Saudi Journal of Biological Sciences 30 (2023) 103737

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Efficacy of Saudi propolis and bee pollen in the reduction of oxidative stress induced with CCl4 in a testis mice model

Khulud Mohammed Alshehri

Department of Biology, Al-Baha University, Baljurashi, Saudi Arabia

ARTICLE INFO

Article history:
Received 25 March 2023
Revised 27 June 2023
Accepted 8 July 2023
Available online 13 July 2023

Keywords:
Antioxidative
Propolis
Bee pollen
Oxidative stress
Testes

ABSTRACT

Low testosterone levels are caused by alcoholism, cigarette smoking, and exposure to toxic chemicals. This work focused on investigating the activities of propolis (PE) and bee pollen (BPE) extracts in reducing the oxidative stress of carbon tetrachloride (CCl4) in male mice models. The 48 male Swiss Albino mice weighed 27.5 ± 2.5 g and were divided into: Group1: Control (-) received distilled water only through oral intubation; Group 2: Control (- -) received corn oil by intraperitoneal (i.p.) injection once a day; Group 3: Control (+) received a sublethal dose of CCl4 intraperitoneally the end of the experiment. Group 4: Stander treated with silymarin at a daily dose of 200 mg/kg orally. Group 5: The mice were given 8.4 mg/ kg bw of (PE) orally. Group 6: The mice were given (BPE) extract (140 mg/kg bw) orally. After five consecutive days of treatment, all mice had testis injury in all groups except G1& G2, by a single i. p injection of CCl4 at a dose of 0.5 mL/kg (bw; 20% v/v in corn oil). The result showed a significant increase in luteinizing, follicle-stimulating, and testosterone hormone levels in the serum and semen parameters in the groups treated with PE and BPE. The histological results showed the greatest improvements in testis structures in the BPE group, which was confirmed using (Bcl-2; immunohistochemistry). These results suggest an important role of the antioxidative effects of PE and BPE in the attenuation of CCl4 oxidative stress.

1. Introduction

Infertility is a major health issue, and approximately 30% caused by male factors (Rowe, 2006). There are a number of factors that may influence spermatogenesis, sperm quantity and quality, and male reproductive hormones. Many illnesses and diseases, including chronic liver disease, diabetes mellitus, coronary heart disease, chronic smoking, alcohol intake, prolonged exposure to air pollution, industrial and pesticide toxins, and inadequate vitamin intake, have harmful effects on the production of normal sperm and spermatogenesis (Halliwell et al., 1990; Khan et al., 2010). Male sexual dysfunction is characterized by a number of issues such as sperm concentration deficiency, abnormal motility, and imbalance levels of sex hormones, which are carried on by alcoholism, drug addiction, old age, cigarette smoking, antidepressant medications, and exposure to a harmful substance (Javadi et al., 2011; Brock et al., 2002). In experimental animals, the industrial solvent carbon tetrachloride (CCl4) damages the kidneys, lungs, and testicles (Abraham et al., 1999). Honeybees create a broad range of products with several physiologically active and useful biochemical ingredients, including vitamins, minerals, and polyphenols (Kaškonienė et al., 2018). For the last 40 years, these chemicals have provided prevention and benefit, and they have also been employed in apitherapy (Ahuja et al., 2010). Therefore, the simplicity, convenience, and availability of apitherapy as self-health care have made this type of treatment promising for dealing with periodontal diseases (Gupta et al., 2014). The four insect species that create bee products are honeybees (Apis), stingless bees, honey wasps, and honey ants. Royal jelly, beeswax, pollen, propolis, beeswax, and bee venom are some of the materials produced by bees (SELAMOGLU, 2019). Hepatocellular necrosis and fat accumulation are signs of CCl4 hepatotoxicity. Hepatocellular necrosis, which surpasses the liver's capacity to regenerate, is brought on by high toxic dosages of CCL4, which frequently results in liver failure. CCl4 lethal dosages cause nonspecific toxicity in multiple organs, which causes death. Cytochrome p450 enzymes, primarily CYP2E1, the endoplasmic reticulum (ER) is the site of metabolism

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

E-mail address: kalshehri@bu.edu.sa

https://doi.org/10.1016/j.sjbs.2023.103737
1319-562X/

to produce the very reactive trichloromethyl radical (CCl3). The highly reactive trichloromethyl peroxyl radical (CCl3O2) is created when CCl3 interacts quickly with oxygen. This radical then reacts quickly with lipids to produce products of lipid peroxidation. Free radicals are more likely to oxidize polyunsaturated fatty acids (PUFA) in the endoplasmic reticulum and mitochondria (Weber et al., 2003).

The objective of the current investigation was to ascertain the antioxidative activities of Saudi propolis (PE) and bee pollen (BPE) extracts in decreasing carbon tetrachloride's oxidative stress (CCl4) in a male mice model.

2. Material and methods

2.1. Preparation of PE and BPE extracts

Propolis and bee pollen were collected from the Baljurashi district in the Al-Baha Region, southwestern Saudi Arabia, which is located at approximately 19°51′40″N 41°33′40″E, during the summer of 2021. The samples were kept at room temperature, away from light and humidity, until further extraction.

2.1.1. Propolis (PE) extract

The propolis samples used in this investigation were crushed into a fine powder after being frozen at −20 °C. After that, ethanol 70% was used to extract the pulverized propolis (30 mL of ethanol was mixed with 1 g of propolis), which was left at room temperature for 4 days while being constantly stirred. The obtained suspension was then run twice through an extraction process before being filtered using a qualitative filter paper (90 mm, Whatman filter paper). The solvent was then filtered out of the extracts and dried under a vacuum (Mihai et al., 2010).

