0.89 ± 0.01^{d}	1.54 ± 0.03^{ab}	34.80 ± 2.08^{c}	7.32 ± 0.30^{b}	2.35 ± 0.03^{b}
G 9 (200 mg/kg b.wt. from BPA + clove oil)	0.92 ± 0.02^{cd}	1.63 ± 0.04^{a}	37.80 ± 0.59^{c}	6.59 ± 0.26^{b}	2.37 ± 0.03^{b}
L.S.D 0.05	0.0396	0.0684	4.5238	1.5478	0.1014

- Each value represents the mean ± S.E. (Standard Error) and the mean of five replicates.
- TSH (Thyroid Stimulating Hormone), FSH (Follicle Stimulating Hormone), E2 (Estrogen), and PRL (Prolactin).
-Values in the same column with the same letter are not significant at $p \leq 0.05$.

TSH hormone, on the other hand, the results indicated that rats exposure BPA has increased in TSH levels BPA may be decreased triiodothyronine (T3) binding and due to increasing TSH secretion bringing about hyperthyroidism and goiter in rodents and a few people. And that agrees with Wang *et al*. [72] who detailed that there was a (0.13 µIU/ml) decline in TSH (−0.14 to −0.11). (Figure 3.1a).

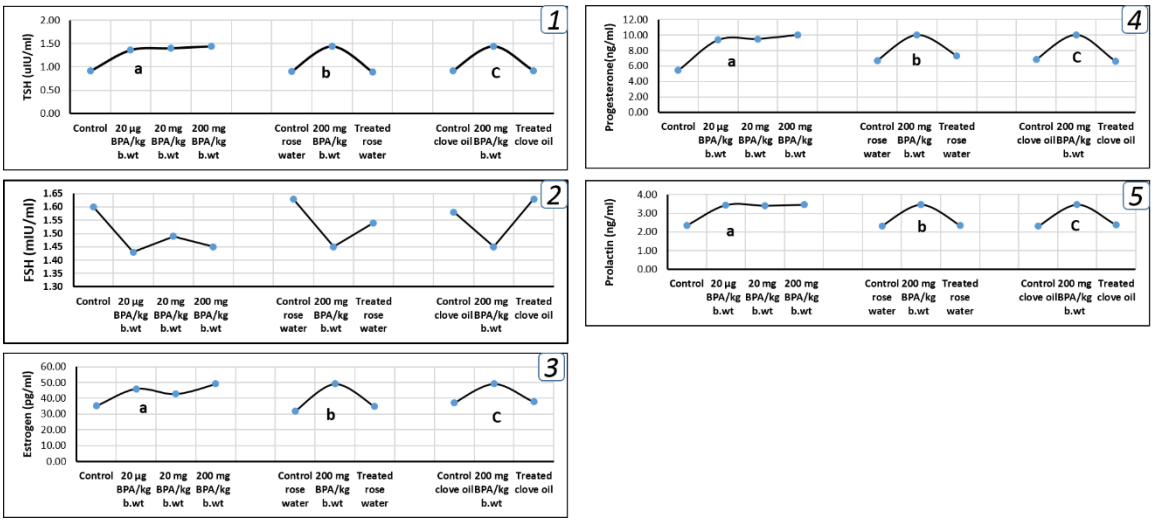

Figure 3. Effect of BPA on Thyroid Stimulating Hormone (TSH) (1), Follicles Stimulating Hormone (FSH) (2), estrogen (3), progesterone (4) and prolactin (5) for 6 weeks in female albino rats.

FSH and estrogen hormones, While an opposite relationship among BPA and FSH was watched between exposed groups which may due to the negative feedback mechanism exerted by estrogen and FSH whereas high estrogen levels observed after BPA exposure caused a decrease in plasma FSH levels, so E2 inhibits either FSH synthesis or secretion reported by [73, 74], as indicated in (Table 4 and Figure 3.2a, 3.3a).

Progesterone hormone, The current study showed the relation between BPA and increased progesterone (Figure 3.4a) that may be due to alteration of prolactin expression following BPA exposure and BPA stimulated tissue changes in the mammary gland were established to incorporate improved estradiol affectability and expanded nearness of progesterone receptor-positive ductal cells which increment progesterone levels [75, 76]. The current result agrees with Mlynarčíková *et al*. [77], who stated that BPA exposure of (0.01–10 lM) raised the basal concentration of progesterone; however, inhibited FSH stimulated estradiol production.

