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Protective effect of propolis on cadmium chloride induced toxicity in male Japanese quails

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Abstract:

One of the biggest concerns in our food supply is heavy metal pollution, which poses a major threat to our ecology. Because of its numerous industrial applications, cadmium (Cd) is a significant heavy metal that is widely dispersed in the environment and recognized as a worldwide source of illness that adversely affects various body systems in humans and animals. Adequate new chelating drugs in conjunction with chemical cleaning and supportive treatment are necessary for patients suffering from cadmium poisoning. Therefore, this study was carried out to investigate the protective effects of propolis on $CdCl_2$-induced toxicity in male Japanese quails. One hundred, twenty-day-old, 100±5 gm weight, male Japanese quails were randomly divided into four equal groups: control (basal feed ad libitum with no additives), propolis-treated group (300 mg/L water), $CdCl_2$-exposed group (5 mg/kg feed), and propolis+$CdCl_2$-treated groups (same previous doses). The quails were exposed for 40 days under a 16/8-hour light/dark cycle. The results declared that $CdCl_2$ lowered the activities of antioxidant system, disturbed the hepatic, and renal biomarkers, and induced a vast array of notable multiorgan

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histopathological alternations involving the liver, kidneys, testes, and lungs. Immunohistocheically, $CdCl_2$ upregulated the hepatic, renal, testicular, and pulmonary expression of caspase3 (casp3) and CYP2E1, while downregulated the expression of Bcl2 in these organs. Supplementation with propolis did not normalize the $CdCl_2$-induced histopathological changes but significantly lowered the severities and frequencies of the tissue lesions and recovered approximately the biochemical parameters toward the typical values. Additionally, propolis significantly regained the casp3, Bcl2, and CYP2E1 tissue expressions toward the values of the control group. It could be assumed that propolis is a promising feed additive for alleviating the $CdCl_2$-induced hepatic, renal, testicular, and pulmonary toxicity.

Introduction:

In recent years, there has been a lot of focus on the pathogenic effects of heavy metals on the ecosystem, human health, and animal health as well as the development of innovative therapeutic approaches to lessen their toxicity **Mitra et al., 2022**. Cd is one of the most hazardous heavy metals that are continuously emitted into the environment, both from natural sources and human-caused sources such industrial processes, waste disposal, and fertilizer manufacturing **Aljohani 2023**. As a result, Cd is discovered in ever-increasing amounts in aquatic bodies, feed, fodder, and livestock tissues, including poultry, in the vicinity of industrial locations

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Lysenko et al., 2021. Humans and animals can be exposed to Cd via breathing air, ingesting food, or drinking water containing the metal **Hayat et al., 2018.** Long-term exposure to Cd, even at very low concentrations, threatens the health of poultry and animals through food chain transmission and bioaccumulation **Zhang t al., 2022; Saedi et al., 2023.** In particular, long-term exposure to Cd reduces the efficiency of feed conversion, growth rate, and egg production in poultry **Aendo et al., 2022.** Regarding the Cd-body system bioaccumulation in poultry, Cd primarily bioaccumulate in the liver, kidneys, lungs, and reproductive organs **Kar et al., 2021**, negatively affects their physiological processes, and enzymatic activities, that necessarily reflected on their serum biochemical parameters **Bokori et al., 1996.** As a result, upon microscopic examination, these organs exhibited numerous degenerative, necrotic, inflammatory, circulatory, neoplastic, and other histopathological alterations **Singh et al., 2016; Peana et al., 2022.** The molecular mechanisms implicating the Cd-induced tissue toxicity are multifactorial **Rahimzadeh et al., 2017**. Most Importantly, Cd impair oxidative phosphorylation and respiration in cells by binding to the mitochondria **Patrick 2003**, disrupts the DNA repair mechanisms **Joseph 2009**, amplify the generation of the reaction oxygen species (ROS) and lipid peroxidation **Lopez et al 2006**, modulate the cellular level of Ca^{2+} and the activities of caspases and nitrogen-activated protein

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kinases **(MRPKs) Brama et al., 2012**, induce non-tissue specific apoptosis **Unsal et al 2020,** disturbs cell division, and differentiation **Waalkes 2003**, decreases mRNA level of Bcl-2 gene **Yang et al., 2021,** and modulates cytochrome P450 systems and Nrf2 mediated antioxidant defense **Guo et al., 2020**.

According to the United Nations Food and Agriculture Organization, around one billion people worldwide suffer from protein insufficiency, and between 691 and 783 million people experienced hunger in 2022 **Villa 2022.** Quails can be a sustainable source of high-quality meat, and egg production **Mukhtar et al., 2022.** When compared to chicken, quail meat has a lower fat level, and a higher content of protein, iron, copper, phosphorus, zinc, potassium, selenium, and vitamins A, C, B2, B3, B6, and B12 **Ioniţă et al., 2010**.

Among the enormous infectious and noninfectious pathologic stimuli that negatively impact the poultry industry, heavy metal poisoning represents a significant hazard with a very limited therapeutic strategies **Aljohani 2023.** Therefore, recently, researchers sought for non-traditional therapeutic agents for the Cd-induced cytotoxicity **Ebrahimi et al., 2023.** Among these agents, propolis (a resin-like material made by honeybees by combining the exudate collected from tree buds, sap flows, and other botanical sources with saliva and beeswax) seems to help in a wide range of pathological conditions including Cd toxicity **Simone-Finstrom,**

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and Spivak 2010; Omar et al., 2023. The molecular basis for the health benefits of propolis have not yet been fully elucidated, but it might be related to its potent antioxidant, anti-inflammatory, antibacterial, and anticarcinogenic contents **Khalil ML (2006); Anjum et al., 2019; Pahlavani et al., 2019.**

Based on the information provided above, we posit that propolis may be useful in reducing the negative effects of Cd. Therefore, we design the current study to ascertain if propolis could mitigate the health risks associated with the build-up of Cd in the liver, kidney, testicles, and lung tissues of quails, focusing on the histopathological, and immunohistochemical analysis. Additionally, a number of factors including body weight gain, degree of oxidative stress, and the hepatorenal functions were investigated.

Material and methods:

Chemicals:

Cadmium dichloride (CdCl2) (MDL number: MFCD00010916, CAS Number: 10108-64-2) was purchased from Sigma-Aldrich Inc. MA, USA. It came as a powder with 99.99% purity. Propolis solution was purchased from Raw Pot

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company, London, United Kingdom. The company guaranteed that propolis was produced using a unique procedure that maintained it exactly as it is found in the hive. It arrived as water based, alcohol free, and entirely raw solution, never been subjected to pasteurization, heat, or boiling, ensuring a 100% bio-available composition. Additionally, the company emphasized that the propolis was obtained from bees that feed exclusively on the same foods they create and pollinate on wild, herbicide, pesticide, and completely chemical-free plants. It came in 50 ml bottles; each ml contains 300 mg organic raw propolis. Both chemicals were used without any further purification.