2.1.2. The bee pollen (BPE) extract

The pollen samples (2 g) were dissolved in 15 mL of ethanol 70% at 70 °C for 30 min with stirred continuously. The solid residue was re-extracted after the supernatant was separated. Subsequently, the ethanol extracts of pollen were combined and kept at 5 °C until use (Carpes et al., 2007).

2.2. Experimental design

The 48 male mice (Swiss Albino) weighing (27.5 ± 2.5 g) were obtained from King Fahd Medical Research Center and were allocated into six groups of eight mice in each group. The mice were kept in typical cages at 20 ± 1 °C, with a cycle of light and darkness, and a humidity level of 65%. The mice received the following treatment in addition to unlimited access to water and regular commercial food:

Group 1: Control (-), the mice served as the first negative control and received distilled water only through oral intubation. Group 2: Control (− −) mice, which served as the second negative control, received corn oil by intraperitoneal (i.p.) injection once a day. Group 3: Control (+), CCl4 was administered intraperitoneally to the mice on day 6 of the experiment at a concentration of 0.5 mL/kg body weight (20% v/v in corn oil). Group 4: Stander, the mice received conventional silymarin treatment at a daily dosage of 200 mg/kg orally. Group 5: The mice were given 8.4 mg/ kg bw of propolis extract (PE) via oral intubation. Group 6: Mice were administered alcoholic bee pollen extract (BPE) (140 mg/kg bw) via oral intubation. For five consecutive days, all treatments were given orally once each day. All animal groups, except G1 and G2, were given a single intraperitoneal injection of 20% CCl4 v/v in corn oil (0.5 mL/kg bw) on the sixth day to cause testis injury. With non-heparinized tubes, the samples of blood were taken from the orbital venous plexus. Clear samples of blood serum were extracted by centrifuging at 2500 rpm for 15 min, then at 80 °C, the samples were maintained. The levels of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol were measured by utilizing kits for enzyme-linked immunosorbent assay (ELISA). The testes and epididymis were used to measure sperm parameters and histological studies were performed after the mice were sacrificed (Tohamy et al., 2014; Al-Sayed et al., 2015).

2.3. Semen parameter

After cutting the epididymal tail and gently pressing the contents onto a glasses slide, the sperm's motility, and it count were evaluated (Seed et al., 1996). To assess the viability of sperm percentage (ratio of alive/dead) and sperm cell normality, eosin-nigrosin staining was performed on seminal smears. (Amann, 1982).

2.4. Sex hormonal assay

According to the manufacturer's instructions, ELISA kits (Human Diagnostic Worldwide, Germany) the levels of testosterone, LH, FSH, prolactin, and estradiol were meagered in serum.

2.5. Histopathological examination

Dissected testicles were preserved in a 10% neutral formalin solution. The fixed specimens underwent trimming, washing, and dehydration in escalating alcohol concentrations. Hematoxylin and eosin (H&E) were used to stain these specimens after they had been cleaned in xylene, embedded in paraffin, and sectioned at 4–6 μm thick. Masson's trichrome was used as a special stain, and the testis's slides were examined microscopically (Presnell et al., 1997).

2.6. Immunohistochemistry examination

The testes samples were embedded in paraffin, deparaffinized with xylene, and rehydrated with gradual ethanol. Following the manufacturer's instructions (Vectastain Elite ABC Kit).

2.7. Statistical analysis

The experiments were performed in triplicate, and all findings are shown as mean ± SEM. The means across the groups were compared using One-way ANOVA, followed by a Tukey's post hoc analysis. Before this analysis, the homogeneity of variances of the groups was checked using the Levene test. The SPSS program was used to examine each data (version 17; SPSS, Inc., Chicago, IL, USA). At $p < 5$, differences were regarded as statistically significant.

3. Results

3.1. Effects of PE and BPE extracts on semen parameters

Table 1 shows the effects of PE and BPE extracts on semen parameters. Morphological normality was significant ($p < 0.05$) in the mice treated with CCl4 compared to the two control groups G1 and G2. The control groups were recorded (97.856 ± 1.45% and (97.910 ± 2.92%, respectively), when the percentage of morphologically normal sperm was significantly increased in G5 and G6 groups (86.970 ± 2.23% and 88.000 ± 3.21%), respectively) compared to the negative control G3. The sperm vitality ratio of sperm

