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Research Article
Western Algerian Propolis Alcohol Extract Effects Against Disruption of Testes Function Induced by Cadmium-sulfate in Male Rats

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Abstract

Background and Objective: Propolis, a natural product derived from plant resins collected by honeybees has been used for thousands of years in traditional medicine all over the world. The composition of the propolis depends upon the vegetation of the area from where it was collected and on the bee species. This study aimed to assess the preventive effects of Algerian propolis against reproductive toxicity of Cadmium Sulfate ($CdSO_4$) in male rats. **Materials and Methods:** Animals were divided into four groups; group 1 was a control, group 2 received 15 mg $CdSO_4$ kg^{-1} b.wt., for consecutive 35 days, Group 3: exposed to $CdSO_4$ then treated with 200 mg propolis kg^{-1}, Group 4: treated with propolis then was administered $CdSO_4$ in the same conditions. **Results:** The results showed that $CdSO_4$ caused a decrease in body weight gain, testes weight and testosterone level. In the $CdSO_4$-treated group, histopathologic examinations revealed apparent alterations in the testes, where it induced marked lesions in seminiferous tubules. Group 4, treated with propolis showed an increased plasma testosterone. **Conclusion:** Through these findings, propolis antagonized the harmful effects of $CdSO_4$. This was proved histopathologically by the great improvement in testes. Propolis could be effective in the prevention against the reproductive toxicity of $CdSO_4$.

Key words: Propolis, cadmium sulfate, testes, reproductive toxicity, male rats

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cadmium (Cd), toxic heavy metal at low dose, is widely present in soil and ecosystems with half-life about 15 years[1]. Cd is used in industry to produce pesticides and batteries, also found in food (mollusks, crustacean and cocoa powder) and in tobacco smoke[2]. About 25000 t of Cd was released every year in environment[3]. According to the International Agency of Research on Cancer (IARC), Cd is classified as a human carcinogen. Cd toxicity promotes the tumorigenesis through cell oxidative stress and inhibition of repair of oxidative DNA damage[4]. Cd toxicity impeded apoptosis and impelled cell proliferation leading to phenotype malignancy[5]. Studies suggested that Cd toxicity induced reactive oxygenated species (ROS) or free radical synthesis and consequently increased redox disruption[6]. Following an intestinal absorption, Cd bind to metalloproteins (MPP) and accumulate in different tissues such as lungs, liver, kidney, testis and prostate[7]. Cd disturb mitochondrial oxidative phosphorylation and inhibit cell respiration. Chronic occupational exposures to Cd reduce the fertility of workers[7,8]. Cilenk *et al.*[8] showed, in animals exposed to $CdCl_2$ at 1 mg kg^{-1}, significant decrease in sperm concentration, sperm motility, normal and live sperm[8]. The treatment of Cd toxicity is only symptomatic.

Propolis is natural resinous hive product. It is synthetized by honeybees, mixing their own waxes and salivated secretions with resins collected from the cracks in the bark of trees and leaf buds[9]. Propolis is consisted of 50% resin and balsam, 30% wax, 10% essential oils, 5% pollen and 5% other substances including organic compounds and minerals such as phenolic acids, flavonoids, terpenes, β-steroids, alcohols and sesquiterpenes[10]. Propolis, used in folk medicine, has biological activities like anti-microbial, anti-tumor and anti-oxydant properties[10]. Recent studies suggested that caffeic acid phenethylester (CAPE), bioactive compound found in propolis has anticancer properties[11]. This molecule did not neutralize the prostate cancer but it slows its progression. Signaling transducer proteins (70S6 protein kinase and Akt), intermediates in steps of PI3K/mTOR signaling pathway, promote cell proliferation and angiogenesis[12]. Propolis, through its magical molecule CAPE, inhibit Akt and 70S6 protein kinase activities and empelled cell death or apoptosis[13]. The present study aimed to assess the reproductive toxicity of Cadmium sulfate in adult male rats. Also, to evaluate the preventive effects of western Algerian propolis against the eventual testis dysfunction induced by Cadmium sulfate.

MATERIALS AND METHODS

Obtaining of propolis: Propolis samples were harvested by farmers at Rebahia area, located in the Saida region (western Algeria). The most abundant plant, as source of propolis, in this region was *Pinus halepensis*. Then propolis was purchased at local market, mainly at store specializing in sales of bee products.

