


ORIGINAL ARTICLE

## Defensive impact of propolis against CCl<sub>4</sub> actuated rats' testicular damage

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### ABSTRACT

**Objective:** The paper's primary goal is to report the devastating impact of carbon tetrachloride (CCl<sub>4</sub>) on rat testicular tissue and the possible protecting function of propolis against CCl<sub>4</sub> based on its free radical scavenging and inflammatory relief properties.

**Materials and Methods:** A total of 24 adult male albino rats had been classified into four groups (six rats/group). Rats of group 1 served as control, whereas groups 2–4 received propolis (200 mg/kg/day), CCl<sub>4</sub> (3 ml/kg/day), and propolis/CCl<sub>4</sub>, respectively. After 4 weeks, the collected sera were applied for the estimation of lipid profile and sex hormones. Also, histopathological picture, malondialdehyde, and tumor necrosis factor alpha (TNFα) gene profile was measured in collected testicular tissues.

**Results:** The present information revealed a noteworthy change ( $p < 0.05$ ) in lipid profile, decrease in testicular weight, testosterone, antioxidants values along with a prominent increase ( $p < 0.05$ ) in estradiol, lipid peroxidation values, and expression of TNFα in rats administrated with CCl<sub>4</sub> compared to control. Moreover, the histopathological profile showed the degeneration of the epithelium. Interestingly, propolis attenuated the destructive effect of CCl<sub>4</sub> on rat testes.

**Conclusion:** The examined dose of propolis reduced oxidation, and inflammatory reactions resulted from CCl<sub>4</sub> exposure and proved that it might have a helpful part in free radicals interceded diseases.

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### KEYWORDS

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### Introduction

Carbon tetrachloride (CCl<sub>4</sub>) is a compound that damages the testes, kidneys, liver, brain, and lungs [1]. Testicular toxicity had been reported as a consequence of oxidative stress (OS) state, following CCl<sub>4</sub> exposure [2,3]. CCl<sub>4</sub> is metabolized within the liver, creating exceedingly reactive and deadly trichloromethyl free radicals like carbon trichloride (CCl<sub>3</sub>). These free radicals are changed over to trichloromethyl peroxyradical through the cytochrome P450 oxygenase enzyme-producing OS condition. CCl<sub>4</sub> starts the autoxidation of lipids through binding to the cytoplasmic membrane polyunsaturated fatty acids, resulting in membrane damage, reducing enzyme activity, disrupting sexual hormones, and finally inducing necrosis [4]. During initial inflammatory processes, OS is associated with elevated cytokines as tumor necrosis factor alpha (TNFα) and Interleukine-1 (IL-1) [5,6]. Inflammation takes place in nearly all disease processes, involving immunological and vascular complications, cancer, sepsis, and metabolic

damage. Kupffer cells activated by CCl<sub>4</sub> start a pathway of inflammatory mediators through the upregulation of TNF-α, IL-1b, and IL-6 expressions [7]. TNF-α is a pro-inflammatory cytokine that regulates a broad range of physiological events, including apoptosis and inflammatory processes [8] and impacting various diseases as diabetes [9,10].

Testicles include an extensive amplification of antioxidant enzymes and free radical scavengers against OS. This antioxidant shield is of significant importance because peroxidative damage is currently considered the most critical reason behind impaired testicular functioning. It supports the pathological consequences of many conditions, from testicular torsion to diabetes xenobiotic exposure. The body can be protected against various oxidative stresses caused by free radicals through body and food antioxidants [11].

Egyptians, Greeks, and Romans used propolis as a portion of human medication for thousands of years, benefiting from its properties [12]. Propolis is considered