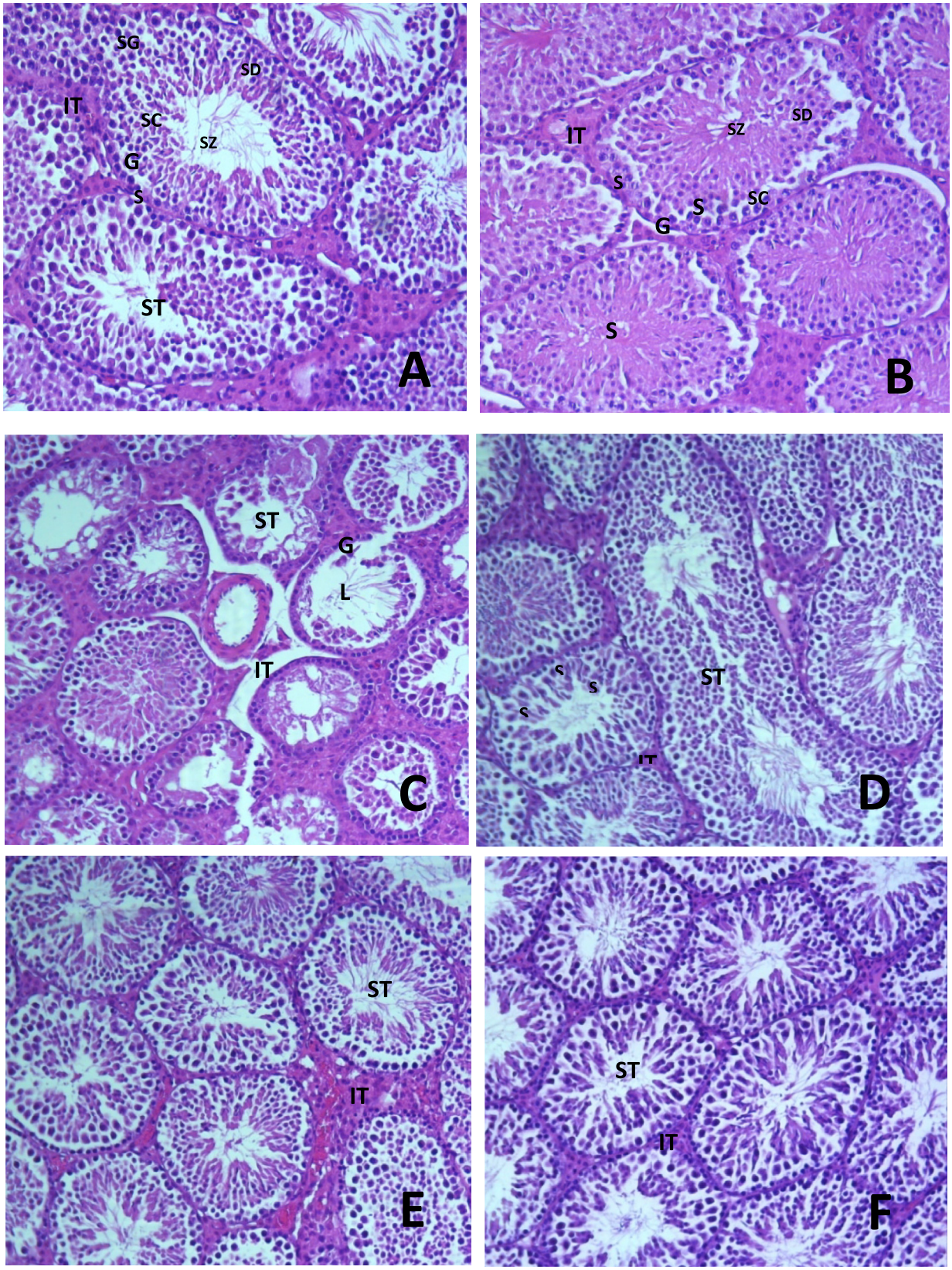

Fig. 1. A&B. The image displays a typical appearance of the seminiferous tubules, which appear to be tightly coiled and lined with a germinal epithelium (G). The interstitial tissue contains a thin layer of loose connective tissue and Leydig cells (IT). The normal cellular composition comprises Sertoli cells (S) along with a regular sequence of spermatogonial stages, such as spermatogonia (SG), spermatocytes (SC), spermatids (SD), and spermatozoa (SZ). **C:** Seriously damaged and mild disturbance in seminiferous tubule structure (ST) and increased luminal diameter (L) with loss of interstitial tissue (IT), the necrotic germinal epithelium (G), and reduction in the number of the spermatogenic cells. **D:** semi-normal arrangement of seminiferous tubules with increased wall thickness, different stages of spermatogenic cells. **E:** A partially normal appearance of the seminiferous tubules with improvement in its structure. **F:** normal seminiferous tubules organization with normal spermatogenesis cells. (H&E × 100).

Table 1
The effects of PE and BPE extracts on semen parameters.

	Morphologically normality %	Sperm vitality (Live:Death ratio)	Motility %	Sperm concentration (10^6 /ml)
Control Group 1	97.856 ± 1.45^{c,d}	16.430 ± 3.10^{c}	85.330 ± 9.07^{a}	37.130 ± 3.94^{b}
Corn oil Group 2	97.910 ± 2.92^{d}	15.580 ± 4.35^{b,c}	84.103 ± 11.07^{a}	35.51 ± 6.56^{b}
CCl_4 Group 3	74.646 ± 5.91^{a}	4.200 ± 1.126^{a}	62.360 ± 12.83^{a}	22.25 ± 2.85^{a}
Silymarin Group 4	91.903 ± 4.11^{c,d}	11.000 ± 2.44^{b,c}	81.350 ± 9.75^{a}	31.193 ± 4.02^{b}
PE Group 5	86.970 ± 2.23^{b}	7.160 ± 2.10^{a}	70.736 ± 5.66^{a}	26.303 ± 2.44^{b}
BPE Group 6	88.000 ± 3.21^{b}	8.043 ± 3.61^{b}	76.953 ± 11.16^{a}	29.1 ± 3.01^{b}

According to Tukey's HSD test, (Mean ± SD) followed by different litters are significantly different. Mean ± SD followed by seem litters are not significantly different.

was not considerably different between the silymarin and BPE treated groups contrasted to the control groups with (11.000 ± 2. 44 and 8.043 ± 3.61, respectively), while the same groups showed significant differences ($p < 0.05$) compared to G3. Sperm motility showed non-significant results in all groups. The sperm concentrations in all control and treated groups were significant compared to the CCl4 group G3.

