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Research Article

The Possible Protective Role of Aqueous Extract of Propolis against Hepatotoxicity, Renal and Testicular Toxicity Induced by Cadmium Chloride in Male White Rats

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Abstract | Cadmium chloride (CC) is a harmful contaminant produced through the manufacturing of batteries, dyes, plastics, fertilisers, and cigarette smoke. Also, CC exposure causes decreased liver, kidney, and testicular functioning, as well as significant histopathological abnormalities. Therefore, the present study investigated the toxic effect of CC and the protective effect of Propolis aqueous extract on CC-induced toxicity in male rats. A total of 24 Wistar albino rats were included in this study, divided into four groups; each group containing 6 rats and administered the treatment doses orally by gavage for 28 days. Control, treated with a physiological solution (5.0 ml/rat). CC-treated group, treated with CC 15 mg/kg/bw. CC+propolis, treated with CC 15 mg/kg/bw + 30 mg/kg/bw propolis. Propolis-treated group, treated with 30 mg/kg/bw propolis. Blood samples were collected for biochemical analysis, and tissue samples (liver, kidney, and testes) were collected for histopathological examination. The results showed that the presence of CC increased (P<0.001) the levels of liver enzymes (ALT; alanine aminotransferase and AST; aspartate aminotransferase), creatinine and urea in the blood serum and lipid peroxidation product (TBARS). While decreased (P<0.001) the total antioxidant capacity (TAC) and number of spermatozoa compared with control group. The histological examination of the kidney revealed alterations induced by CC, including atrophy, tubular necrosis, enlargement of the glomeruli, dilatation and congestion of the glomerular capillaries (G) and the blood vessels around the renal tubules, atrophy in some renal glomeruli, hemorrhage, and around edema interstitial the blood vessels. It is concluded that utilizing propolis with CC considerably reduced the biochemical and histological damage produced by CC, and it is suggested that propolis could be utilized as a cytoprotective agent against CC pathological toxicity.

Keywords | Propolis, Inflammation, Oxidative stress, Antioxidant, Cadmium chloride toxicity, Histopathology, Herbal medicine, Biochemical indices

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INTRODUCTION

Worldwide, natural products and medicinal plants have been used for thousands of years. Particularly, the extracts and oils from these plants have been employed in a number of pharmacological, complementary medicine, and natural therapy applications (Ibrahim et al., 2018; Abdel-Ghfar et al., 2022). Furthermore, medicinal products became more popular than synthetic ones. Antioxidant-rich compounds have been discovered as possible therapeutic agents in this area (Ahmed et al., 2022b; Al-Syaad et al., 2023a).

The word propolis has been derived from Greek in which Pro means in front of and polis means community or city, which means hive defensive substance (Anjum et al., 2019). It is also known as Bee-glue, and it is a natural resin (wax-like) substance found in bee hives that Honey bees use as a cementing agent to bind open areas and fissures in their hives (Pahlavani et al., 2020). The overall chemical composition of most propolis is quite similar. It is composed of resins (50%), bee wax (30%), essential and aromatic oils (10%), pollen (5%), and other organic components (5%), according to (Gómez-Caravaca et al., 2006; Anjum et al., 2019). Esters, flavonoids, terpenes, beta-steroids, aromatic aldehydes, and alcohols are among the important organic components discovered in propolis (Huang et al., 2014). Propolis contains vitamins B1, B2, and B6, as well as minerals magnesium, calcium, potassium, iron, and a few enzymes; succinic dehydrogenase, glucose-6-phosphatase, adenosine triphosphates, and acid phosphatase (Lotfy, 2006). The phenolic compounds contribute to the antioxidant, antimicrobial, antiviral, anti-inflammatory, antifungal, wound healing and cardio protective functions (Huang et al., 2014; Ali et al., 2019; Hussein et al., 2022).