Animals and study design:

One hundred, clinically healthy, twenty-day-old, 100±5 gm weight, male Japanese quails were purchased from Quesna quail farms, Egypt. The birds were randomly divided into four equal groups (1) control group (feed on quail ration supplied by a local manufacturer, Aleman feed company, Egypt, and consisted of yellow corn 46%, soybean meal 25%, gluten 8%, wheat fish meal 7%, wheat bran 5%, clover powder 3%, yeast 2%, limestone 2%, bone meal 1%, vitamins 0.5%, and mineral salts 0.5%), (2) propolis group (feed the same diet with adding propolis in a dose of 300 mg/L of drinking water; **Nna et al., 2018**), (3) $CdCl_2$-exposed group (feed the same diet with adding CdCl2 in a dose of 5 mg./kg ration; **Akter et al.,**

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2019), and propolis + $CdCl_2$-treated group (feed the same diet with adding propolis in a dose of 300 mg/L of drinking water and $CdCl_2$ in a dose of mg./kg ration). The quails were exposed daily for 40 days under a 16/8-hour light/dark cycle. They were kept under standard laboratory conditions with ad libitum access to feed and water in well-ventilated places. All the animal experiments were conducted in accordance with the Ethical Norms on Animal Care and Use approved by Faculty of veterinary Medicine, Aswan University, Egypt.

Biochemical analysis:

Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase levels were measured according to **Reitman and Frankel (1957); Bowers and McComb (1972).** The blood urea nitrogen and serum creatinine were measured spectrophotometrically Using commercial diagnostic kits (Sigma Diagnostics, Cairo, Egypt). Additionally, malondialdehyde (MDA) as a lipid peroxidation marker was measured according to the method of **Aebi 1884, and the** total antioxidant capacity (TAC) as a biomarker for oxidative stress were measured per the method of **Koracevic et al., 2001.**

Histopathological investigations of the liver, kidneys, testes, and lung

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Following the updated guidelines for poultry necropsy and diagnosis, **Lucio-Martinez and Korich 2010**, ten quails per each group were randomly selected, humanly euthanized by decapitation at the scheduled time, necropsied recording any gross lesions, and representative tissue specimens were sampled from the liver, kidneys, testes, and lungs. Immediately, the tissue specimens were immersed for 72 hours in a 10% neutral buffered formalin solution. After being fixed, the tissue samples were impregnated, embedded in paraffin wax, dehydrated using increasing concentrations of ethyl alcohol, cleared with Histo-Choice® (Sigma-Aldrich, St. Louis, USA) clearing agent, sectioned at 5 μm thick, and stained with hematoxylin and eosin dyes **Suvarna et al. 2018**, and examined by light microscope recording any histopathological changes. Subsequently, the hepatic, renal, testicular, and pulmonary tissue sections underwent morphometric analysis and multiparametric numerical lesion scoring. The outcomes were reported as percentages (means ± SE). In summary, for each bird, using an AmScope digital imaging system, ten randomly chosen nonoverlapped, fixed-size, 10× microscopic fields (100 images/organ/group) were captured. Next, these images were interpreted and statistically analyzed to determine the severities and frequencies of the lesions. A five-point rating system was used to assess the lesion's severity (or extent): zero represented the lesion's absence; 1, focal distribution; 2, multifocal distributions; 3, locally extensive

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distribution; and 4, diffuse distribution. The following formula was used to calculate the frequency, or the number of times the lesion occurs: $F\% = N_{lesion} \div N_{total} \times 100$. Where N_{lesion} is the total number of images that showed a particular lesion, and N_{total} is the total number of images in the group (100). In the end, the outcomes were given as percentages (means ± SD).

Immunohistochemical investigation of the caspase 3 (casp3), Bcl2, and cytochrome P450 2E1 (CYP2E1)

For each quail, three formalin-fixed paraffin-embedded five-micron thick tissue sections per organ were prepared and immunostained according to the avidin-biotin-peroxidase complex technique developed by **Hsu et al., 1981**. The first sections were immunostained for the detection of the Casp3 antigen using the rabbit monoclonal anti-caspase-3 primary antibody [EPR18297] (ab184787, abcam Inc.) at 1/1000 dilution. The second set of sections were immunostained for Bcl2 using mouse monoclonal BCL-2 primary antibody [100/D5] (ab692, abcam Inc.) at 1/100 dilution. The third set of sections were immunostained for the detection of cytochrome P450 2E1 (CYP2E1) using rabbit polyclonal anti-cytochrome P450 2E1 primary antibody (ab53945, abcam Inc.) at dilution 5 µg/ml. Harris hematoxylin was employed as the counterstain and 3,3′-diaminobenzidine (DAB) as the chromogen. Then, ten high-power microscopic fields (40×) per biomarker per organ

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per quail (100 images/biomarker/organ/group) were taken at the same exposure period, and the color deconvolution plugin in the open-source ImageJ program **(Schneider et al., 2012)** (http://rsb.info.nih.gov/ij) was used to analyze these images to quantify the caspase 3, cytochrome P450 2E1, and Bcl2 immunoexpressions by determining the percentages of the brown color area fraction to the total areas of the images, and the results were expressed as mean + ±SD.

Statistical analysis:

Using the IBM® SPSS® Statistics software, version 25, the statistical analysis was performed. Analysis of variance (ANOVA) test was used to examine the data, and Duncan's post hoc test was used to identify the important variations between the experimental collectives. The results are presented as the mean± standard error (SE). Every variation amongst groups were regarded as statistically significant at $P < 0.05$.