3.2. Effect of PE and BPE on male reproductive hormones

Table 2 shows the effects of PE and BPE extracts on the male reproductive hormones testosterone, FSH, LH, and estradiol. The negative control group G3, administered CCl4, demonstrates a significant decrease ($p < 0.05$) in the hormonal levels of testosterone, FSH, LH, and estradiol. The hormonal levels in mice treated with silymarin showed significant results in all hormonal tests ($p < 0.05$) compared to the negative control G3. The testosterone hormone level in group G6 treated with BPE was not significant (3.055 ± 0.55) in the silymarin group, whereas it was significant in the negative control G3. In contrast, FSH levels were significantly increased after treatment with BPE and PE (2.414 ± 0.33 and 2.03 6 ± 0.19, respectively). The LH levels were significantly increased after treatment with silymarin, BPE, and PE (1.731 ± 0.19, 1.489 ± 0.16, and 1.202 ± 0.14, respectively). Estradiol levels were significantly decreased after treatment with silymarin, BPE, and PE (18.172 ± 1.49, 21.211 ± 0. 31, and 29.133 ± 1.18, respectively).

3.3. Histopathological overview of testis

3.3.1. H&E stain

G1 and G2: The lining of the Seminiferous tubules is made up of the germinal epithelium, which contains a large number of dividing cells. This epithelium includes Sertoli Cells, which are large columnar cells that extend through the entire thickness of the germinal epithelium. The germinal epithelium consists of a single layer of germ cells called spermatogonia, which rest on the basement membrane. The spermatogonia undergo division to produce Primary Spermatocytes, which cross from the basal epithelial layer to the luminal compartment of the germinal epithelium. The Primary Spermatocytes produce Secondary Spermatocytes, which rapidly divide to produce Spermatids. These Spermatids undergo spermiogenesis, ultimately transforming into sperm as shown in (Fig. 1A & B).

G3: The section shows serious damage and mild disturbance in seminiferous tubule structure with loss of interstitial tissue and irregular basement membrane, necrotic germinal epithelium, tubular deformation and degeneration of reduced in size of seminiferous tubules, increased luminal diameter, and reduced Leydig cell population. Reduction in the number of spermatogenic cells (Fig. 1C).

G4: The image displays the histological structure of seminiferous tubules that are mostly normal, but with an augmented thickness of their walls and diameter. There is a typical range of spermatogenic cells at different stages, including spermatogonia (SG), spermatocytes (SC), and spermatids (SD). Additionally, there is an elevation in the number of interstitial cells within the intertubular tissue (IT), as shown in (Fig. 1D).

G5: The section shows a partially normal appearance of the seminiferous tubules, improvement in the seminiferous tubule structure with increased interstitial tissue thickness, and degeneration of different stages of spermatogenic cells (Fig. 1E).

G6: the section shows the normal seminiferous tubule organization with normal spermatogenesis and basement membrane, and increased interstitial tissue thicknesses similar to those in the control groups (Fig. 1F).

3.3.2. Masson's trichrome

This staining was positive for collagen fibers in the tubular tissue and interstices of the *peri*-seminiferous tubule.

G1 and G2: The observation revealed the presence of a small amount of compact collagen fibers that were situated around the seminiferous tubules, the basement membrane, and the blood vessels, as depicted in (Fig. 2A & B).

G3: Collagen fibers were sloughed around the seminiferous tubules and destruction of walls of the seminiferous tubules (Fig. 2C).

G4: The image illustrates the typical quantity of compact collagen fibers surrounding the seminiferous tubules, basement membrane, and blood vessels, as shown in (Fig. 2D).

G5: The image depicts the magnitude of compact collagen fibers encircling the seminiferous tubules, basement membrane, and blood vessels, as illustrated in (Fig. 2E).

Table 2
The effects of PE and BPE extracts on the male reproductive hormones.

	Testosterone	FSH	LH	Estradiol
Control Group 1	4.448 ± 011^{c}	3.945 ± 0.32^{c}	2.877 ± 0.45^{c}	15.794 ± 0.36^{a}
Corn oil Group 2	4.455 ± 0. 31^{c}	3.811 ± 0.33^{c}	3.038 ± 0.25^{c}	16.587 ± 0.51^{ab}
CCl_4 Group 3	1.332 ± 0.13^{a}	1.060 ± 0.091^{a}	0.939 ± 0.08^{a}	34.062 ± 0.46^{e}
Silymarin Group 4	3.317 ± 0.35^{b}	3.207 ± 0.34^{c}	1.731 ± 0.19^{b}	18.172 ± 1.49^{b}
PE Group 5	2.587 ± 0.48^{b}	2.036 ± 0.19^{b}	1.202 ± 0.14^{b}	29.133 ± 1.18^{d}
BPE Group 6	3.055 ± 0.55^{b}	2.414 ± 0.33^{b}	1.489 ± 0.16^{b}	21.211 ± 0 31^{c}

According to Tukey's HSD test, (Mean ± SD) followed by different litters are significantly different. Mean ± SD followed by seem litters are not significantly different.