Cadmium is an environmental and industrial toxic pollutant produced from the manufacture of batteries, dyes, plastics, fertilizers and cigarette smoke (Genchi et al., 2020). Furthermore, CC-toxicity is due to the fact that humans consume animals and plants that efficiently absorb cadmium and deposit it in their tissues (Fiamegkos et al., 2015; Genchi et al., 2020). However, CC-oxidative stress, which leads to the occurrence of many serious pathological conditions, as a result of its long-term accumulation in specific tissues, particularly the liver and kidneys, at a rate of up to 75% of the total quantity of cadmium in the body (Bellinger et al., 2004; Stohs and Bagchi, 1995). Järup (2002) indicated that exposure to cadmium leads to impaired liver and kidney functions, with serious histopathological changes.

Nonetheless, there has been little research on propolis's protective capacity against hepatotoxicity, renal toxicity, and testicular toxicity. Therefore, the current study seeks to explore the efficacy of aqueous propolis extract in reducing the toxic effects of cadmium chloride poisoning through histological and histochemical changes in white rats' hepatic, renal, and testicular tissues.

MATERIALS AND METHODS

Study location

This study was conducted at the Physiology Lab., Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia.

Animals and experimental design

Twenty four male Wistar albino rats (*Rattus norvagicus*) apparently healthy and clinically free of diseases, with body wight ranged form150 to 200 g, and aged 8-10 months, were included in this study. The animals were obtained from the Animal house, Department of Biology, College of Science, King Khalid University. The rats housed in plastic cages, lighting cycle was organized under standard conditions (12 hours) daily lighting, and water and feed were provided to them *ad libitum*, and ventilation was set appropriately. The floor of those cages was covered with dry sawdust to facilitate the cleaning process and to bring the experimental animals closer to the natural environment. The rats were randomly divided into four groups (n - 6 per group). G1; Control group, received physiological saline solution (0.5 ml/rate). G2; CC-treated group, the rats treated with cadmium chloride 15 mg/kg/bw according to Al-Gebaly (2017). G3; CC+ propolis-treated group, treated with 15 mg/kg//bw cadmium chloride + 30 mg/kg/bw propolis. G4; Propolis-treated group, treated with 30 mg/kg/bw propolis according to Tatli et al. (2020). The study lasted for 28 days according to Ognjanović et al. (2007). The aqueous propolis extract was perpetrated according to Ozsoy-Sacan et al. (2005).

Blood biochemical analysis

Blood samples were obtained from rats during the slaughter process at the end of the experimental period. Blood samples were centrifuged at 3,500 rpm for 10 min, and then plasma samples were harvested and stored at–20°C until further assays. liver Enzymes (ALT and AST) were estimated according to (Belfield and Goldberg, 1971; Bannerjee et al., 1979). Total antioxidant capacity (TAC) was measured according to (Diab et al., 2020). TBARS were measured according to (Svingen et al., 1979).

Lipid peroxidation product assay

The liver tissue was crushed in a phosphate buffered saline (PBS) solution with a pH of 7.4, and the level of lipid peroxidation product (TBARS) in the liver tissue was determined using the method provided by (Buege and Aust, 1978).

Histopathology

At the end of the experimental period, fresh liver, kidney, and testes tissues were quickly dissected and fixed in neutral buffered formalin (10%). Using an automatic tissue processor (Sakura, Japan), the fixed specimens were dehydrated with several grades of ethanol (70, 80, 90, 95, and 100%), cleared in two changes of xylene, and impregnated with two changes of molten paraffin wax at 60 °C, as described by El-Refaiy and Eissa (Vickers, 2017). A microtome was used to cut serial sections of 4-5 µm thickness, and an auto stainer was used to stain the sections with hematoxylin and eosin. Histochemical staining

was done with hematoxylin and eosin and periodic acid schiff methods (Suvarna et al., 2018). Using an optical microscope, the mounted specimens were evaluated for changes in the hepatic tissues of each rat under study.

Statistical analysis

The biochemical parameters were expressed in terms of mean standard deviation (SD). The significance of differences in mean values across groups was assessed using a one-way analysis of variance followed by Duncan's test, and P 0.05 was declared significant using the statistical analysis system (SPSS, 1996).