Prolactin hormone (PRL), The results found that a positive relationship between BPA exposure and increasing prolactin levels, which may occur due to BPA, bind to membrane estrogen receptors (α, β, γ (mER)) which are capable of non-genomic steroids action. On the other hand, BPA can likewise stimulate gene expression of prolactin and cell multiplication in (cells GH3 cells and primary anterior pituitary) and also stimulate estrogen which has a direct role in stimulating PRL gene expression to increase the level of serum PRL (Figure 3.5a) [78, 79]. Watson *et al*. [80] stated that GH3/B6 producing calcium flux, which leads to prolactin release.

The Current results (Figure 3.5a) confirm with Stoker *et al*. [81], Goloubkova *et al*., [82] and Helal *et al*., [83] whose revealed that there is a positive relation between BPA exposure level and prolactin when injecting Fisher 344 rats by (15 mg/kg/day of BPA) brought about an increase in serum prolactin levels and also the treatment of (ovariectomized Wistar) rats with (11–250 mg/kg of BPA) every day motivated hyperprolactinemia (Goloubkova *et al*., [82].

Finally, it was noticed that the low dose (20 µg/kg b.wt.) of BPA, as well as the high dose (200 mg/kg b.wt.) have the same disruption effect on sex hormones and TSH in rats. This might indicate that the very low dose of BPA could be harmful since it could be found as a pollutant in food, feed, and drinks.

3.2. Prevention of bisphenol A effects using rose water and clove oil.

3.2.1. Total antioxidant activity (TAA).

Data in Table 5, showed the antioxidant activity in both rose water and clove oil, noting that antioxidants in clove oil are higher than rose water. This may indicate that the antioxidant activity is not the only factor affecting prevention from adverse effects of BPA since rose water better results than clove oil. It was suggested that another compound could have presented an effect against BPA. So it was decided to determine the chemical compounds of both rose water and clove oil using GC/MS.

Table 5. Total antioxidant activity (TAA) of rose water and clove oil.

Type	TAA (ppm)
Rose water	40.470 ± 0.20
Clove oil	156.995 ± 0.25

- Values are expressed as mean ± standard error (n = 3).
- Total antioxidant activity (TAA) was expressed as µmol of ascorbic acid/g of extract.
- Different letters in the same column indicate a significant difference ($P < 0.05$).

3.2.2. GC/MS/MS of rose water and clove oil.

Results were carried out by GC/MS/MS analysis, and rose water and clove oil are given (Table 6, 7).

3.2.2.1. Rosewater.

GC/MS/MS results of rose water (Table 6) showed the presence of many antioxidant compounds that included phenols and flavonoids (17.80 %) such as (3,6,2',4',5'-Pentahydroxyflavone), (6,4'-Dimethoxy-7-hydroxyisoflavone), (Quercetin 3,5,7,3',4'-pentamethyl ether). There are mono and sesquiterpene (50.35 %) such as trans-Sesquisabinene hydrate, Farnesol, α-Himachalene, Caryophyllene, Citronellyl laurate, Patchoulane and some volatile constituents like Rose oxide, Camphene, Linalool, cis-Geraniol, and Isopulegol. The predominant aliphatic hydrocarbons (23.68%) were Heptacosane, Eicosane, Heneicosane, and Nonacosane.

These results presented that rose water rich with antioxidant compounds and used *Damask roses*, which industrially developed for the creation of (rose oil and rose water) after steam distillation of rose concrete and total rose outright after extraction [84]. The result agrees with Mirza and Najafpour [85] and Babu and Kaul [86] who showed rose oil contend ecosane, citronellol, geraniol, Farnesol, hexacosane, and nonacosane. The results indicated that compounds of rosewater could be as considered antioxidant, and this agrees with Cai *et al.* [87], who reported that petal extracts of the other Rosa species were contained components demonstrated good antioxidant activity.

Table 6. Chemical compounds of rose water.