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Results:

Biochemical findings:

The biochemical findings were presented in table (1)

Histopathological, and the immunohistochemical findings

a. Liver

The light microscopic examination declared that the livers of the control and propolis-treated groups had normal histology with absence of any pathological lesions (Fig. 1 A, and B). The livers of the $CdCL_2$-treated group revealed numerous hepatopathic alterations, primarily of degenerative, necrotic, and inflammatory nature such as vacuolar, and hydropic degeneration, fatty change, single-coagulative necrosis, vascular congestion, and leukocytic infiltration particularly with mononuclear cells (Fig. 1 C). Supplementation with propolis significantly rescued the hepatic parenchyma against the CdCL2-induced hepatopathic alterations, yet it did not maintain the normal liver histology. Some hepatopathic alterations were evident in the livers of the propolis+$CdCL_2$ group including cytoplasmic vacuolation, fatty degeneration, and mononuclear cell infiltration (Fig. 1 D). For precise histopathological evaluation, a detailed multiparametric lesion scoring for all recorded lesions in the hepatic tissue sections of all groups was presented in table 2. Immunohistochemically, the image analysis confirmed that no significant difference

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was found between the control and propolis groups regarding the expression of casp3, Bcl2, and CYP2E1. In contrast, exposure to $CdCL_2$ significantly upregulated the expression of casp3, and CYP2E1 and downregulated the expression of Bcl2 compared to either the control or propolis groups. The hepatic tissue sections of the propolis+ $CdCL_2$ group showed significant downregulation to the expression of casp3 and upregulation to the Bcl2 compared to the $CdCL_2$ group, but the expression did not regain to those of either the control or propolis group. Additionally, there was no difference in the expression levels of CYP2E1 between the $CdCL_2$ and propolis+ $CdCL_2$ groups. The immunoexpression of three biomarkers in the hepatic tissue sections of all groups was shown in Figs. 1 E–H, I-L and M-P, respectively and statistically summarized in table 3.

b. Kidneys

The microscopic investigation of the kidney sections of the control and propolis group revealed normal histological architecture without any histopathological lesions (Fig. 2 A and B). Meanwhile, the kidneys of the $CdCL_2$ group exhibited a vast array of nephropathic changes involving the glomeruli (congestion, collapse, and necrosis), the tubules (marked vacuolation, nuclear pyknosis, single-cell necrosis, and cast formation), and the interstitium (congestion of the interstitial blood vessels, and the peritubular capillaries, edema,

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and inflammatory cell infiltrate particularly with mononuclear cells) (Fig. 2 C). The nephroprotective effects of propolis against the $CdCL_2$-induced kidney tissue injury was average as the same nephropathic alterations seen in the $CdCL_2$ group were evident the kidneys of the propolis+$CdCL_2$ group but were milder and less frequent to those seen in $CdCL_2$ group (Fig. 2 D). For precise histopathological evaluation, a detailed multiparametric lesion scoring for all recorded lesions in the renal tissue sections of all groups was presented in table 2. Immunohistocheically, the image analysis declared that the effects of propolis and $CdCL_2$ on the expressions of casp3, Bcl2, and CYP2E1 in the kidneys were the same to those observed in the hepatic tissue sections. Concisely, the immunoexpression of three biomarkers in the kidney tissue sections of all groups was shown in Figs. 2 E–H, I-L and M-P, respectively and statistically summarized in table 3.

c. Testes

The panoramic microscopical investigation declared normal histology in the testicular tissue sections of the control and propolis treated groups (Fig. 3 A and B), where few but serious histopathological changes were seen in the testes of the $CdCL_2$ group. The basic alteration in this group was notable germ cell depletion not restricted to a specific germ cell type or stage (Fig. 3 C), accompanied with marked reduction in the heights of the germinal epithelium. Other alterations such as

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interstitial vascular congestions, and edema were noticed with almost absent to the inflammatory response. Interestingly, propolis supplementation exerted remarkable protective effects on the testicular histology against the $CdCL_2$-induced germ cell depletion, as very mild reductions in the heights of the germinal epithelium with absence to the pathological changes in the testicular interstitium was seen in the testes of the propolis+$CdCL_2$ group (Fig. 3 D). For precise histopathological evaluation, a detailed multiparametric lesion scoring for all recorded lesions in the testicular tissue sections of all groups was presented in table 2. Regarding the immunoexpressions of the casp3, Bcl2, and CYP2E1, it was obvious that exposure to $CdCL_2$ significantly upregulated the expression of casp3, and downregulated the expression of Bcl2, with no effect of the CYP2E1 compared to the control or propolis groups. Supplantation with propolis succeeded to regain the normal expression values of the three biomarkers in the testicular tissue sections of the propolis+$CdCL_2$ group. Briefly, the immunoexpression of three biomarkers in the testicular tissue sections of all groups was shown in Figs. 3 E–H, I-L and M-P, respectively and statistically summarized in table 3.

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d. Lungs

Normal histological findings were seen in the lungs of the control, and propolis groups upon microscopic examination (Fig. 4 A and B). Exposure to $CdCL_2$ adversely affected the pulmonary tissue as a group of histopathological alterations including pulmonary congestion, hemorrhage, and infiltration with mononuclear cells were seen in the lungs of the $CdCL_2$ group (Fig. 4 C). The protective effects of propolis against the CdCL2-induced lung injury was average as almost the same pneumopathic alterations seen in the CdCL2 group were seen in the lungs of the propolis+CdCL2 group but were slightly milder and with lower (Fig. 4 D). For precise histopathological evaluation, a detailed multiparametric lesion scoring for all recorded lesions in the pulmonary tissue sections of all groups was presented in table 2. Immunohistocheically, the image analysis emphasized that the effects of propolis and $CdCL_2$ on the expressions of casp3, Bcl2, and CYP2E1 in the lungs were the same to those observed in the hepatic, and renal tissue sections. Concisely, the immunoexpression of three biomarkers in the lung tissue sections of all groups was shown in Figs. 2 E–H, I-L and M-P, respectively and statistically summarized in table 3.

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Discussion

Worldwide, the shortage of foods containing good quality protein was well recognized and represents a global major challenge **Boliko 2019**. Therefore, the search for cheap, and mass-produced sources of protein is a top priority for many countries. Quail can contribute greatly to solving this problem, as they are prolific, easy to raise, and good source of high-quality meat **Mukhtar et al., 2022**. However, heavy metal contamination of the air, water, soil, and food has become a challenge for many poultry farmers, including quail breeders, in recent times **Aljohani, A.S., 2023.** Due to its extensive natural distribution, high level of toxicity that can cause harm to many organs even at low exposure levels, its carcinogenic potential, and its annual deaths both in animals and humans, Cd is considered one of the priority metals that is significant for public health **Tchounwou et al., 2012.** In the current study, an effort was made for precise evaluation of the Cd cytotoxicity on the liver, kidney, testis, and lungs in quails, then we assessed the potential contribution of co-administration of propolis in abating the Cd-induced biochemical and histopathological alterations in these organs. The results of the current study revealed that all the investigated organs; liver, kidney, testis, and lugs, were vulnerable to Cd toxicity. The only difference was the degree of toxicity, where the most affected organs were the kidneys, followed by liver, followed by the testes,

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followed by the lungs. Previous researchers have announced similar findings **Rahimzadeh et al 2017; Akter et al., 2019; Aljohani, A.S., 2023.**

Biochemically, exposure to Cd has increased the liver enzymes (AST, and ALT), increased the kidney injury biomarkers (BUN, and creatinine), increased the lipid peroxidation (MDA), and induced oxidative stress condition evidenced by a significant decrease to the total antioxidant capacity. The cause for the leakage of hepatic enzymes (ALT and AST) into the bloodstream in related directly to the Cd-induced lysosome instability within the hepatocytes **Șlencu et al., 2014,** while the rising of BUN, and creatinine levels was due to the Cd-induced tubular dysfunction **Swaddiwudhipong et al., 2007.** The elevation of the MDA levels and the notable decrease in the total antioxidant capacity were caused by the binding of Cd to the mitochondria and consequent inhibition of both the cellular respiration and oxidative phosphorylation even at very low concentration **Patrick 2003**. Even more, Cd induce depletion of reduced glutathione, binds sulfhydryl groups with protein, and causes to enhance production of reactive oxygen species, and inhibits the activity of antioxidant enzymes, such as manganese-superoxide dismutase, catalase, and copper/zinc-dismutase **Filipic 2012**.