RESULTS AND DISCUSSION

Blood biochemical

The animals treated with Cadmium chloride (CC) had a significant increase (P<0.01) in the levels of lipid peroxidation products (TBARS), liver enzymes (ALT, AST), creatinine and urea compared with the other treatment groups (Table 1). Meanwhile, rats treated with CC and propolis had improvement in the liver and renal function, but the levels of liver enzymes (ALT and AST) creatinine and urea were significantly lower than propolis-treated group and control groups. In contrast, ALT and AST enzyme levels were significantly higher in samples treated with CC (Table 1).

The TAC level in the serum of the CC group was significantly lower (P< 0.01) than the other treatment groups. Furthermore, in the CC + propolis-treated group, there was a decrease (P > 0.05). There was also a non-significant rise in the propolis-treated group versus the control group. However, a non-significant (P > 0.05) rise in TBARS, TAC, liver enzymes (ALT and AST), urea and creatinine were seen in the propolis-treated group compared to the control group (Table 1).

Sperm count X 10⁶/ml

The concentration of spermatozoa (x10⁶/ml; mean ± SEM) was significantly higher (p < 0.01) in the CC+Propolis-treated group and Propolis-treated group (72.55±6.18 and 92.88±5.58) than that in the CC group (61.71±5.64), respectively. While, there were non-significant decrease (P > 0.05) was observed in the control and treated group. However, there were a significantly higher (p < 0.01) decrease in the CC group than that in the control treated group (61.71±5.64 Vs 89.21±7.35), respectively.

Histopathological results

Testicular tissue

Microscopic examination of the control group's testicular tissue (Figure 1a, b, c) indicated normal testicular tissue with regular organization of the seminiferous tubules (ST)

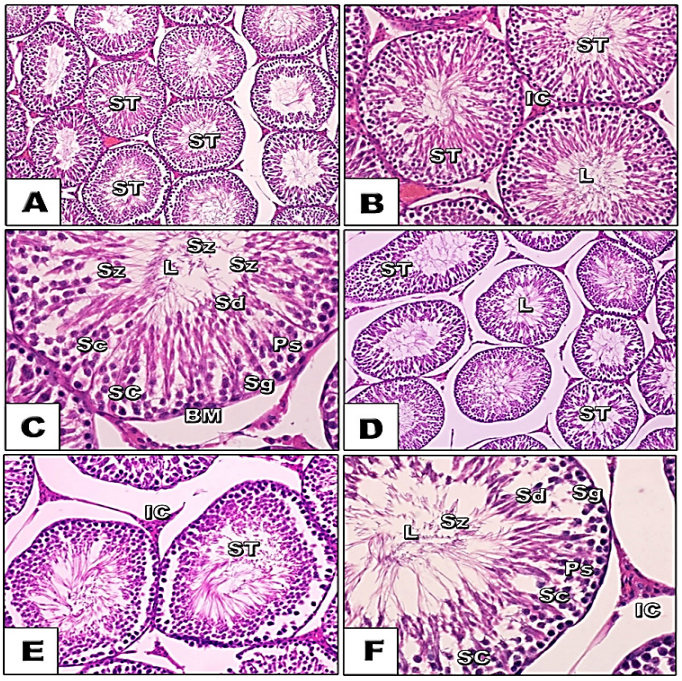

Figure 1: Schematic diagram showing the histopathology of rats' testicular tissues from the control group (A, B and C) and CC-treated group (D, E and F). ST = seminiferous tubules; IC = interstitial cells; L = lumen of the seminiferous tubule; BM = basement membrane; SC = Sertoli cells; Sg = spermatogonia cells. H & E X200, H & E X400.

Table 1: The TBARS liver tissues, TAC, ALT, AST liver enzemes, Creatnine, Urea and Sperm concentration in the control and treated groups.