No.	R.t.	Name	Area sum %
1	4.348	Nopol (terpene)	0.07
2	4.536	Isosakuranetin	0.20
3	5.694	α-Pinene oxide	0.09
4	5.844	(S)-(-)-Citronellic acid	0.15
5	6.141	3',5'-Dimethoxy-3,5,7,4'-tetrahydroxyflavone	0.10
6	6.814	α-Pinene	0.16
7	6.944	3-Carene	0.25
8	7.299	Isopulegol	0.17

No.	R.t.	Name	Area sum %
9	7.504	Camphene	1.92
10	7.659	γ-Terpinene	0.64
11	7.826	4-Terpinenyl acetate	0.82
12	7.997	Allo-Ocimene	1.72
13	8.152	3,6,2',4',5'-Pentahydroxyflavone	1.08
14	8.307	α-Terpinene	0.46
15	8.411	Myrtenal	0.20
16	8.574	2,6-Di-tert-butyl-4-methoxyphenol	0.18
17	8.637	(-)-β-Pinene	0.35
18	8.691	Linalool	0.37
19	8.838	Rose oxide	3.77
20	9.314	Fraxidin	2.31
21	9.461	3-Hydroxy-7,8,2'-trimethoxyflavone	0.21
22	9.565	Humulene	0.43
23	9.703	Caryophyllene oxide	0.42
24	9.908	γ-Elemene	0.35
25	10.247	Citronellol	6.08
26	10.351	Sabinen	0.65
27	10.422	cis-Geraniol	0.95
28	10.489	Citronellol acetate	0.62
29	10.606	Isogeraniol	1.71
30	10.887	Farnesol	0.45
31	11.150	4'-Hydroxy-2,3',5,5'-tetramethoxychalcone	0.85
32	11.238	Eugenol	2.43
33	11.422	Geranic acid	1.01
34	11.526	Methyleugenol	2.16
35	11.664	α-Guaiene	0.30
36	11.869	Epicubebol	0.20
37	11.936	α-Muurolene	0.47
38	12.070	α-Himachalene	1.43
39	12.216	Isovitexin (apigenin 6-C-glucoside)	0.87
40	12.354	Caryophyllene	0.58
41	12.488	trans-Sesquisabinene hydrate	1.32
42	12.576	α-Bulnesene	1.04
43	12.672	α-Cedrene	0.45
44	12.789	cis-α-Bisabolene	0.14
45	12.906	Nerolidol	0.76
46	13.090	Hexadecanoic acid, ethyl ester	0.23
47	13.245	Heptacosane	0.31
48	13.295	Squalene	0.59
49	13.538	Widdrol	0.65
50	13.742	1-Tetradecanol	0.74
51	13.830	8-Heptadecene	0.99
52	13.993	Eicosane	1.60
53	14.148	geranyl-α-terpinene	0.60
54	14.345	Santalcamphor	1.02
55	14.449	Hexa-hydro-farnesol	0.57
56	14.545	Geranyl oleate	1.38
57	14.683	Phytol	0.85
58	14.876	Retinoic acid	1.19
59	15.235	1-Octadecene	3.47
60	15.361	Heneicosane	2.77
61	15.545	β-Santalol	1.12
62	15.683	6,4'-Dimethoxy-7-hydroxyisoflavone	2.30
63	15.837	Genkwanin	1.41
64	16.005	Nonacosane	1.18
65	16.235	2-Hexadecanol	1.09
66	16.373	Thunbergen	0.47
67	16.498	9-Tricosene, (Z)-	0.85
68	16.623	1-Heneicosanol	0.74
69	16.682	1-Eicosene	1.32
70	16.895	.β-Eudesmol	2.60
71	17.100	Chrysoeriol	3.61
72	17.322	Quercetin 3,5,7,3',4'-pentamethyl ether	1.27
73	17.585	6-Octadecenoic acid	0.28
74	17.878	17-Pentatriacontene	0.95