Histopathologically, Cd induced multiparametric multisystemic morphological alterations, evidenced by a wide array of degenerative, necrotic, circulatory,

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and inflammatory responses in the hepatic, renal, testicular, and pulmonary tissues. Previous animal and human studies had typical findings **Rahimzadeh et al., 2017, Suljevic et al., 2019.** The main common feature in all pathological lesions caused by Cd toxicity in this study model was the multiplicity nature, as the lesions did not restrict to a special tissue or a specific immune response but involved multi mechanisms involved degenerative, apoptotic, necrotic, inflammatory, and circulatory responses. This could be related to the mechanisms of Cd toxicity which basically involve dysfunction of DNA repair mechanisms **Rani et al., 2014,** induction of chromosomal aberrations **Joseph 2009**, generation of reaction oxygen species **Filipic 2012,** modulating the Ca2+ cellular level, and activating the cellular caspases and nitrogen-activated protein kinases (MRPKs) **Brama et al., 2012.** These mechanisms of toxicity enable Cd to induce multiparametric multisystemic lesions which involved the liver (vacuolar, and hydropic degeneration, fatty change, single-coagulative necrosis, vascular congestion, and leukocytic infiltration particularly with mononuclear cells), kidneys (glomerular congestion, collapse, and necrosis, tubular vacuolation, necrosis, and cast formation, and interstitial congestion, edema, and inflammatory cell infiltrates), testes (germ cell depletion, with marked reduction in the heights of the germinal epithelium, and interstitial vascular congestions, and edema), and lungs (pulmonary congestion, hemorrhage, and infiltration with

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mononuclear cells). Almost the same findings were reported by previous studies **Rahimzadeh et al 2017; Akter et al., 2019; Aljohani, A.S., 2023.**

Since the reports that included studying the toxicity of Cd in quails are not too much, we deliberately performed immunostaining to the caspase3 as a biomarker for apoptosis **Ward et al., 2008,** Bcl2 as antiapoptotic biomarker **Hata et al., 2015,** and CYP2E1 as biomarker for xenobiotic toxicity **García-Suástegui ETA AL., 2017,** in a trial to understand the molecular basis of Cd toxicity in quails. A key finding of the present study is the significant upregulation of the expression of the casp3, and CYP2E1 and the significant downregulation of the Bcl2 expression in the $CdCL_2$ group compared to either the control or propolis groups in the hepatic, renal, testicular, and pulmonary tissues. This activation of caspases explains the single-cell necrosis in the examined tissues as caspase-3, is essential effector caspase, playing a pivotal role in the caspase-dependent apoptosis **Gao et al., 2013.** The molecular base involved in Cd induced Bcl2 downregulation is not well characterized, but this downregulation makes matters worse and increases the apoptotic pathways **Kim et al 2000.** The upregulation of CYP2E1 in the hepatic, renal, and pulmonary tissues related to the ability of Cd to increase the expression of the CYP2 isoforms **Guo et al., 2020**. This upregulation of CYP2E1 occurred as an inherited cellular mechanism

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to detoxify xenobiotics including Cd, in a trial to restore cellular hemostasis **Manikandan and Nagini 2018**.

Based on the above, searching for an economic, and field applicable therapy for Cd toxicity in the affected poultry farms has become an urgent necessity. By exploring, the database of the published papers, it become clear that propolis is one of the natural materials that used to ameliorate the heavy metal toxicity, with reliable and promising results, almost nil harmful effects, and potent antioxidant, anti-inflammatory, antibacterial, and anticarcinogenic contents **Khalil ML (2006); Anjum et al., 2019; Pahlavani et al., 2019.** Bees create propolis, sometimes known as "bee glue," during the building and upkeep of their colonies. Bees use a mixture of saliva and beeswax to make propolis, which serves as the hive's defense system **Cornara et al., 2017.**

Propolis's anti-inflammatory and antioxidant qualities stem from its composition of bioactive phytochemicals. The exact composition of a propolis sample usually vary between hives, location and seasons, but generally, propolis contains many chemicals such as alcohols, aromatic aldehydes, esters, fatty acids, amino acids, lignans, diterpenes, sesquiterpenes, flavonoids, itamins, and minerals **Batista et al., 2012**. When it comes to the protective effects of propolis against the Cd-induced hepatic, renal, testicular, and pulmonary toxicity in quails, it was

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obvious that propolis significantly decreased the biochemical alterations induced by Cd, and notably reduced the histopathological changes in the liver, kidneys, testes, and lungs of quails. Similar findings were previously reported in quails **Akter et al., 2029**. Additionally, propolis significantly regained the immunoexpression of the caspase3, Bcl2 and CYP2E1 in the liver, kidneys, testes, and lungs toward the normal expression values, yet it did nor normalize it.

Conclusion:

In summary, we conclude that propolis gave a very promising results in abating the Cd-induced cytotoxicity, and can be applied as an economic, and easily applicable food supplement to poultry feeding systems. In addition, this work adds knowledge to the field of natural nutrition research in reducing the harmful components found in the environment. Therefore, more study is required to add to our understanding of propolis' nutritional properties and assist explaining how it works.