Item	Control	Treated group		
		CC	CC+Propolis	Propolis
TBARS (n mol/g tissue)	40.42±3.22	89.51±4.02***	50.08±4.11	39.26±3.92
TAC (µm/l)	190.41±7.6	109.33±6.33**	160.23±5.92	195.04±7.32
ALT (mg/dl)	82.11±7.52	171.22±8.24***	112.04±7.57*	79.07±6.77
AST (mg/dl)	52.42±4.31	111.26±7.25***	81.14±5.75*	47.33±6.03
Creatinine (mg/dl)	0.69±0.13	1.25±0.15***	0.82±0.07*	0.64±0.14
Urea (mg/dl)	42.51±4.13	77.15±5.64***	54.3±4.34*	43.9±4.52
Sperm count X 10⁶/ml	89.21±7.35	61.71±5.64***	72.55±6.18	92.88±5.58

Data are expressed as mean ± S.D, *Means in the same row differ significantly (p ≤ 0.05); **Means in the same row differ significantly (p ≤ 0.01); ***Means in the same row differ significantly (p ≤ 0.001).

and interstitial cells (IC), and primary and secondary, spermatids, and sperm filling the lumen of the seminiferous tubule (L). Spermatocytes are dependent on basement membrane (BM), in addition to supporting columnar cells (Sertoli cells). In contrast, the CC group, microscopic examination revealed interstitial edema between the seminiferous tubules as well as shedding and desquamation of sperm cells into the lumen of the seminiferous tubule, as well as vacuolar degenerative changes in the seminiferous tubule tissue, deformation, and buckling basement membrane (Figure 1d, e, f).

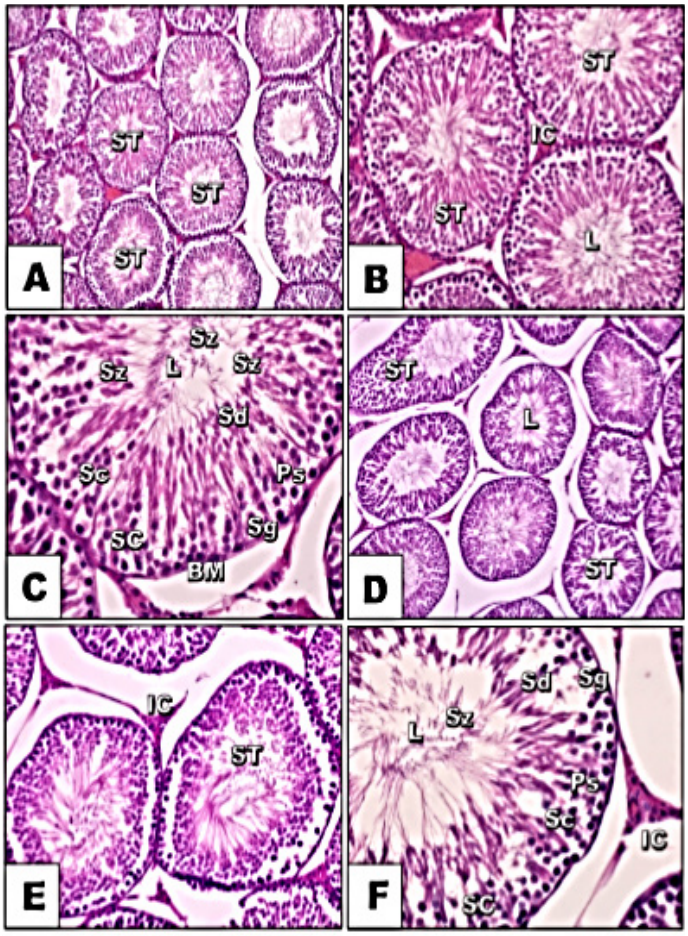

Figure 2: Schematic diagram showing the histopathology of rats' testicular tissues from the CC+proplise-treated group (A, B and C) and proplise-treated group (D, E and F). ST = seminiferous tubules; IC = interstitial cells; L = lumen of the seminiferous tubule; BM = basement membrane; SC = Sertoli cells; Sg = spermatogonia cells. H & EX200, H & EX400.

The histological results showed the remarkable improvement in the texture of the seminiferous tubules and an increase in the number of cells lining them in the CC+propolis-treated group (Figure 2a, b, c). However, the propolis-treated group (Figure 2d, e, f) exhibited healthy testicle, seminiferous tubule is lined with a complex epithelial tissue consisting of spermatogonia cells, primary and secondary spermatocytes, and spermatogonia in addition to supporting columnar cells called Sertoli cells. Sperm cells are based on a basement membrane.