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a powerful antioxidant used in the preparation of functional foods and food supplements. It may help prevent and in the dietary control of patients with chronic diseases caused by OS [13]. Propolis is regarded as a resinous material harvested by honeybees from distinctive plants. It contains many biochemical constituents, like polyphenols, flavonoids, aglycones, phenolic, and ketones, with several biological and pharmacological properties [14]. It has gastroprotective, hepatoprotective, immunomodulatory, wound healing, antidiabetic, and antineoplastic functions, owing to propolis' antioxidant, anti-inflammatory, and antimicrobial properties [15,16]. Propolis inhibits the generation of various inflammatory markers, such as nitric oxide, IL-1 and IL, due to its high flavonoid content [12]. Propolis' defensive capacity is brought about from its modulator impact on antioxidant enzymes, suppressing free radicals initiation, and reducing subsequent damage [17]. Studies on mammals clarified that propolis elevated testosterone levels in rats [18,19] and markedly increased rabbit body weight (B.W.), the relative weight of the testes, and epididymis [20]. Caffeic acid phenyl ester (CAPE), a propolis active compound, has been proven to inhibit nuclear factor kappa B and TNF $\alpha$  [21,22]. Testosterone level, lipid profile, and pathological alterations in testes of male rats induced by different chemicals / pesticides as chlorpyrifos were estimated by ElMazouly et al. [19]; however, estradiol level, OS, and antioxidants, TNF  $\alpha$  were not measured too. The current study aimed to elucidate the defensive impact of propolis on CCl<sub>4</sub> testicular damage in male rats based on its antioxidant and anti-inflammatory properties reflecting on selected biochemical, histopathological, and molecular indices.

## Materials and Methods

### Ethical approval

All experimental procedures are maintained and carried out by national guidelines and protocols, authorized by the Alexandria University Institutional Animal Care and Use Committee (3082020).

### Chemicals and reagents

CCl<sub>4</sub> was purchased from Central Drug House, India. Olive oil and bee propolis extract capsules were obtained from Best Naturals, USA. Kits for Total Cholesterol (TC), and triacylglycerol (TAG) were obtained from Vitro Scient Co, Egypt. High-density lipoprotein cholesterol (HDL-c) kit had been received from Spectrum Co, Egypt. Testosterone and estradiol kits were obtained from Cayman Chemical Co., USA. Malondialdehyde (MDA), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPX), and superoxide dismutase (SOD) kits were obtained from Bio diagnostic Co, Egypt. RNeasy Mini Kit (Catalogue #74104)

and Quantitect SYBR green PCR kit (Catalogue # 204141) were obtained from Qiagen, USA.

### Experimental animals

This investigation was carried out with strict rules to maintain and defend animal welfare without subjecting them to any degree of endurance or stress. Twenty-four male rats weighing 150  $\pm$  10 gm were purchased from an Egyptian company to produce vaccine, sera and drugs. Animals were harbored in clean metal cages with a 12 h day-night cycle, the temperature of 22  $\pm$  2.0 and humidity of 45%  $\pm$  1. The rats were fed commercial diet pellets and allowed food and water *ad libitum* for an adaptation period of 2 weeks. To investigate the damage of CCl<sub>4</sub>, animals were distributed randomly into four groups (six rats/group) during the 4-week experiment period. Group I (control): injected i.p. with olive oil as a vehicle (3 ml/kg B.W./twice/week). Group II (Propolis) injected i.p., with olive oil and treated orally with propolis (200 mg/kg B.W./daily) [23]. Group III (CCl<sub>4</sub>) injected i.p. with CCl<sub>4</sub> (3 ml/ kg B.W./ twice/week) 1:1 diluted with olive oil [24]. Group IV (propolis/ CCl<sub>4</sub>) was treated with propolis and CCl<sub>4</sub>, as mentioned before.

### Blood collection

After 4 weeks, rats fasted for 12 h, anesthetized, and blood samples were drawn from the eye's retro-orbital sinus. The blood samples were let to coagulate, centrifuged at 3,000 rpm for 10 min, and kept frozen at -20°C until used to determine serum lipid profile and sexual hormones (testosterone and estradiol).

### Tissue preparation

Animals were anesthetized and sacrificed by cervical dislocation directly after gathering blood samples. Testes were rapidly removed, washed by the ice-cold saline buffer, blotted in filter papers, weighted, and split into three parts. The first part was directly soaked up in 10% formalin solution for histological studies. Expression of TNF $\alpha$  was determined using the second portion which was frozen quickly in liquid nitrogen and kept at -80°C. The third part was weighed and homogenized, using tissue lyser with ice-cold saline preparing 25% w/v homogenate and kept frozen at -20°C to measure the MDA level antioxidant enzyme activities.