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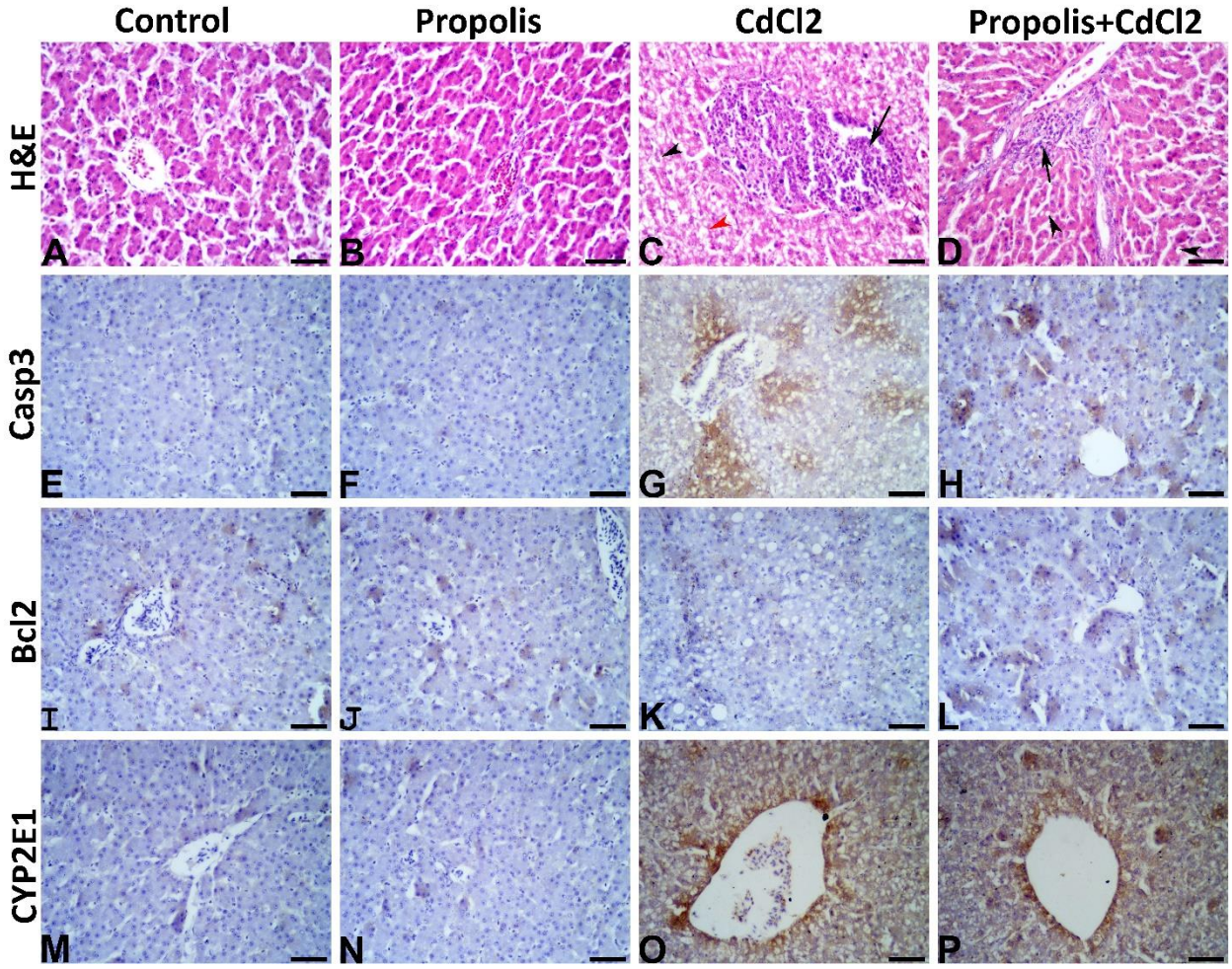

Figure 1|

A-D: Representative photomicrograph of the H&E-stained liver tissue sections showing normal quail liver histology in the control (**A**), and the propolis (**B**) groups. The liver of the $CdCL_2$ group shows focal necrotic area replaced by mononuclear cells (black arrow), vacuolation of the hepatocytes (black arrowhead), and single-cell necrosis (red arrowhead) (**C**). The liver of the propolis+ $CdCL_2$ group shows mild portal mononuclear cell infiltration

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(black arrow), and microsteatosis to numerous hepatocytes (black arrowheads) (**D**). The scale bars =25 microns (objective 40).

E-P: Representative photomicrograph of casp3 (**E-H**), Bcl2 (**I-L**), and CYP2E1 (**M-P**) immunostained hepatic tissue sections manifesting (1) no detectable differences in the expressions of the three biomarkers between the control and propolis groups, (2) significant upregulation of the casp3, and CYP2E1 expression and significant downregulation of the Bcl2 expression in the CdCL2 group compared to either the control or propolis groups, (3) significant downregulation to the expression of casp3 and upregulation to the Bcl2 the propolis+$CdCL_2$ group compared to the CdCL2 group, and (4) no difference in the expression levels of CYP2E1 between the $CdCL_2$ and propolis+ CdCL2 groups. The scale bars =25 microns (objective 40).