Liver tissue

A microscopic examination of the livers of control rats revealed a normal, well-preserved architecture, with normal histological components of the hepatic lobules and portal areas, a central vein (CV), hepatocytes (H), polygonal cells with circular nuclei (N), and hepatocytes arranged in the form of hepatic cords that radiate regularly from the central vein to the lobule borders. Small sinusoids (S) in the hepatic cords separate them (Figure 3a, b, c). Histopathological assessments of the CC-treated group's hepatic tissues revealed cellular necrosis (Nc) necrosis and hemorrhage with shrinkage of hepatic cell nuclei (Py) pyknosis with inflammation spread and numerous immune cell clusters in the liver tissue (ICI) inflammatory cell infiltrations (Figure 3d, e, f). Hepatic tissue from the CC-propolis group and the propolis group showed normal hepatic architecture, including normal central vein (CV) and hepatocytes (H). Small (S), highlighting the absence of pathogenic alterations (Figure 4a, b, c, d, e, f).

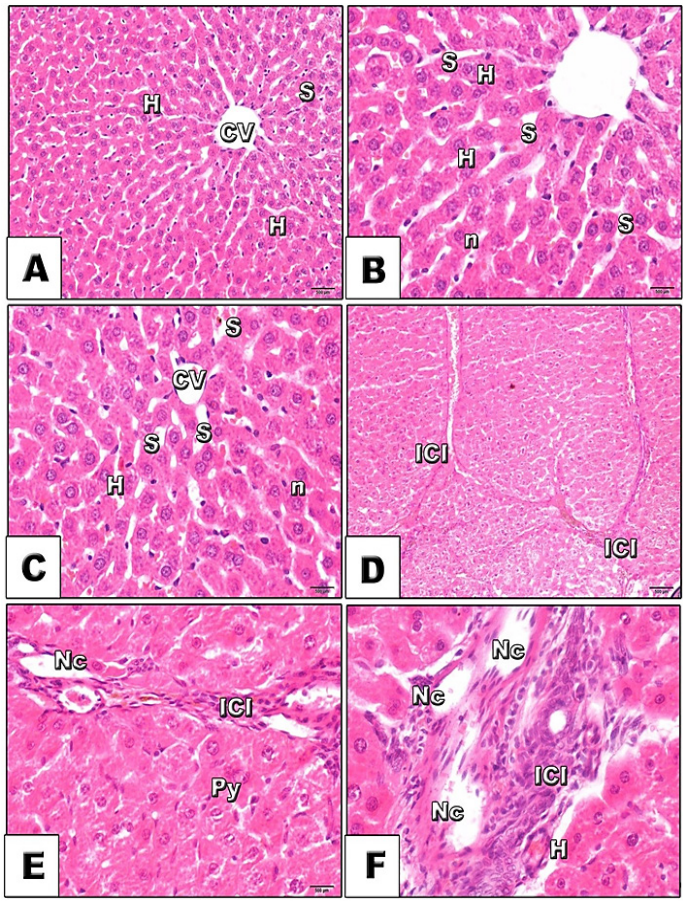

Figure 3: Schematic diagram showing the histopathology of rats' testicular tissues from the CC+proplise-treated group (A, B and C) and proplise-treated group (D, E and F). CV = central vein; H = hepatocytes; N = nuclei; S = sinusoids; Bc = necrosis; Py = pyknosis; ICI = inflammatory cell infiltrations. H & E X200, H & E X400.