No.	R.t.	Name	Area sum %
75	18.225	1-Octadecene	1.70
76	18.392	Cetene	1.47
77	18.451	Citronellyl tiglate	1.50
78	18.994	β Carotene	0.52
79	19.162	Octanoic acid, pentadecyl ester	0.97
80	19.354	Docosanoic acid, 1,2,3-propanetriyl ester	0.61
81	19.567	Erucic acid	1.09
82	20.358	Citronellyl laurate	1.72
83	20.512	Nonanoic acid, tetradecyl ester	1.26
84	20.784	Octacosanol	1.21
85	21.052	2,6-Dimethyl 2,6-octadiene	1.33
86	21.574	Oleyl oleate	0.48
87	21.884	Pentadecyl nonanoate	1.93
88	22.306	Astilbin	0.52
89	23.021	7-Acetoxy-4-methylcoumarin	2.52
90	23.368	5,7-Dimethoxyflavanone	0.37
91	23.506	9-Octadecen-1-ol, (Z)-	2.59
92	23.832	Patchoulane	0.52
93	24.100	β-Phenylethyl butyrate	0.70

3.2.2.2. Clove oil.

Table 7 clove oil GC/MS/MS analysis has shown many antioxidant compounds such as phenols, flavonoids (34.93 %), and terpenes (65.06 %). The major components are as follows: Eugenol (13.99%), (E)-Isoeugenol (5.76%), Caryophyllene (2.12%), Caryophyllene oxide (4.07 %) Farnesol (4.70%) and Dehydrodieugenol (4.38 %). Also, clove oil consists of phenols (Methyl vanillate, Ferulic acid, Gentisic acid) and flavonoid (Vitexin, 6, 2', 4'-Trimethoxyflavanone, Quercetin 3, 5, 7, 3', 4'-pentamethyl ether).

The results showed rose water and clove oil were contend flavonoids such as quercetin, its most effective antioxidant of flavonoids present in foodstuff. Quercetin preventing oxidative damage by a free 3-OH substitute, which is believed to sugar the stability of the flavonoid radical [88]. Clove oil content eugenol which used as medicines due to it exhibits anti-inflammatory activity, anticancer and antioxidant properties [89, 90].

The obtained result agrees with Tomaino *et al*. [91] and Lee and Shibamoto [92] whose revealed that clove oil contains essential oil such as eugenol, eugenyl acetate, and caryophyllene.

Table 7. Chemical compounds of clove oil.

No.	R.t.	Name	Area sum %
1	3.206	3,4'-Dimethoxy-2-hydroxychalcone	0.16
2	4.435	3,4-Dimethylacetophenone	0.24
3	4.586	6,2',4'-Trimethoxyflavanone	0.17
4	6.104	Dimethoxycurcumin	0.34
5	7.149	Gentisic acid	0.31
6	7.249	β-Resorcylic acid	0.14
7	7.354	Quercetin 3,5,7,3',4'-pentamethyl ether	0.16
8	7.467	β-Myrcene	0.36
9	7.663	m-Hydroxymandelic acid	0.18
10	7.843	β-Cymene	0.55
11	7.973	Cineole	1.10
12	8.123	α-Pinene	0.25
13	8.282	4-Terpinenyl acetate	0.15
14	8.583	p-Cymenene	0.22
15	8.621	Terpinolene	0.29
16	8.667	Linalool	0.61
17	9.114	Carveol acetate	0.28
18	9.545	Terpinen-4-ol	0.78
19	9.662	Caryophyllene	2.12
20	10.189	Syringic acid	0.89