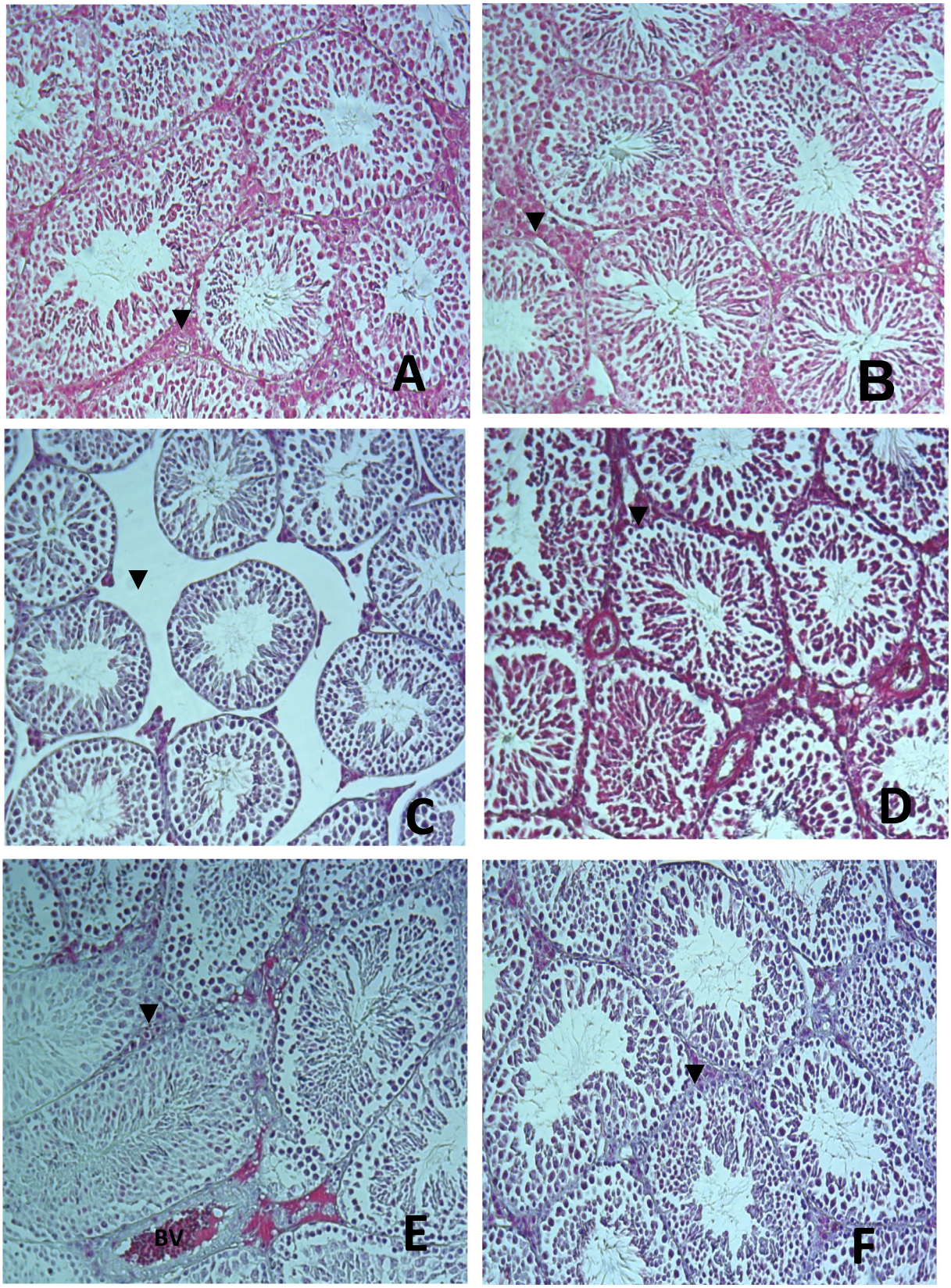

Fig. 2. A & B. The observation revealed the presence of a small number of compact collagen fibers located around the seminiferous tubules, basement membrane, and blood vessels, which are marked with a downward arrow (▼). In image C, there is an indication of the shedding of collagen fibers around the seminiferous tubules, as well as the damage of the walls of the seminiferous tubules (▼). Image D demonstrates the typical amount of dense collagen fibers around the seminiferous tubules, marked with a downward arrow (▼). Image E displays the magnitude of compact collagen fibers surrounding the seminiferous tubules, also marked with a downward arrow (▼). Finally, Image F reveals the presence of a small number of compact collagen fibers around the seminiferous tubules (▼), basement membrane, and blood vessels (BV) as identified through Masson's trichrome staining under a 100x magnification.