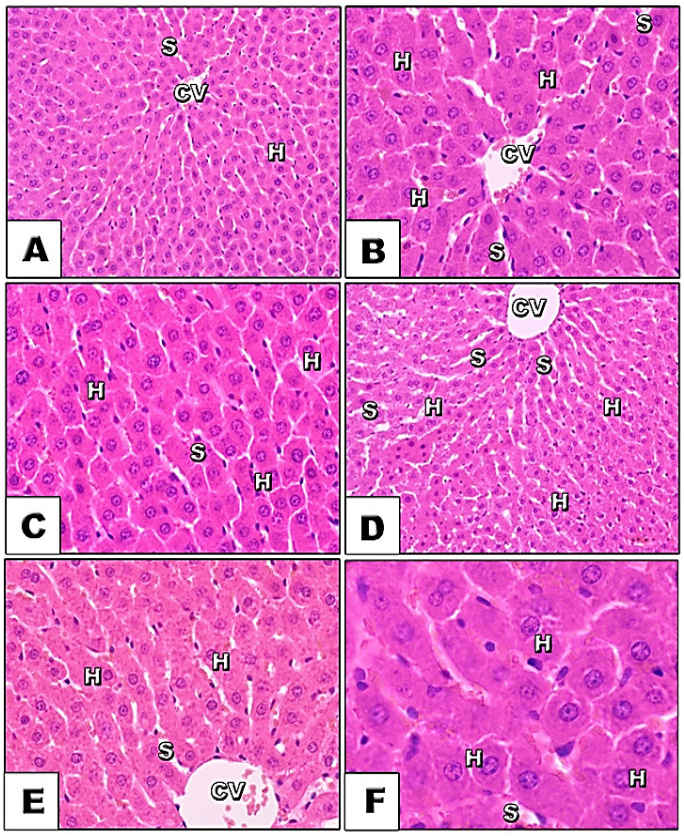

Figure 4: Schematic diagram showing the histopathology of rats' testicular tissues from the CC+proplise-treated group (A, B and C) and proplise-treated group (D, E and F). CV = central vein; H = hepatocytes; N = nuclei; S = sinusoids; Bc = necrosis; Py = pyknosis; ICI = inflammatory cell infiltrations. H & E X200, H & E X400.

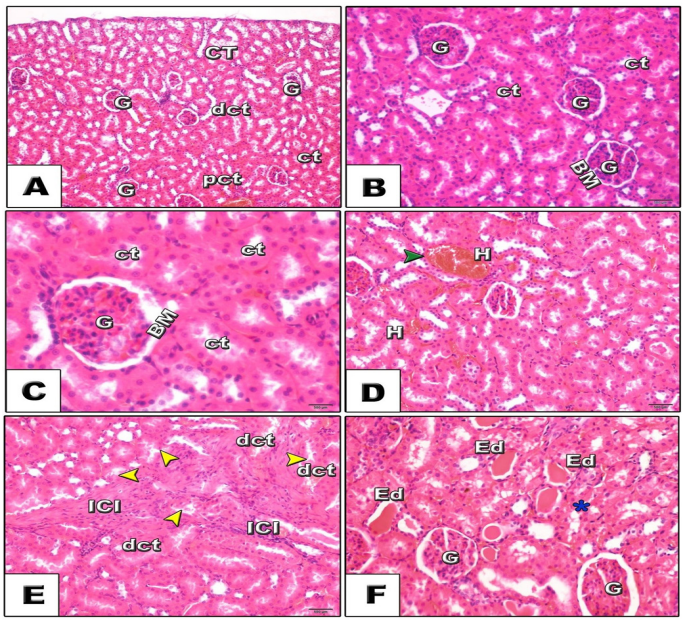

Figure 5: Schematic diagram showing the histopathology of rats' testicular tissues from the CC+proplise-treated group (A, B and C) and proplise-treated group (D, E and F). G = glomeruli; M = Bowman's capsule; PCT = proximal convoluted tubules DCT = distal convoluted tubules. H & E X200, H & E X400.

Kidney tissues

Histopathological alterations of the control group's kidney tissues revealed the renal glomeruli (G) interstitial by Bowman's capsule (BM), as well as the proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) in their normal condition. We also see normal blood arteries between the renal tubules (Figure 5a, b, c). In addition, represented a cross-sectional section of the cortical kidney's tissue of the kidney CC group (Figure 5d, e, f), illustrating renal dysfunction represented by dilatation and congestion of the glomerular capillaries (G) and the blood vessels between the renal tubules, atrophy in some renal glomeruli, necrosis, and edema or inflammatory edema, with some bleeding around renal blood vessels, infiltration of inflammatory cells and focal fibrosis in between the renal tubules were also seen. However, the kidney cortical of the CC+propolis-treated group and the propolis-treated group exhibited normal renal architecture, including normal PCT, DCT, and peritubular blood vessels. The two sections show the renal glomeruli (G) interstitial by Bowman's capsule (BC), the proximal and distal convoluted tubules (PCT), and the distal convoluted tubules (DCT) in normal form, as well as the presence of normal blood vessels between the tubules Nephrotic with the absence of pathological (Figure 6a, b, c, d, e, f).