No.	R.t.	Name	Area sum %
21	10.343	p-Cumic aldehyde	0.69
22	10.561	α-Guaiene	0.71
23	10.787	Tetra-O-methylfisetin	1.59
24	11.309	Eugenol	13.99
25	11.619	(E)-Isoeugenol	5.76
26	11.748	Methyl vanillate	0.79
27	11.907	α-Himachalene	1.05
28	12.079	Caryophyllene oxide	4.07
29	12.279	Longifolene	2.65
30	12.397	Cubebol	0.20
31	12.526	Aceteugenol	3.59
32	12.723	Aromandendrene	1.20
33	12.836	Cedrenol	0.80
34	13.003	Farnesol	4.70
35	13.195	Nerolidol	2.82
36	13.283	(-)-Spathulenol	0.89
37	13.438	β-Ionone, dihydro-	0.24
38	13.484	Longiborneol	0.93
39	13.588	Thujopsene	1.57
40	13.726	7-epi-cis-sesquisabinene hydrate	2.01
41	13.814	Longipinocarveol, trans-	1.36
42	13.877	3,5-di-tert-Butyl-4-hydroxybenzyl alcohol	0.67
43	14.086	Ferulic acid	4.21
44	14.303	Methoxyeugenol	0.97
45	14.433	Nootkatone	0.78
46	14.646	β-Gurjunene	0.58
47	14.743	Ledol	0.39
48	14.835	Antioxidant BKF	0.28
49	14.947	Dimethylfraxetin	0.70
50	15.182	Cedrol	0.34
51	15.617	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	0.56
52	15.738	7,3',4',5'-Tetramethoxyflavanone	0.57
53	15.926	3-(3,4-Dimethoxyphenyl)-7-methoxy-4-methylcoumarin	0.52
54	16.043	Dimethyl caffeic acid	0.28
55	16.29	Astilbin	0.34
56	16.449	Sclareol	0.83
57	16.821	(S)-(-)-Citronellic acid	0.40
58	17.176	Propyl gallate	0.25
59	17.858	Chrysoeriol	0.25
60	18.619	Vitamin E	0.23
61	19.384	Genkwanin	0.19
62	20.191	3,2',4',5',6-Pentamethoxyflavone	0.29
63	20.605	Vitexin	9.70
64	20.986	3,6,2',3'-Tetramethoxyflavone	5.19
65	21.241	7,8-Dihydroxy-4-methylcoumarin	0.76
66	21.5	3'-Benzyloxy-5,6,7,4'-tetramethoxyflavone	0.43
67	21.651	Scrophulein	0.99
68	21.86	2,4-Di-t-butyl-6-(4-methoxybenzyl)phenol	1.19
69	22.09	Morin	0.24
70	22.303	Vanillic acid	0.61
71	22.458	Phenol, 4,4'-isopropylidenebis[2,6-dichloro-	0.66
72	22.738	Dehydrodieugenol	4.38
73	23.164	Gardenin	0.58
74	23.277	Rosmarinic acid	0.45
75	24.21	2,4-di-tert-Butyl-6-(4-methoxybenzyl)phenol	0.23

3.3. Histopathological studies.

3.3.1. Testes.

The histopathological examination of testes for groups is given in (Figure 4). Testes of a negative control group (G1) demonstrating the naturalist histological structure of interstitial tissues and seminiferous tubules. Testes in a group of rats received rose water (G2) revealed normal spermatogenic series and normal interstitial tissue, cells of Leydig, and formation of

mature sperms in the lumen. Rats group (G3), which received clove oil revealing normal histoarchitecture of the seminiferous tubules with normal spermatogenic series, interstitial cells of Leydig, and formation of mature sperms. Testes from a group of rats received tween (G4), revealing the normal histological structure of the seminiferous tubules.

Rats group (G5), which received the BPA (20 µg/kg b.wt.) low dose, elucidate impairment of the spermatogenic cell series with a reduction in the formation of mature spermatids, necrosis of some cells of Leydig. Testes from rats received 20 mg/kg b. wt. from BPA (G6), showing interstitial edema, with impairment of the spermatogenic cell series in some seminiferous tubules with a moderate reduction in mature sperms and destruction of Leydig. Rats group (G7) which treated by 200 mg/kg b. wt. from BPA showing severe destruction and necrosis of spermatogenic series in some seminiferous tubules and reduction in mature sperms and interstitial edema with necrosis of some Leydig cell.