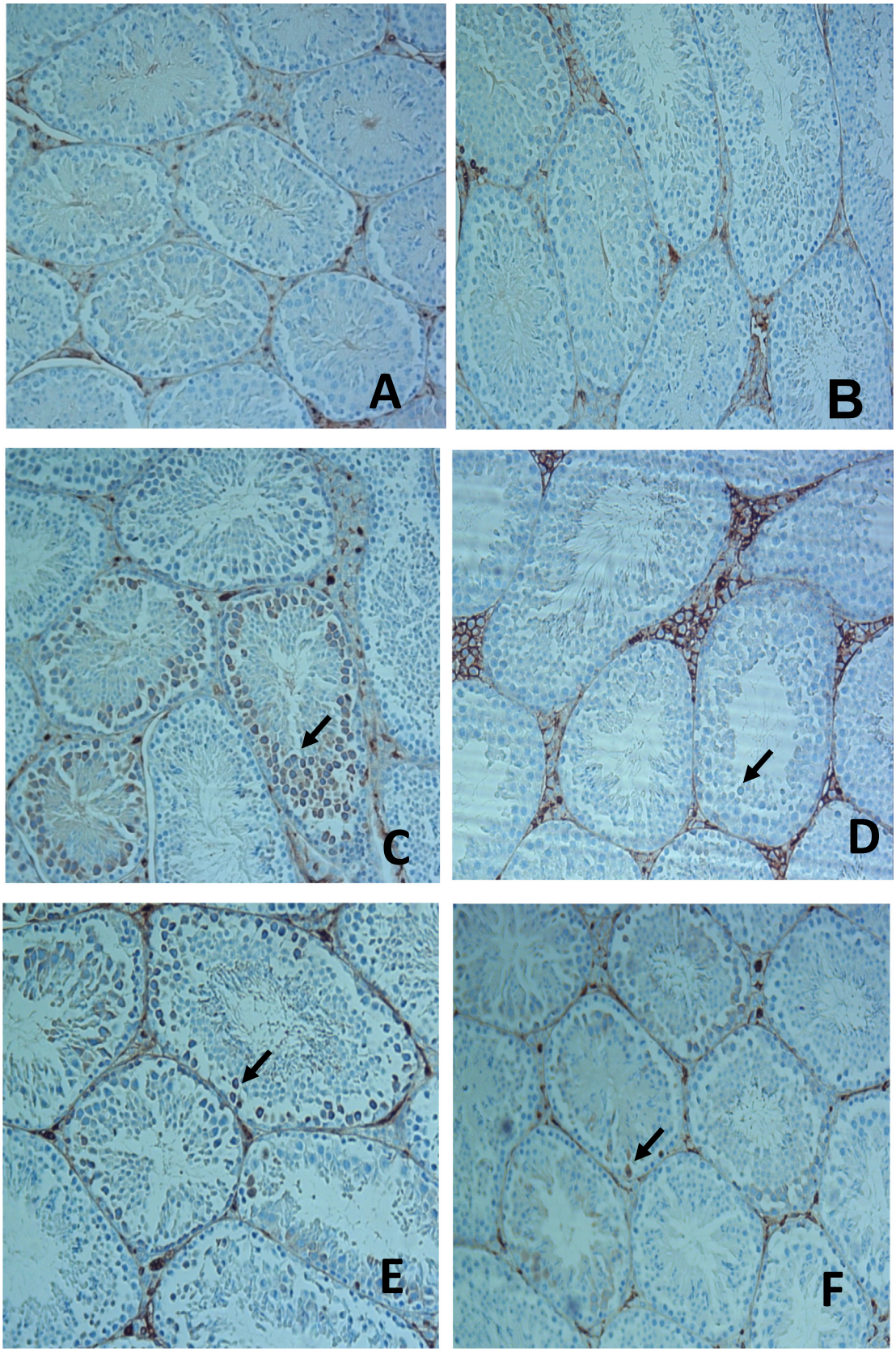

Fig. 3. A & B. showed Bcl-2-negative cells**. C:** Bcl-2-positive cells with brown immunostaining (arrow). **D:** rare number of positive Bcl-2-cells (arrow). **E:** a few Bcl-2-negative cells (arrow). **F:** very rare positive Bcl-2-cells (arrow), (Bcl-2 × 100).

G6: The observation revealed a small number of compact collagen fibers located around the seminiferous tubules, basement membrane, and blood vessels, as shown in (Fig. 2F).

3.3.3. The immunohistochemistry (of B-cell lymphoma 2 (Bcl-2)

Bcl-2 shows anti-apoptotic activity and was still traced in germ cells and seminal balls.

G1 and G2: the Bcl-2 protein expression in testicular control mice showed Bcl-2-negative cells (Fig. 3A & B).

G3: Bcl-2 protein expression in testicular treated with CCl4 showed Bcl-2-positive cells with brown immunostaining (Fig. 3C).

G4: The Bcl-2 protein expression in testicular mice showed Bcl-2-negative cells (Fig. 3D).

G5: The Bcl-2 protein expression in testicular mice showed decreased positive Bcl-2 cells (Fig. 3D).

G6: The Bcl-2 protein expression in testicular mice showed rare positive Bcl-2 cells (Fig. 3F).