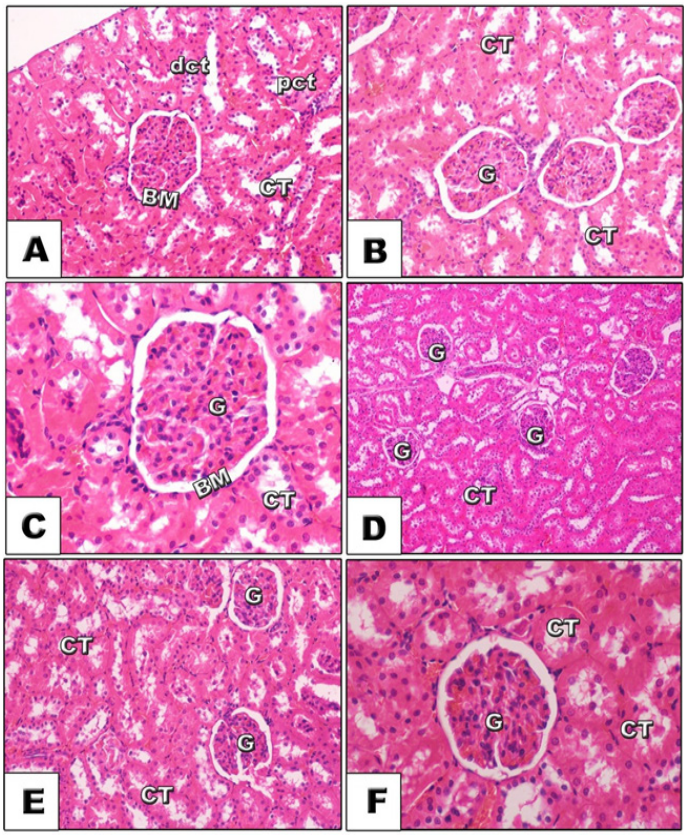

Figure 6: Schematic diagram showing the histopathology of rats' testicular tissues from the CC+proplise-treated group (A, B and C) and proplise-treated group (D, E and F G = glomeruli; M = Bowman's capsule; PCT = proximal convoluted tubules DCT = distal convoluted tubules. H & E X200, H & E X400.

The intake of cadmium in rats led to a significant decrease in the level of antioxidants such as (superoxide dismutase, catalase, glutathione peroxidase and Glutathione S-transferase) in liver and kidney tissues when compared to the control group, and cadmium also led to a significant increase in the level of lipid peroxidation in the tissues of the liver and kidney when compared to the control group due to its production of many free radicals, which reduces the total antioxidants capacity due to their consumption in dealing with free radicals, leading to a significant increase in the level of oxidative stress and this is considered the cause of hepatic and renal toxicity of cadmium (Shrivastava et al., 2017; Hussein et al., 2022; Ahmed et al., 2023).

Cadmium toxicity occurs by different mechanisms and showed that cadmium stimulates the production of free radicals, which leads to the destruction of fats, proteins and DNA, and thus leads to many pathological conditions in humans and animals. Chronic exposure to cadmium leads to the accumulation of this heavy metal in the liver, kidneys, and other tissues, which leads to metabolic and histological changes, destruction of cell membranes, changes in gene expression, and programmed cell death (Waisberg et al., 2003). The use of cadmium chloride led to an increase in the levels of ALT and AST in the blood serum compared to the control group. Also, a decrease in the level of total antioxidants (TAC) in the blood serum accompanied by an increase in the rate of fat oxidation (TBARS) was observed in the liver tissue of the cadmium chloride group compared to the control group. It was declared by (Kovac et al., 2015; Al-Syaad et al., 2023b) that exposure of rats to cadmium led to liver toxicity, as evidenced by the significant increase in the levels of enzymes (ALT and AST) in the blood serum compared to the control group. The reason for this is that cadmium leads to the production of a large amount of Free radicals that lead to damage to proteins, cell membranes and DNA. Also, cadmium intake led to a significant increase in the rate of lipid peroxidation in comparison to the control group, which leads to damage to liver cells and thus the release of a large amount of enzymes (ALT and AST). Also, the intake of cadmium in rats led to a significant increase in the level of creatinine and urea in the blood serum compared to the control group, which reflects the occurrence of renal toxicity due to cadmium (Shati, 2011).