### Determination of biochemical parameters

Enzyme-linked immunosorbent assay kits were used for the determination of testosterone and estradiol concentrations [25]. The concentrations of TC, TAG, and HDL-c have been estimated according to the procedures reported previously [26-28]. The concentrations of very low-density lipoprotein cholesterol (VLDL-c) and low-density lipoprotein cholesterol (LDL-c) were calculated as mentioned

previously [29]. The level of MDA was evaluated in testicular tissues according to Satoh [30]. GSH, GPX, CAT, and SOD activities were determined in testicular tissue by methods described earlier [31–34].

#### Gene expression analysis of TNF- $\alpha$ using quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from testicular tissues using the RNeasy mini kit (Qiagen, USA) according to the manufacturer's guides. qRT-PCR using 2 $\times$  QuantiTect SYBR Green PCR Master Mix was carried out to estimate relative quantitative determination of the gene expression TNF $\alpha$  mRNA level using a real-time PCR machine (Stratagene MX3005P). Amplification was carried out using the following protocol: 94°C for 15 sec for both  $\beta$ -actin and TNF- $\alpha$ ; 55°C for 30 sec for B-actin and 60°C for 30 sec for TNF- $\alpha$ ; and 72°C for 30 sec for both genes. Relative changes in mRNA levels were determined by the “ $\Delta\Delta Ct$ ” method, as stated previously [35]. Table 1 shows the sequence of the used primer.

#### Histopathological examination

Immediately after sacrificing, testes were quickly immersed in 10% buffered formalin solution for 48 h. After that, the samples were transformed through the paraffin embedding method and were stained with hematoxyline and eosin (H&E) according to the procedures mentioned previously [36].

#### Statistical analysis

The analysis was carried out using a one-way analysis of variance. Data were expressed as means  $\pm$  standard error of the mean.  $p < 0.05$  was set as statistically significant [37].

**Table 1.** Primer sequences of the amplified genes.

Gene	Primer sequence(5'-3')	Reference
Rat $\beta$ -actin	TCCTCTGAGCGCAAGTACTCT	[69]
	GCTCAGTAACAGTCCGCTAGAA	
TNF- $\alpha$	ACT GAA CTT GGG GGT GATTG	[70]
	GCT TGG TGG TTT GCT ACG AC	

TNF- $\alpha$ : Tumor necrosis factor alpha

## Results

The data concluded in Table 2 illustrate that testes weight and testes/B.W. (%) were markedly increased ( $p < 0.05$ ) in CCl<sub>4</sub>-treated rats (group III) in comparison with group I. On the other hand, rats protected with propolis (200 mg/kg/B.W.) successfully reduced ( $p < 0.05$ ) the increased testes weight and testes/B.W. (%) over group III. Rats in group II showed no significant impact on previous values as compared to rats in group I. CCl<sub>4</sub> administration induced a prominent decrease ( $p < 0.05$ ) in serum testosterone and considerable elevation in estradiol concentration (10.13  $\pm$  0.21a) over group I. Propolis reversed alterations of CCl<sub>4</sub> in both serum level of testosterone and estradiol. Moreover, it boosted the testosterone level over control rats.

The outcomes shown in Table 3 revealed that serum TC, TAG, and LDL-c levels were increased markedly ( $p < 0.05$ ); however, serum HDL-c was significantly decreased ( $p < 0.05$ ) in group III as compared to group I. Propolis induced a pronounced hypolipidemic activity as reflected in a significant decrease ( $p < 0.05$ ) of serum TC, TAG, VLDL-c, and LDL-c levels and a significant increase ( $p < 0.05$ ) of HDL-c compared to group III. Serum HDL-c increased significantly, while LDL-c level was decreased markedly ( $p < 0.05$ ) in group II compared to group I.

Data in Table 4 reveal that CCl<sub>4</sub> significantly increased ( $p < 0.05$ ) the testicular level of MDA and significantly decreased ( $p < 0.05$ ) the GSH levels and activities of GPX, GSH, CAT, and SOD in contrast to group I. Propolis successfully decreased ( $p < 0.05$ ) MDA concentration and showed significant antioxidant proprieties as reflected in the prominent increase ( $p < 0.05$ ) in GSH level and antioxidant enzymes activities (GPX, CAT, and SOD) compared to group III. Besides, propolis significantly improved ( $p < 0.05$ ) the antioxidant capacity and attenuated peroxidation in group II in comparison with group I.