4. Discussion

This research's aim was to examine the efficacy of Saudi propolis and bee pollen extracts on oxidative stress in the testes. Toxicity was demonstrated by CCl4, which induced different changes in histological and biochemical parameters in experimental mice. The current results showed high deficiency in the negative control group, which was treated with CCl4 only, compared to the positive control in the semen parameters, including sperm motility, vitality, morphological normality, concentration, and decrease in sperm density in seminiferous tubule lumen. In addition, histopathological results showed severe necrosis, atrophy, and abnormal organization of seminiferous tubules in the testes of the CCl4 group. The toxic agents used in this study were CCl4- produced chloride and trichloromethyl radicals (CCl3) that reacted with O2 to generate CCl3O2. Fatty acids are bound by CCl3 to produce alkoxy and peroxy radicals, which are the principal initiators of testicular lipid peroxidation. The testes consumed glutathione as an outcome of enhanced lipid peroxidation after exposure to CCl4. CCl3 expression increased after CCl4 exposure in the testicles. This could be referred to as the increase in PUFA peroxidation present in the testicular cell membrane, as mentioned In earlier investigations (Bruckner et al., 2002; Park et al., 2005). Abnormal hormone levels were noticed in groups treated with CCl4. However, due to the silymarin antioxidant activity and capacity to defend plasma lipids from oxidation, silymarin was shown to significantly alter the experimental groups (Thomas et al., 1997). A previous study (Oufi et al., 2012) focused on the effect of silybin (One of the structural isoforms of silymarin is silybin) on testicular tissue and proved that this flavonoid can improve testicular parameters such as the diameter of the primary spermatocytes and spermatids, motility of sperm, and percentage of live sperm. In addition, silymarin increased testosterone secretion. In the present study, PE and BPE extracts showed significant changes in male reproductive hormone levels compared to the negative control. Recent results regarding the histopathological structure of testicular and male reproductive hormone levels include testosterone, LH, FSH, and estradiol. This finding showed improved seminiferous tubule organization with semi-normal spermatogenesis and spermatozoa associated with significant changes in semen parameters, such as sperm concentration, motility, sperm vitality, and morphological normality in PE and BPE extracts. The defensive capacity of propolis is brought about by its modulatory impact on antioxidant enzymes, suppressing free radical initiation, and reducing subsequent damage (BARLAK et al., 2015). Studies on mammals have shown that propolis elevates testosterone levels in rats (Yousef et al., 2009; ElMazoudy et al., 2011). The effectiveness of PE extract as a protective treatment against CCl4 toxicity was confirmed in a recent study, as well as in a previous report that demonstrated the detrimental effect of CCl4 on lipid profile in rats (Albokhadaim, 2015; Azab et al., 2015). Numerous flavonoids are present in resinous exudates from the surface of *Dalbergia ecastaphyllum* were provided propolis its antioxidant properties (Daugsch et al., 2008; Salatino et al., 2018). This study indicated that BPE resulted in significant changes in histological, hormonal, and semen parameters. The group treated with BPE showed improvement in seminiferous tubules accompanied by a normal appearance of spermatogenesis with the existence of spermatozoa in the seminiferous tubules' lumen. In a previous study, BPE showed an ameliorative effect on the testes of diabetic rats. Moreover, the improvement in spermatogenesis is evident by a significant enhancement in the cells number in different spermatogonial stages, and Sertoli cells as compared to control negative rats, and an increase in the number of interstitial Leydig cells (Mohamed et al., 2018). However, bee pollen extract is utilized for its antioxidant properties because it contains higher amounts of bioactive compounds (Denisow et al., 2016). Bee pollen accumulates various different compounds including nutrients, and amino acids. Leucine, isoleucine, and valine-branched exogenous amino acids are abundant in bee pollen, along with fatty acids, vitamins, minerals, phenolic organic substances such as flavonoids, and phenolic acids, certain organic acids, and inorganic components, have been detected (Rzepecka-Stojko et al., 2015; Chantarudee et al., 2012; Denisow et al., 2016; Kalaycıoğlu et al., 2017).

5. Conclusion

This study demonstrated the ability of Saudi propolis and bee pollen extracts in the oxidative stress of CCl4 in a testis mouse model, as evidenced by the ameliorative effects on the histopathological structure of seminiferous tubules, as well as semen parameters including sperm concentration, motility, vitality, and morphological normality. The significant reversal of all male reproductive hormone levels, testosterone, LH, FSH, and estradiol, provided promising natural products with considerable therapeutic properties with semen and sex hormonal improvers, as well as efficient antioxidative properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was funded by the Deanship of Scientific Research at Al-Baha University, Al-Baha, KSA under grant number (No. 1440/13).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103737.

References

Abraham, P., Wilfred, G., Cathrine, S.P., 1999. Oxidative damage to the lipids and proteins of the lungs, testis and kidneys of rats during CCl4 intoxication. Clin. Chim. 9, 177–289.

Ahuja, A., Ahuja, V., 2010. Apitherapy–A sweet approach to dental diseases-Part I: Honey. J. Adv. Dent.al Res. I 1, 81–86.