At the present time, people's attention has increased to herbal treatment and natural products as a complementary medicine or an alternative to conventional medicines, due to the lack of side effects of herbal medicines (El-Zamkan et al., 2021; Ahmed et al., 2022a). Many studies showed that the use of antioxidants can modify or prevent the toxic effects of many toxic substances (Ibrahim et al., 2022). Propolis is widely used in ethno-medicine and as a supplement, owing to its medical and therapeutic benefits, such as treating of infectious diseases and cancer. Studies have also shown that taking antioxidants, such as propolis, can modify toxicity resulting from pollutants and heavy metals (Beydilli et al., 2015). On light of the above, significant improvement was observed in the measured parameters affected by cadmium toxicity of liver function, kidney function, testicular toxicity, oxidative stress, antioxidant level and sperm count in the cadmium chloride + propolis group compared to the control group.

Several studies proved that antioxidants, including natural and plant extracts, provide protection against hepatotoxicity resulting from environmental and chemical pollutants and heavy metals such as cadmium, by preventing lipid peroxidation and increasing the activity of antioxidant enzymes (Fernando and Soysa, 2016). Propolis acts as an antioxidant agent, owing to its phenolic compounds especially flavonoids which acts as free radical scavengers and protect the tissues from their toxicological effects. Also, (Coskun et al., 2005; Ali et al., 2022) proved that propolis and its components has a protective role against the toxic effects of diabetes in rats, as the levels of liver enzymes ALT and AST and lipid peroxidation products (lipid peroxidation) represented by elevated TBARS decreased significantly in serum in the group treated with propolis. As well as a significant increase in the level of antioxidants enzymes such as (superoxide dismutase, catalase, glutathione peroxidase and Glutathione S-transferase) in the liver, kidney and heart compared with the diabetic group.

CONCLUSIONS AND RECOMMENDATIONS

The current study showed that the use of aqueous extract of propolis has a protective effect and reduces cadmium toxicity on the liver, kidneys and testes of white rats, so we recommend using this natural product on a daily basis. Future pharmacological studies are required for studying the comparative effects of using different concentrations of propolis extract, and the possibility for conducting experiments on different animals such as mice, guinea pigs or rabbits. Moreover, further studies are needed to conduct the experiment for a period longer than 28 days and using another natural substance with propolis.

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NOVELTY STATEMENT

The present study highlights the ability of the aqueous

extract of propolis in reducing the toxic effects on the liver, kidney and testis of white rats after they were subjected to cadmium chloride poisoning.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical consideration

All experiments were reviewed and approved following the rules and regulations of the Research Ethics Committee, King Khalid University. All experiments were carried out in the Physiology Lab., Department of Biology, College of Science, and King Khalid University, Saudi Arabia. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

List of appreciations

CC = cadmium chloride ALT = alanine aminotransferase; AST = aspartate aminotransferase; TAC = total antioxidant capacity; TBARS = lipid peroxidation product; ST = seminiferous tubules; IC = interstitial cells; L = lumen of the seminiferous tubule; BM = basement membrane; SC = Sertoli cells; Sg = spermatogonia cells; CV = central vein; H = hepatocytes; N = nuclei; S = sinusoids; Bc = necrosis; Py = pyknosis; ICI = inflammatory cell infiltrations; G = glomeruli; M = Bowman's capsule; PCT = proximal convoluted tubules; DCT = distal convoluted tubules.

Conflict of interest

The authors have declared no conflict of interest.

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