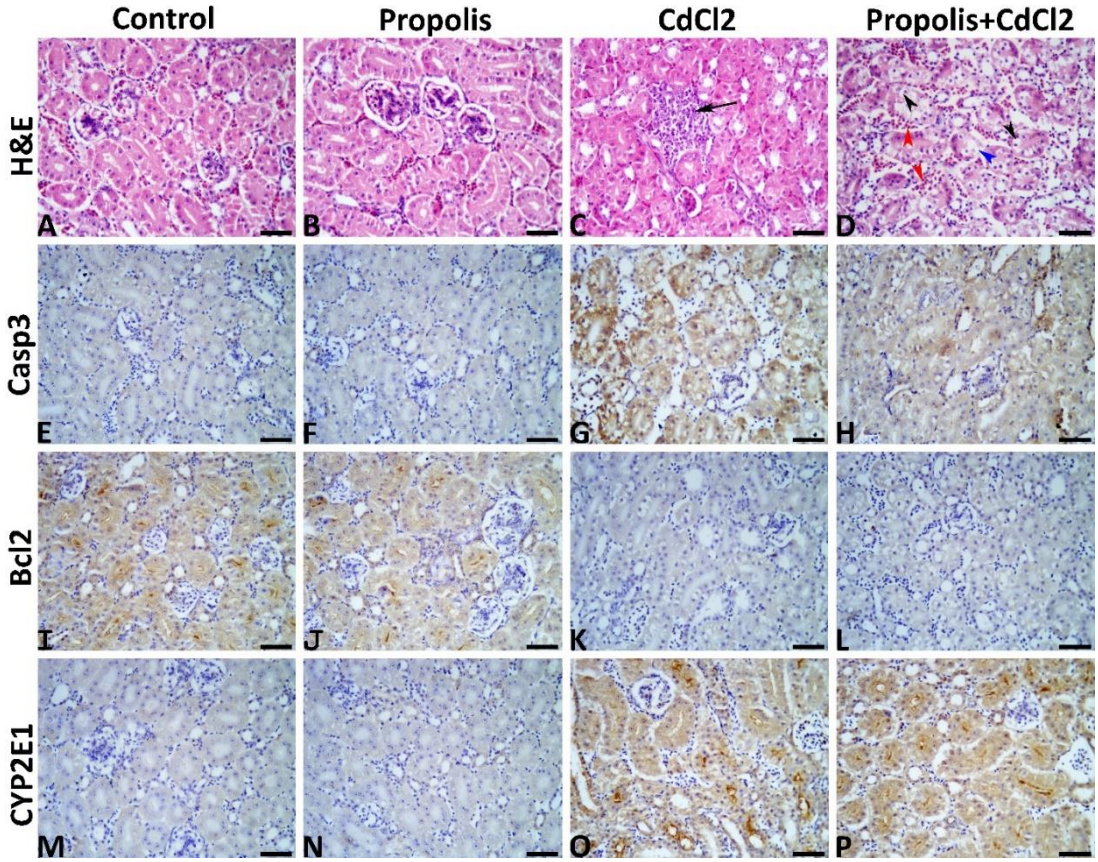

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Figure 2|

A-D: Representative photomicrograph of the H&E-stained kidney tissue sections showing normal quail kidney histology in the control (**A**), and the propolis (**B**) groups. The kidney of the $CdCL_2$ group shows focal necrotic area replaced by mononuclear cells (black arrow) (**C**). The kidney of the propolis+ $CdCL_2$ group shows congestion of the peritubular capillaries (red arrowheads), tubular vacuolations (black arrowheads), and single-cell necrosis (black arrowhead) (**D**). The scale bars =25 microns (objective 40).

E-P: Representative photomicrograph of casp3 (**E-H**), Bcl2 (**I-L**), and CYP2E1 (**M-P**) immunostained kidney tissue sections manifesting (1) no detectable

differences in the expressions of the three biomarkers between the control and propolis groups, (2) significant upregulation of the casp3, and CYP2E1 expression and significant downregulation of the Bcl2 expression in the CdCL2 group compared to either the control or propolis groups, (3) significant downregulation to the expression of casp3 and upregulation to the Bcl2 in the propolis+CdCL2 group compared to the CdCL2 group, and (4) no difference in the expression levels of CYP2E1 between the $CdCL_2$ and propolis+ $CdCL_2$ groups. The scale bars =25 microns (objective 40).

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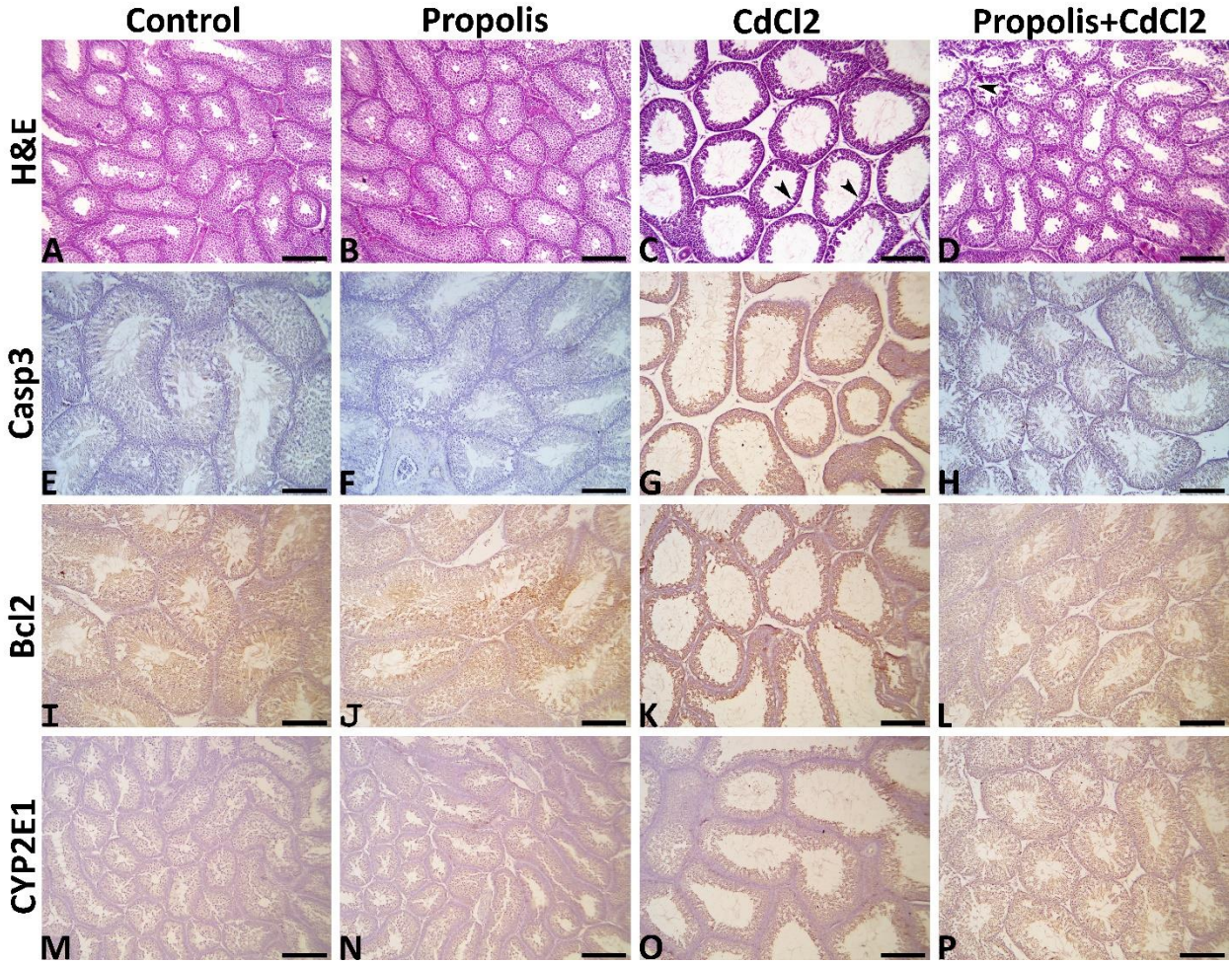

Figure 3|

A-D: Representative photomicrograph of the H&E-stained testes tissue sections showing normal quail testis histology in the control (**A**), and the propolis (**B**) groups. The testis of the $CdCL_2$ group shows marked germ cell depletion with shortening of the heights of the germinal epithelium (black arrowheads) (**C**). The testis of the propolis+ $CdCL_2$ group shows mild germ cell depletion (black arrowhead) (**D**). The scale bars =50 microns (objective 10).

E-P: Representative photomicrograph of casp3 (**E-H**), Bcl2 (**I-L**), and CYP2E1 (**M-P**) immunostained testes tissue sections manifesting (1) no detectable

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differences in the expressions of the three biomarkers between the control and propolis groups, (2) significant upregulation of the casp3, and significant downregulation of the Bcl2 expression, with no effect on the CYP2E1 expression in the CdCL2 group compared to either the control or propolis groups, (3) regaining the expression of the three biomarkers in the testicular tissue sections of the propolis+CdCL2 group to almost the values of the control and propolis group. The scale bars =50 microns (objective 10).