Albokhadaim, I., 2015. Influence of dietary supplementation of propolis on hematology, biochemistry and lipid profile of rats fed high cholesterol diet. J. Adv. Vet. Anim. Res. 2, 56–63.
Al-Sayed, E., Abdel-Daim, M.M., Kilany, O.E., Karonen, M., Sinkkonen, J., 2015. Protective role of polyphenols from Bauhinia hookeri against carbon tetrachlorideinduced hepato-and nephrotoxicity in mice. Renal Failure. 37, 1198–1207.
Amann, R.P., 1982. Use of animal models for detecting specific alterations in reproduction. Fundam. Appl. Toxicol. 2, 13–26.
Azab, E.A., Algridi, M.A., Lashkham, N.M., 2015. Hypolipidemic and antiatherogenic effects of aqueous extract of Libyan propolis in lead acetate intoxicated male albino mice. IJSR 4, 1060–1068.
BARLAK, Y., DE\uGER, O., Ucar, M., ÇAKIRO\uGLU, T.N.I., 2015. Effects of Turkish propolis extract on secretion of polymorphonuclear elastase following respiratory burst. Turk. J. Biol. 39, 194–201.
Brock, G.B., McMahon, C.G., Chen, K.K., Costigan, T., Shen, W., Watkins, V., Anglin, G., Whitaker, S., 2002. Efficacy and safety of tadalafil for the treatment of erectile dysfunction: results of integrated analyses. J. Urol. 168, 1332–1336.
Bruckner, J.V., Ramanathan, R., Lee, K.M., Muralidhara, S., 2002. Mechanisms of circadian rhythmicity of carbon tetrachloride hepatotoxicity. J. Pharmacol. Exp. Ther. 300, 273–281.
Carpes, S.T., Begnini, R., Alencar, S.M. de, Masson, M.L., 2007. Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. Ciência e agrotecnologia. 31, 1818–1825.
Chantarudee, A., Phuwapraisirisan, P., Kimura, K., Okuyama, M., Mori, H., Kimura, A., Chanchao, C., 2012. Chemical constituents and free radical scavenging activity of corn pollen collected from Apis mellifera hives compared to floral corn pollen at Nan, Thailand. BMC Complement. Alternat. Med. 12, 1–12.
Daugsch, A., Moraes, C.S., Fort, P., Park, Y.K., 2008. Brazilian red propolis—chemical composition and botanical origin. Evid. Based Complement. Alternat. Med. 5, 435–441.
Denisow, B., Denisow-Pietrzyk, M., 2016. Biological and therapeutic properties of bee pollen: a review. J. Sci. Food Agric. 96, 4303–4309.
ElMazoudy, R.H., Attia, A.A., El-Shenawy, N.S., 2011. Protective role of propolis against reproductive toxicity of chlorpyrifos in male rats. Pestic. Biochem. Physiol. 101, 175–181.
Gupta, R.K., Stangaciu, S., 2014. Apitherapy: holistic healing through the honeybee and bee products in countries with poor healthcare system. Beekeeping for poverty alleviation and livelihood security. Springer, Dordrecht, pp. 413–446.
Halliwell, B., Gutteridge, J.M.C., 1990. [1] Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. Elsevier 186, 1–85.
Javadi, L., Farzadi, L., Fathiazad, F., Nouri, M., et al., 2011. Anti-oxidative effects of citro flavonoids on spermatogenesis in rat. Afr. J. Pharm. Pharmacol. 5, 721–725.
Kalaycıoğlu, Z., Kaygusuz, H., Döker, S., Kolaylı, S., Erim, F.B., 2017. Characterization of Turkish honeybee pollens by principal component analysis based on their individual organic acids, sugars, minerals, and antioxidant activities. LWT 84, 402–408.
Kaškonienė, V., Katilevičiūtė, A., Kaškonas, P., Maruška, A., 2018. The impact of solid-state fermentation on bee pollen phenolic compounds and radical scavenging capacity. Chem. Pap. 72, 2115–2120.
Khan, R.A., Khan, M.R., Sahreen, S., 2010. Evaluation of Launaea procumbens use in renal disorders: A rat model. J. Ethnopharmacol. 128, 452–461.
Mihai, C. M.; M\uarghita\cs, L. Al, 2010: Antioxidant capacity of Transylvanian propolis. *Bulletin of the University of Agricultural Sciences \& Veterinary Medicine Cluj-Napoca. Animal Science \& Biotechnologies*., **67**.
Mohamed, N.A., Ahmed, O.M., Hozayen, W.G., Ahmed, M.A., 2018. Ameliorative effects of bee pollen and date palm pollen on the glycemic state and male sexual dysfunctions in streptozotocin-Induced diabetic wistar rats. Biomed. Pharmacother. 97, 9–18.
Oufi, H.G., Al-Shawi, N.N., Hussain, S.A.R., 2012. What are the effects of silibinin on testicular tissue of mice? J. Appl. Pharmaceut. Sci. 2, 9–13.
Park, W.H., Lee, S.K., Kim, C.H., 2005. A Korean herbal medicine, Panax notoginseng, prevents liver fibrosis and hepatic microvascular dysfunction in rats. Life Sci. 76, 1675–1690.
Presnell, J.K., Schreibman, M.P., Humason, G.L., 1997. Humason's animal tissue techniques. Johns Hopkins University Press.
Rowe, T., 2006. Fertility and a woman's age. J. Reprod. Med. 51, 157–163.
Rzepecka-Stojko, A., Stojko, J., Kurek-Górecka, A., Górecki Michałand Kabała-Dzik, A., Kubina, R., Moździerz, A., Buszman, E., 2015. Polyphenols from bee pollen: structure, absorption, metabolism and biological activity. Molecules 20, 21732–21749.
Salatino, A., Salatino, M.L.F., 2018. Brazilian red propolis: legitimate name of the plant resin source. MOJ Food Process. Technol. 6, 21–22.
Seed, J., Chapin, R.E., Clegg, E.D., Dostal, L.A., Foote, R.H., Hurtt, M.E., Klinefelter, G.R., Makris, S.L., Perreault, S.D., Schrader, S., et al., 1996. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. Reprod. Toxicol. 10, 237–244.
Selamoglu, Z., 2019. APITHERAPY AND BIOMEDICAL APPLICATIONS. Applied Natural Sciences 2019, 25.
Thomas, M.J., Chen, Q., Franklin, C., Rudel, L.L., 1997. A comparison of the kinetics of low-density lipoprotein oxidation initiated by copper or by azobis (2-amidinopropane). Free Radic. Biol. Med. 23, 927–935.
Tohamy, A.A., Abdella, E.M., Ahmed, R.R., Ahmed, Y.K., 2014. Assessment of anti-mutagenic, anti-histopathologic and antioxidant capacities of Egyptian bee pollen and propolis extracts. Cytotechnology 66, 283–297.
Weber, L.W.D., Boll, M., Stampfl, A., 2003. Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit. Rev. Toxicol. 33, 105–136.
Yousef, M.I., Salama, A.F., 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. Food Chem. Toxicol. 47, 1168–1175.