CCl<sub>4</sub> caused marked inflammation as reflected on significant upregulation ( $p < 0.05$ ) TNF $\alpha$  gene expression in contrast with group I (Table 5). The combined administration of propolis induced the downregulation ( $p < 0.05$ ) of TNF $\alpha$  gene expression compared to group I (Table 5), attenuating the testicular inflammation caused by CCl<sub>4</sub>.

**Table 2.** Effect of propolis on testes weight, testis/ B.W. (%), testosterone, and estradiol in rats treated with CCl<sub>4</sub>.

Groups	Estradiol(ng/ml)	Testosterone(ng/ml)	Testis/body weight (%)	Testis weight (gm)	Final body weight (gm)
Group I	3.23 $\pm$ 0.45d	3.87 $\pm$ 0.13c	2.52 $\pm$ 0.12c	5.77 $\pm$ 0.31c	228.80 $\pm$ 1.77a
Group II	4.10 $\pm$ 0.37d	5.22 $\pm$ 0.11a	2.41 $\pm$ 0.12c	5.54 $\pm$ 0.22c	229.40 $\pm$ 2.25a
Group III	10.13 $\pm$ 0.21a	1.9 $\pm$ 0.09b	1.20 $\pm$ 0.14a	2.56 $\pm$ 0.29a	211.60 $\pm$ 4.15b
Group IV	7.76 $\pm$ 0.08b	3.07 $\pm$ 0.06d	2.13 $\pm$ 0.21c	4.75 $\pm$ 0.39c	223.00 $\pm$ 3.24a

Values are mean  $\pm$  standard errors.

Means in the same column with different letters are significantly different at  $p < 0.05$ .

Group I (control); Group II: Propolis 200 mg/kg B.W.; Group III: CCl<sub>4</sub>-treated rats 2 ml/kg B.W./i.p.; and Group IV: CCl<sub>4</sub> + propolis.

**Table 3.** Effect of Propolis on Serum lipid profile in rats treated with CCl<sub>4</sub>.

Groups	HDL-c (mg/dl)	LDL-c(mg/dl)	vLDL-c (mg/dl)	TAG (mg/dl)	TC(mg/dl)
Group I	48.5 ± 1.59b	34.27 ± 0.6b	16.86 ± 0.66b	84.3 ± 3.32b	99.6 ± 3.15cb
Group II	60.3 ± 1.96c	16.18 ± 0.03c	15.92 ± 1.42b	79.6 ± 7.11b	92.4 ± 3.21c
Group III	22 ± 0.67a	84.4 ± 1.18a	29.2 ± 1.21a	146.0 ± 6.07a	136 ± 3.06a
Group IV	35.2 ± 1.07d	58.4 ± 1.61d	24.4 ± 1.11d	122 ± 5.54d	118 ± 3.79d

Values are mean ± standard errors.

Means in the same column with different letters are significantly different at  $p < 0.05$ .

Group I (control); Group II: Propolis 200 mg/kg. B.W.; Group III: CCl<sub>4</sub>-treated rats 2 ml/kg B.W./i.p.; and Group IV: CCl<sub>4</sub> + propolis.

TC = Total cholesterol; TAG = Triacylglycerol; vLDL-c = Very low-density lipoprotein cholesterol; LDL-c = Low-density lipoprotein cholesterol; HDL-c = High-density lipoprotein cholesterol.

**Table 4.** Effect of propolis on testes lipid peroxidation, GSH, and antioxidant enzymes in rats treated with CCl<sub>4</sub>.

Groups	SOD (IU/gm wt tissue)	CAT (IU/gm wt tissue)	GSH (μmol/gm wt tissue)	GPX (IU/gm wt tissue)	MDA (nmol/gm wt tissue)
Group I	131.23 ± 1.18e	412.12 ± 3.16e	37.89 ± 1.68c	8.27 ± 0.23c	24.40 ± 0.55de
Group II	134.12 ± 1.23e	409.5 ± 3.11e	50.70 ± 2.33a	12.20 ± 0.22a	14.42 ± 0.91a
Group III	85.92 ± 1.1b	135.72 ± 2.3b	19.82 ± 1.61b	3.08 ± 0.25b	60.85 ± 5.88c
Group IV	115.13 ± 1.2c	310.37 ± 2.6c	31.35 ± 2.15e	6.04 ± 0.55e	40.73 ± 4.73b

Values are mean ± standard errors.