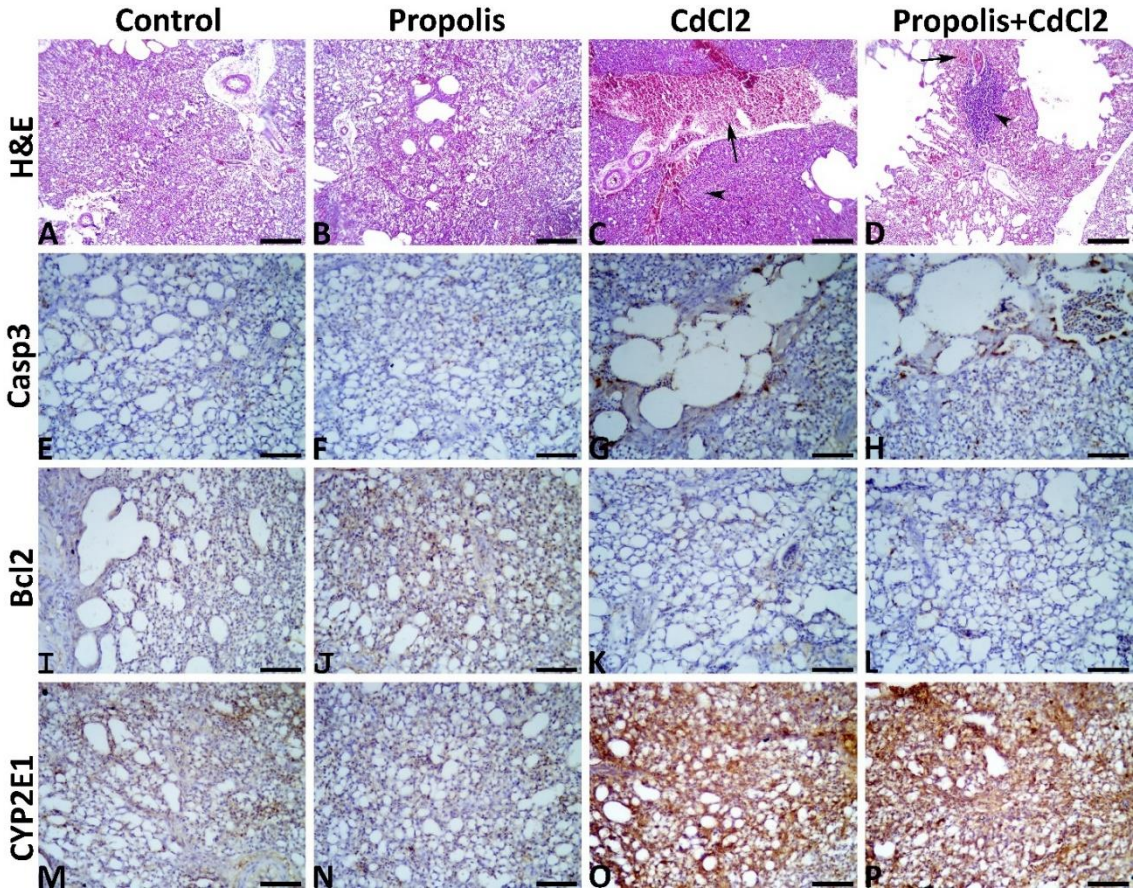

Figure 4|

A-D: Representative photomicrograph of the H&E-stained lung tissue sections showing normal quail lung histology in the control (**A**), and the propolis (**B**) groups. The lung of the $CdCL_2$ group shows notable vascular congestion (black arrow), and diffuse mononuclear cell infiltration to the pulmonary tissue

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(black arrowhead) (**C**). The lung of the propolis+$CdCL_2$ group shows mild vascular congestion (black arrowhead), and focal mononuclear cell aggregation (black arrowhead) (**D**). The scale bars =50 microns (objective 10).

E-P: Representative photomicrograph of casp3 (**E-H**), Bcl2 (**I-L**), and CYP2E1 (**M-P**) immunostained lung tissue sections manifesting (1) no detectable differences in the expressions of the three biomarkers between the control and propolis groups, (2) significant upregulation of the casp3, and CYP2E1 expression and significant downregulation of the Bcl2 expression in the CdCL2 group compared to either the control or propolis groups, (3) significant downregulation to the expression of casp3 and upregulation to the Bcl2 the propolis+$CdCL_2$ group compared to the CdCL2 group, and (4) no difference in the expression levels of CYP2E1 between the $CdCL_2$ and propolis+ CdCL2 groups. The scale bars =25 microns (objective 40).

Table (1) effect of propolis and $CdCl_2$ on the AST, ALT, Creatinine, BUN, MDA, and TAC levels in Japanese quail

	Control	Propolis	$CdCl_2$	Propolis+ $CdCl_2$
AST(U/L)	251 ± 4.16^{b}	245 ± 4.78^{b}	412 ± 6.89^{a}	268 ± 5.37^{b}
ALT (U/L)	6.4 ± 0.48^{c}	5.6 ± 0.41^{c}	18.5 ± 1.35^{a}	9.5 ± 0.96^{b}
Creatinine (mmol/L)	0.49 ± 0.01^{b}	0.47 ± 0.01^{b}	1.2 ± 0.014^{a}	0.64 ± 0.21^{b}
BUN (mmol/L)	5.8 ± 1.21^{c}	5.3 ± 0.98^{c}	10.4 ± 1.94^{a}	7.1 ± 1.05^{b}
MDA (nmol/L)	5.18 ± 0.24^{b}	3.86 ± 0.22^{b}	$14.65\pm0.2.17^{a}$	7.1 ± 1.09^{b}
TAC (U/ml)	180.68 ± 5.36^{a}	218.2 ± 6.47^{a}	95.3 ± 4.89^{c}	161.98 ± 4.37^{b}

Values are represented as the mean ± SE (n=10). The means in the same column with dissimilar superscripts (a, b, c) are significant at $p < 0.05$.