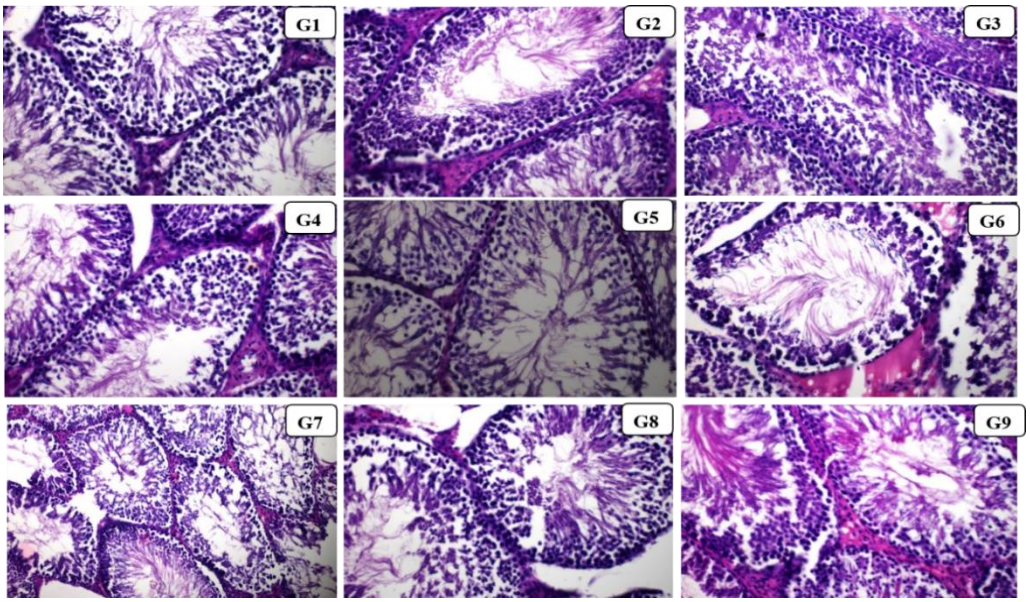

Figure 4. Photomicrographs of testes of male rats. (G1) Control group. (G2) rats received rose water control. (G3) rats received clove oil control. (G4) rats received tween 20 control. (G5) rats received 20 µg/kg b.wt. from BPA. (G6) rats received 20 mg/kg b.wt. from BPA. (G7) rats received 200 mg/kg b.wt. from BPA. (G8) rats received 200 mg/kg b.wt. from BPA + rose water. (G9) rats received 200 mg/kg b.wt. from BPA + clove oil. (X 400). H and E stain.

While rats group (G8) dosed with 200 mg/kg b. wt. from BPA and received in drinking water rose water, showed marked improvement in the histological structure of the seminiferous tubules, and formation of mature spermatid with a mild reduction in sperms. Testes from rats (G9), treated with (200 mg/kg b. wt. from BPA + clove oil) revealing a marked improvement in the histological structure of seminiferous tubules, with mild edema in the interstitial tissue.

These results demonstrated that BPA exposure caused many toxic effects on testes such as necrosis of some cells of Leydig may be due to BPA caused interference of proliferative activity, development of Leydig cells in rat and BPA acts as a mitogen, which leads to decrease in plasma testosterone level in BPA treated rats [93]. The present results agree with Nakamura *et al*. [94] who reported that BPA caused a reduction in the number of Leydig cells as long as the testosterone levels following 6 weeks of BPA administration because of BPA suppressed aromatase gene expressions which lead to reduced 17β-oestradiol biosynthesis and aromatase is an enzyme necessary for the aromatization of testosterone to17β-oestradiol.

BPA causes impairment of the spermatogenic cell series, probably due to low testosterone level, which caused the failure of spermatogenesis and disruption of the

seminiferous epithelium and whereas the process of spermatogenesis begins with the differentiation of spermatogenic which requires testosterone action [95]. Takahashi and Oishi [53] expressed that BPA caused diminish the weights of (testes and epididymis), which might be inhibition of spermatogenesis, steroidogenic enzyme activity, and diminished elongated spermatids.

The present study showed that BPA exposure caused necrosis of some cells of Leydig and interstitial edema in tested, this might be due to BPA caused oxidative stress and the production of free radicals lead to organ toxicity through upsetting the harmony among reactive oxygen species (ROS) and antioxidant defenses system in testes of rats and damaging the vital organs [42, 74]. Further, BPA disrupts fertility, spermatogenesis, steroidogenesis and testis maturation, and spermatogenesis [96-98].

These results confirmed with Hanafy *et al*. [99] who showed that a chain of structural changes in the left testicles happened results from exposure (1 mg and 10 mg from BPA) after 6 weeks caused seminiferous tubules to appear irregularly arranged and with mild edema of the interstitial space and scattered congested blood vessels in-between tubules and hemorrhage. Similarly, Tamilselvan *et al*. [100] revealed that testes sections were showed varying morphological changes in 200 mg/kg b.wt. BPA exposed rats for 30 days, which include extensive seminiferous tubules damage, which was evidenced by the necrotic changes.