Means in the same column with different letters are significantly different at  $p < 0.05$ .

Group I (control); Group II: Propolis 200 mg/kg. B.W.; Group III: CCl<sub>4</sub>-treated rats 2 ml/kg B.W./i.p.; and Group IV: CCl<sub>4</sub> + propolis.

MDA = Malondialdehyde; GPX = Glutathione peroxidase; GSH = Reduced glutathione; CAT = Catalase; SOD = Superoxide dismutase.

**Table 5.** Effect of propolis on testes gene expression of TNF-α in rats treated with CCl<sub>4</sub>.

Groups	Expression fold change (2-ΔΔct)
Group I	1 ± 0.02e
Group II	0.35 ± 0.01a
Group III	3.7 ± 0.12b
Group IV	2.1 ± 0.11c

Values are mean ± standard errors.

Mean in the same column with different letters are significantly different at  $p < 0.05$ .

Group I (control); Group II: Propolis 200 mg/kg B.W.; Group III: CCl<sub>4</sub>-treated rats 2 ml/kg B.W./i.p.; and Group IV: CCl<sub>4</sub> + propolis. TNF-α: Tumor necrosis factor alpha.

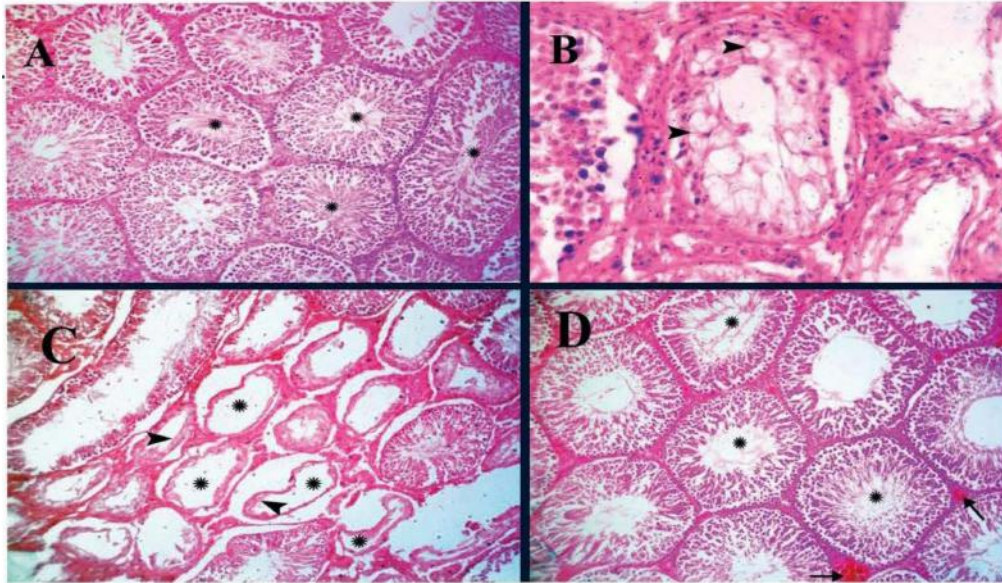
As indicated in Figure 1A, no histopathological alterations have been recorded in rat testes of group I and group II. Also, the seminiferous tubules and interstitial tissue showed standard histological criteria (Fig. 1A). On the contrary, the rats' testes in group III revealed congestion in testicular blood vessels and interstitial capillaries. The interstitium showed edema as it was mildly extended by homogenous eosinophilic material. Multifocal, moderate numbers of seminiferous tubules exhibited marked degeneration of lining epithelial cells characterized by swollen pale discrete large vacuoles, usually replacing the cytoplasm and displaced the nucleus periphery of the cell (Fig. 1B). Occasionally, the degenerated tubules showed

collapse, with thick buckled basement membrane and lined by single or two layers of degenerated germ cells. These changes were frequently accompanied by reduced spermatogenesis and spermatozoa's absence in the degenerated tubules lumen (Fig. 1C). Testes of rats received combined doses of propolis and CCl<sub>4</sub> (group IV) showed the normal histological appearance of seminiferous tubules, mild congestion of testicular blood vessels, and moderate active spermatogenesis (Fig. 1D)

## Discussion

Normal cellular function is maintained through keeping equilibrium between the reactive oxygen species (ROS) and the antioxidants [38]. ROS formed either typically during cellular metabolism or particular chemicals exposure [39]. Spermatozoa are permanently exposed to the "oxygen paradox", resulting in excessive generation of ROS involved in male infertility [40].