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Table (2) effect of propolis and CdCl2 on the histology of the liver, kidneys, testes, and lungs of Japanese quail

Organ	Lesion	Control		Propolis		$CdCL_2$		Propolis+$CdCL_2$	
		S	F	S	F	S	F	S	F
Liver	Vacuolar degeneration	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.25±0.12[a]	6.25±0.9[a]	0.56±0.07[b]	2.31±0.47[b]
	Hydropic degeneration	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.96±0.35[a]	13.52±2.08[a]	1.01±0.04[b]	4.25±0.97[b]
	Microsteatosis	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.35±0.47[a]	9.25±1.78[a]	0.5±0.02[b]	2.27±0.27[b]
	Macrosteatosis	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.4±0.03[a]	4.27±0.91[a]	0.1±0.02[b]	1.07±0.04[b]
	Single-cell necrosis	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	2.25±0.71[a]	9.18±0.1.3[a] 6	0.8±0.33[b]	3.25±0.77[b]
	Necrotic foci	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.12±0.42[a]	3.25±0.44[a]	0.51±0.06[b]	0.72±0.08[b]
	Intralobular inflammatory cell infiltration	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.92±0.43[a]	7.26±1.07[a]	1.04±0.07[b]	3.07±0.48[b]
	Portal inflammatory cell infiltration	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	2.35±1.03[a]	15.32±2.17[a]	1.46±0.92[b]	7.07±1.03[b]
	Sinusoidal congestion	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.34±0.07[a]	9.11±1.09[a]	1.02±0.02[b]	4.25±0.82[b]
	Central veins and portal congestion	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	2.17±0.92[a]	13.23±2.41[a]	1.17±0.33[b]	8.29±1.91[b]
	Hemorrhages	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.02±0.05[a]	2.35±0.07[a]	0.3±0.01[b]	0.95±0.02[b]
	Cholestasis	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.02±0.01[a]	1.03±0.02[a]	0.02±0.01[b]	1.03±0.02[b]
Kidneys	Glomerular congestion	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.22±0.05[a]	10.11±1.74[a]	1.01±0.04[b]	6.25±1.02[b]
	Glomerular collapse	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.42±0.61[a]	5.23±1.02[a]	0.78±0.09[b]	2.22±0.34[b]
	glomerular necrosis	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.65±0.04[a]	3.26±0.78[a]	0.07±0.02[b]	1.26±0.42[b]
	Tubular vacuolations	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	3.25±1.25[a]	27.91±4.79[a]	2.04±0.97[b]	13.7±2.35[b]
	Tubular necrosis	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	3.21±0.95[a]	24.25±3.39[a]	2.21±1.04[b]	11.35±2.11[b]
	Cast formation	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	0.0±0.0[c]	1.18±0.34[a]	9.56±1.88[a]	1.2±0.15[b]	6.36±1.21[b]

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	Vascular congestion	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	3.1±1.21 a	39.25±5.68 a	2.44±0.9 b	22.45±3.96 b
	Hemorrhage	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.02±0.55 a	7.69±1.23 a	0.53±0.04 b	2.36±0.07 b
	Interstitial edema	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.21±0.01 a	3.22±0.41 a	0.01±0.01 b	1.25±0.02 b
	Interstitial leukocytic infiltration	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.21±0.04 a	9.36±1.2v	0.99±0.07 b	7.23±1.11 b
Testes	ST with vacuolated epithelium	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.11±0.25 a	12.24±2.24 a	0.05±0.03 b	9.36±1.27 b
	STs with depleted germ cells	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	3.41±1.17 a	71.12±9.68 a	1.83±0.91 b	27.07±4.23 b
	ST with necrotic epithelium	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.25±0.63 a	13.58±2.15 a	0.68±0.07 b	7.23±0.61 b
	STs with multinucleated giant cells	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.2±0.01 a	4.23±0.28 a	0.00±0.00 b	0.00±0.00 b
	Vascular congestion	0.0±0.0 a	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.35±0.01 a	7.25±1.08 a	0.14±0.08 b	3.25±0.27 b
	Interstitial hemorrhage	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
	Interstitial leukocytic infiltration	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Lungs	Pulmonary consolidation	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
	Congestion	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	2.36±0.93 a	52.07±7.26 a	1.25±0.04 b	21.51±4.26 b
	Hemorrhages	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.22±0.07 a	14.23±2.25 a	0.26±0.07 b	4.26±0.75 b
	Septal thickening	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.17±0.71 a	11.28±2.71 a	0.07±0.01 b	5.23±1.12 b
	Edema	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a	0.25±0.02 a	2.14±0.02 a	0.00±0.00 b	0.00±0.00 b
	Emphysema	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.23±0.44 a	7.25±1.33 a	1.22±0.41 b	6.35±1.27 b
	Leukocytic infiltration	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	0.0±0.0 c	1.23±0.04 a	14.25±3.25 a	0.87±0.05 b	6.35±1.75 b

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S= severity, F= frequency (%), STs= seminiferous tubules.

Values are represented as the mean ± SE (n=10). The means in the same column with dissimilar s uperscripts (a, b, c) are significant at $p < 0.05$.

Table (3) effect of propolis and CdCl2 on the immunoexpression of casp3, Bcl2, and CYP2E1 of the liver, kidneys, testes, and lungs of Japanese quail

Organ	Biomarker	Control	Propolis	$CdCL_2$	Propolis+$CdCL_2$
		Area fraction %	Area fraction %	Area fraction %	Area fraction %
Liver	Caspase 3	0.00±0.00	0.00±0.00^{c}	14.25±3.68^{a}	5.26±1.39^{b}
	Bcl2	9.35±1.25^{a}	10.18±1.35^{a}	4.23±1.35^{c}	6.32±1.25^{b}
	CYP2E1	3.14±0.96^{c}	3.48±0.67^{c}	19.19±4.25^{a}	18.36±3.97^{b}
kidneys	Caspase 3	0.00±0.00^{c}	0.00±0.00^{c}	21.36±4.56^{a}	11.25±2.36^{b}
	Bcl2	14.25±3.25^{a}	14.08±3.36^{a}	2.51±0.08^{c}	4.36±1.25^{b}
	CYP2E1	2.24±0.95^{c}	2.87±1.04^{c}	19.35±4.25^{a}	16.35±4.14^{b}
Testes	Caspase 3	0.00±0.00^{c}	0.00±0.00^{c}	31.25±5.89^{a}	6.35±1.74^{b}
	Bcl2	21.25±4.56^{a}	23.54±4.15^{a}	8.69±2.15^{c}	12.25±3.65^{b}
	CYP2E1	5.36±1.25^{a}	5.98±1.28^{a}	6.35±1.45^{a}	6.98±1.24^{a}
Lungs	Caspase 3	0.00±0.00^{c}	0.00±0.00^{c}	4.25±0.09^{a}	2.32±0.08^{b}
	Bcl2	6.35±1.23^{a}	7.26±1.98^{a}	2.36±0.45^{c}	4.26±1.75^{b}
	CYP2E1	9.36±2.75^{b}	9.89±2.45^{b}	36.25±5.45^{a}	34.25±6.38^{a}

Values are represented as the mean ± SE (n=10). The means in the same column with dissimilar superscripts (a, b, c) are significant at $p < 0.05$.