While rosewater and clove oil showed improvement in histological structure in testes might occur due to antioxidant compounds, which important to reduce the BPA induced toxicity [101]. For this reason, rosewater and clove oil was used because of their have phenolic and flavonoids compounds and modification of gene expression through inhibiting nuclear-factor-kappa B (NF-Kb) activation [102]. Polyphenols compounds interact in several ways, such as receptive oxygen, redox-active transition metal chelators, enzyme modulators, and nitrogen species scavengers [103]. Flavonoid has high reactivity of the (OH) substituent, and a number of (OH groups) on the (B-ring) being relates to ROS scavenging efficiency [104, 105].

The current study showed an improvement in steroid hormone and histopathology structure; this might be the job of flavonoids like (Quercetin) which prevents oxidative damage and stress raised by free radicals at the tissue in different body organs like male reproductive system. Likewise, quercetin in rose water and clove oil have effectiveness on testes by activating the testes and epididymis during stimulating testosterone hormone secretion [106]. Quercetin was likewise responsible for the synthesis of testosterone by stimulates the enzymes that transport of cholesterol to Leydig cells [107].

3.3.2. Ovary.

Figure 5 demonstrated that the transverse section of the ovary of female rats. Negative control (G10) revealed normal histological structure; the cortex contains ovarian follicles in varying stages of development. A corpus luteum is also present to contain granules lutein cells and primordial follicles. Ovary from control rose water (G11), revealed normal histological structure with varying stages of ovarian follicles development and formation of corpus luteum. Rats group (G12), which received clove oil showing normal histoarchitecture, the cortex contains ovarian follicles in varying stages of development with corpus luteum formation and highly cellular stroma of loose connective. Ovary control of rats received Tween (G13), showed the cortex contains ovarian follicles in varying stages of development and corpus luteum with granules lutein cells. The stroma is highly cellular of loose connective tissue.

Whereas ovary of rats from a group (G14) dosed 20 µg / kg b. wt. from BPA showed severe congestion of blood vessels and hemorrhage with a reduced number of ovarian follicles. Rats group (G15) dosed with (20 mg/kg b. wt. from BPA), showing severe congestion, hemorrhage with a reduced number of ovarian follicles, and few inflammatory cells infiltration. Ovary of rats from the group (G16) dosed 200 mg/kg b.wt. BPA revealed inactive ovary with severe congestion, hemorrhage, few inflammatory cells in the stroma, and reduce the number of ovarian follicles.

In contrast, The ovary of rats from a group (G17) which dosed with 200 mg/kg b. wt. from BPA and treated with rose water, showing most of the ovarian cortex contain corpus luteum with granulose lutein cells with dilated blood vessels. Rats from a group (G18) dosed with (200 mg/kg b. wt. from BPA and treated with clove oil), exhibiting marked improvement in the histological structure of ovary with normal ovarian follicles in various stages of development.

The sensitive organ of hormones is the ovary, which creates steroid hormones. It has been highly recommended that BPA exposure is connected to numerous sicknesses, which are impacted by the variety in estrogen levels, for example, ovarian disease [108]. This section summarizes the findings of BPA exposure in the ovary, where BPA caused congestion of blood vessels, hemorrhage, reduced number of ovarian follicles, and few inflammatory cells infiltration in female rats. On account of BPA has influences on fertility by disturbing estrogen signaling. Also, BPA bind to ERβ higher than ERα [109], in spite of the fact that its coupling liking for the two receptors is more prominent than (1000–10000) fold lower than that for E2 [110].