Cell damages induced by ROS could be slowed or prevented by antioxidants [41]. Propolis has many biological activities as anti-inflammatory, anticancer, antioxidant, antibiotic, and antifungal activities [20,42]. Recently, propolis was stated as a potent scavenger of ROS [43]. The observed significant reduction of testes weight, testes/B.W. ratio, and testosterone concentration in rats were administered with CCl<sub>4</sub> compared to control ones caused by the CCl<sub>4</sub> toxic effect. CCl<sub>4</sub> can generate free radicals leading to



**Figure 1.** The histopathological findings of rat testes. (A): Groups (I and II) showing a normal histological appearance of seminiferous tubules (asterisk); H&E stain  $\times 200$ . (B): Testis of rat from  $\text{CCl}_4$ -treated group (III) showing testicular degeneration characterized by swollen pale discrete large cytoplasmic vacuoles (arrowhead) in the germinal epithelium; H&E stain  $\times 400$ . (C) Testis of rats from the group (III) showing degeneration of the lining epithelium (arrow) of few seminiferous tubules accompanied by reduced spermatogenesis and absence of spermatozoa in the lumen (asterisk); H&E stain  $\times 100$ . (D): Group (IV) showed normal histological criteria of the seminiferous tubules with moderate active spermatogenesis (asterisk) and mild congestion of testicular blood vessels (arrow); H&E stain  $\times 200$ .

OS affecting testicular germline, testicular weight, or even testosterone level. Male infertility could be caused directly by free radicals or indirectly through disrupting male hormonal balance leading to reproductive dysfunction [44]. A pronounced drop in testosterone confirmed alterations in rats' reproductive physiology. The reduction in testosterone production may activate P450 aromatase, which favors estrogen production from androgen, thereby decreasing androgen levels [19]. The recorded significant increase of estradiol level may indicate dysregulation of the pituitary function as an insult of  $\text{CCl}_4$  exposure [45–47]. The current finding regarding testicular weight, testosterone, and estrogen concentrations confirmed by histopathological findings of the testis indicated a degeneration of testicular tissue and the lining epithelium of a few seminiferous tubules accompanied by reduced spermatogenesis and absence of spermatozoa in group III. A previous report [48] stated that administration of  $\text{CCl}_4$  to normal rats induced a marked reduction in body weight, testes, seminal vesicles, and prostate glands; moreover, there was marked testicular degeneration, lowered semen quality and quantity, a marked reduction of testosterone, luteinizing hormone and follicle-stimulating hormone concentrations with a prominent increase of rat serum concentration of prolactin and estradiol.

The propolis defensive role against the detrimental impact of  $\text{CCl}_4$  in testes weight, testes/B.W. ratio, and sex

hormones (testosterone and estradiol) may be explained by the antioxidant capacities of phenols and flavonoids or other described constituents [49–53] protecting the reproductive system against toxicity. Other reports [20,54] revealed that propolis provides elevation in testosterone level, body, and testes weights with reductions of oxidants levels. The observed antioxidant effects came in harmony with histopathological findings that indicated the typical architecture of testes of propolis-treated rats.

The significant increase of TC, TAG, LDL-c, and VLDL-c and pronounced drop of HDL-c in rats treated with  $\text{CCl}_4$  following the previous reports [55,56] demonstrated that  $\text{CCl}_4$  increased peroxidation of polyunsaturated fatty acid and oxidants concentrations. The increased level of cholesterol is owed to reduced androgen concentration, as mentioned in Table 2, which agrees with ElMazoudy et al. [19]. The observed ameliorative impact of Propolis on the detrimental effect of  $\text{CCl}_4$  on lipid profile has been documented earlier in rats [57,58], particularly TAG level. Propolis hypolipidemic role might be exhibited through lipase stimulation and modulation of metabolism of lipid [59]. Moreover, Alves et al. [60] cited that propolis showed hypocholesterolemic effect attributed either directly to the hepatic role or indirectly to the thyroid hormones, as these hormones affect the fat metabolism.

Antioxidants relieve stress conditions produced from increased oxidants over antioxidants through either

enzymatic or non-enzymatic pathways. Excessive ROS could produce lipid peroxidation, damage DNA, RNA, and denatured protein in the spermatozoa [44,45,61]. Testicular lipid peroxidation was increased after CCl<sub>4</sub> exposure as the testicular MDA was increased parallel to depletion of GSH content and the reduction activities of GPX, CAT, and SOD. This could be referred to as the increase in polyunsaturated fatty acids peroxidation that presents in the testicle cell membrane. The phase activates the CCl<sub>4</sub> I cytochrome P450 system to form reactive metabolic trichloromethyl radicals (CCL3), which is the principal initiator of lipid peroxidation. CCl<sub>4</sub> consumed GSH as an outcome of enhancing lipid peroxidation, as mentioned by Park et al. [62]. In the current research, the antioxidant system (SOD, CAT, GPX, and GSH) in CCl<sub>4</sub>-treated groups was prominently reduced compared to group I. CCl<sub>4</sub> generated cellular ROS subsequently depleted of the antioxidant cellular system as mentioned by El-Boshy et al. [63]. OS indicated by elevated testicular MDA may also be caused by elevated estradiol in group III due to high estradiol testicular fatty acid degeneration [47]. Chronic inflammation is linked with OS as ROS is considered as second messengers that propagate pro-inflammatory signals that's why rats treated with CCl<sub>4</sub> showed upregulation of TNF $\alpha$  expression compared to control. This was confirmed by current histopathological findings that cleared inflammatory testicular degeneration characterized by swollen pale discrete large cytoplasmic vacuoles and edema as indicated by expanded interstitium due to homogenous eosinophilic material.

Infertility has been associated with testicular OS recommending the need to develop powerful antioxidant therapies facing this condition [64]. Preceding results cleared that propolis treatment ameliorated the CCl<sub>4</sub>-induced imbalance in the oxidant-antioxidant system of testes due to its antioxidant properties and successfully increased the intracellular concentration of glutathione. Glutathione has crucial participation in maintaining antioxidant and down-regulating cytokine transcription and biosynthesis. Therefore, pre-treatment of propolis downregulated expression of TNF- $\alpha$  and successfully increased SOD, CAT, and GPX activities in CCl<sub>4</sub>-treated rats. These findings came in parallel with other works [7,65,66], which stated that propolis increased concentration of reduced glutathione and SOD activity and reduced lipid peroxidation processes in plasma, liver, lungs, and brain of mice in a dose and tissue-dependent manner. Propolis showed potent anti-inflammatory properties due to CAPE, a chief element of propolis, and may act through suppressing pro-inflammatory cytokines or mediators like TNF $\alpha$  and interleukins [67,68]. Validation of approach of the present research as clinical therapies has to be performed in the future. Besides expression of antioxidants and cytokines genes during propolis supplementation in CCl<sub>4</sub> intoxicated rats has to be conducted.

## Conclusion

The current research concluded that the used dose of propolis (200 mg/kg/B.W.) is safe and capable of ameliorating CCl<sub>4</sub> testicular damage in rats. Propolis showed a protective effect. It successfully improved antioxidants and alleviated OS conditions, reduced TNF $\alpha$ , corrected hormone levels, and replaced damaged tissues with healthy ones; however, constructive studies must be accomplished to approve these trials as clinical treatments.

## List of abbreviations

CCl<sub>4</sub>: Carbon tetrachloride, TNF $\alpha$ : Tumor necrosis factor alpha, CCL3: trichloromethyl radical, IL-1: Interleukine-1, CAPE: Caffeic acid phenyl ester, TC: Total cholesterol, TAG: TriAcylGlycerol, HDL-c: High-density lipoprotein cholesterol, MDA: Malondialdehyde, GSH: Reduced glutathione, GPX: Glutathione peroxidase, SOD: Superoxide dismutase, VLDL-c: Very low-density lipoprotein cholesterol, LDL-c: Low-density lipoprotein cholesterol, CAT: Catalase, qRT-PCR: Quantitative real-time polymerase chain reaction, H&E: Hematoxyline and eosin, OS: Oxidative stress, ROS: Reactive oxygen species.

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## Conflict of interest

The author declares that there is no conflict of interest related to this research.

## Author's contribution

ASH is responsible for designing, experimentation, analysis, and manuscript preparation and finalization.

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