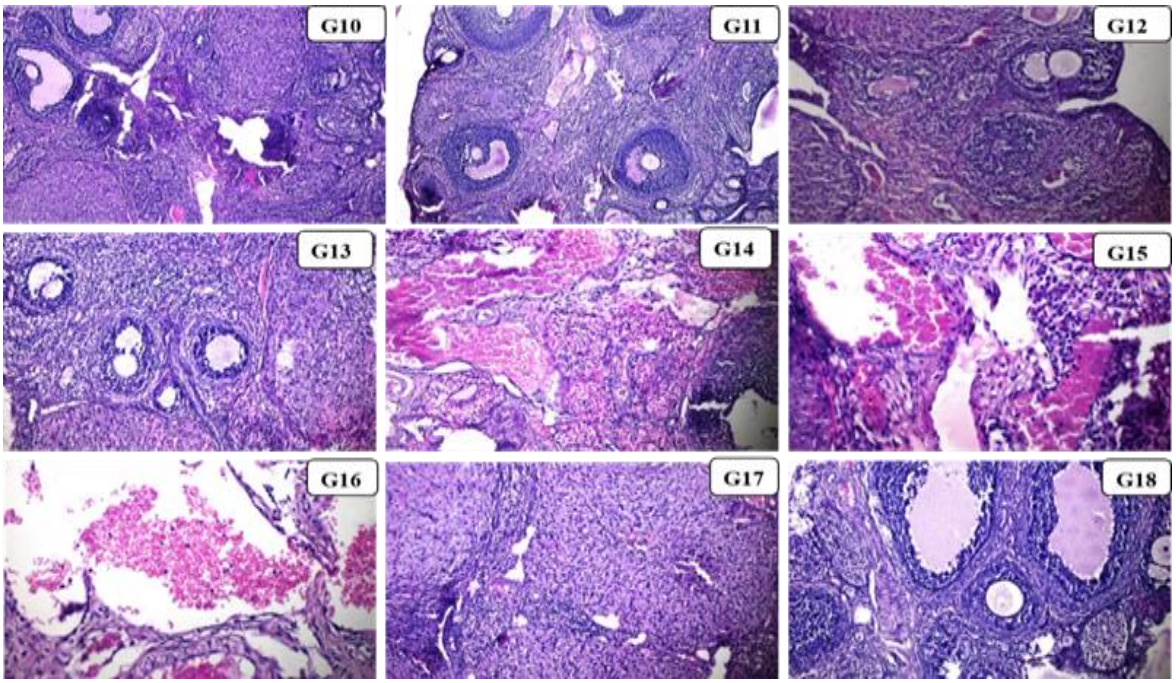

Figure 5. Photomicrographs of the ovary of female rats. (G10) Control group. (G11) rats received rose water control. (G12) rats received clove oil control. (G13) rats received tween 20 control. (G14) rats received 20 µg/kg b.wt. from BPA. (G15) rats received 20 mg/kg b.wt. from BPA. (G16) rats received 200 mg/kg b.wt. from BPA. (G17) rats received 200 mg/kg b.wt. from BPA + rose water. (G18) rats received 200 mg/kg b.wt. from BPA + clove oil. (X 400). H and E stain.

The results showed that BPA caused inflammatory cells in the stroma which may be due to there is the relationship between BPA exposure and endometrium linked disorders which

due to BPA stimulate inflammatory signals and caused oxidative stress in cultured endometrial stromal cells (ESCs) through ER-α reported by Cho *et al*., [111].

BPA-induced multinucleated and hemorrhagic tissue [112]. Lee *et al*. [113] stated that a low concentration of BPA caused more apoptosis in ovarian follicles that were combined with increased degrees of the apoptotic protein (caspase-3). Rats exposure to BPA through the early postnatal period had a diminished in (ovarian follicular store, a decay in the supplies primordial follicles), increment in (antral atretic follicles, higher happening of various oocyte follicles (MOFs)) and lower ovarian weight [114, 115].

4. Conclusions

The Present study revealed that exposure of BPA for 6 weeks causes oxidative stress in experimental animals through upsetting the harmony among ROS and antioxidant protection system in testis which leads to a reduction in fertility, and decreased total and free testosterone in male, while the increase in female hormones like TSH, progesterone, estrogen and prolactin and decrease in FSH compared to control groups and reduced number of ovarian follicles in the ovary. The present study showed that rose water and clove oil have a strong antioxidant activity and a major role in the treatment of biological damage brought about via BPA exposure. Rosewater and clove oil exposure due to modification of whole histological injury saw by BPA disturbance impacts.

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Conflicts of Interest

The authors declare no conflict of interest.

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