Preparation of propolis alcohol extract: This experiment step was conducted according to Franchi's protocol[14]. Amount of 50 g of the propolis was cut into small pieces and extracted with 600 mL of 80% ethanol at 60°C for 30 min. Mixture was centrifuged and the supernatant was evaporated at 40°C. Dried residue was kept at 4°C. Aqueous suspension of propolis was prepared and orally administered to the animals for 35 days in a dose of 200 mg kg^{-1} b.wt.[15].

Experimental design: Twenty male adult rats (average weight 180-200 g) were used in the experiments. Animals were obtained from Pasteur Institute, Algiers, Algeria. The scientific committee of Research Laboratory of Water Resources and Environment, Faculty of Science, University of Saida, Algeria, approved the design of the experiments and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were caged in groups and given feed and water *ad libitum*. After one week of acclimation, animals were divided into four equal groups. The toxic dose of Cd used in the experiments was 15 mg kg^{-1} b.wt.[16]:

Group 1: Normal animals used as controls

Group 2: Experiment control animals orally administrated with $CdSO_4$ (15 mg kg^{-1} day^{-1})

Group 3: Animals, initially exposed to $CdSO_4$ were orally treated with propolis extract (200 mg kg^{-1} day^{-1})

Group 4: Animals, initially treated with propolis were orally administered with $CdSO_4$ in the same experiment conditions

Biochemical study: During the experiment period, different blood biochemical parameters were determined. Serum cadmium, prostate specific antigen (PSA) and testosterone were measured using mini-VIDAS automate analyzer (Bio-Merieux, France). The enzyme-linked fluorescent assay (ELFA method) was used to assess serum PSA and testosterone levels. The activity of Glutathione S-transferase (GST) was measured according to Habig's

method[17]. The absorbance was measured at 310 nm using UV-spectrophotometer. The enzyme catalase (CAT) activity was measured spectrophotometrically at 240 nm[18].

The blood samples were centrifuged at 2500 rpm for 10-15 min and the sera isolated were used for estimation of the serum glucose, triglycerides, total cholesterol, creatinine and urea levels. It was added to this study, a measurement of the blood count formula (BCF) that determine the count of all blood cells through controller Coulter STKS®.

Histopathological study: Testis were removed and kept in 10% formol saline for 24 h, dehydrated in ethanol and embedded in paraffin. Sections were cut at 5 micron thickness, mounted on slides and stained with hematoxylin and eosin.

Statistical analysis: Data were expressed as Mean±SD, with a value of $p<0.05$ or $p<0.01$ considered statistically significant. Statistical evaluation was performed by one way analysis of variance (ANOVA) followed by the Tukey's t-test for multiple comparisons. All analysis were made with the statistical software Sigma-Plot (version 11.0).

RESULTS

Results showed significant ($p<0.01$) decrease in body weight and testis weight in animals administrated with $CdSO_4$ at the dose 15 mg kg^{-1} (167.2±4.4 and 2.28±0.1 g, respectively) compared to controls (223.1±9.8 and 2.82±0.56 g, respectively) and animals treated with propolis at the dose 200 mg kg^{-1}; group 3 (190.3±2.8 and 2.64±2.01 g, respectively) and group 4 (176.6±2.7 and 2.4±0.96 g, respectively) (Table 1). Whereas experiments of this present study indicated significant increase in blood glucose (1.83±0.1 g L^{-1}), lipid profile (Triglycerides: 1.76±0.1 g L^{-1} and T-cholesterol: 4.11±0.18 g L^{-1}), kidney parameters (Urea: 0.84±0.07 g L^{-1} and creatinine: 62.89±9.36 mg L^{-1}), blood PSA (9.15±3.62 ng mL^{-1}), testosterone (0.95±0.11 ng mL^{-1}) and serum cadmium (8.80±0.89 µg L^{-1}) in animals treated with cadmium (group 2) compared to controls (1.03±0.11, 0.96±0.05, 0.74±0.06 and 0.17±0.02 g L^{-1}, 10.84±1.21 mg L^{-1}, 2.27±0.28 and 1.31±0.24 ng mL^{-1}, 0.03±0.001 µg L^{-1}, respectively) and animals orally administrated with propolis as follow; group 3 (1.37±0.1, 1.37±0.04, 2.26±0.22, 0.55±0.06 g L^{-1}, 31.65±6.00 mg L^{-1}, 4.69±0.48, 1.06±0.10 ng mL^{-1} and 1.40±0.90 µg L^{-1}, respectively) and group 4 (1.36±0.06, 1.27±0.04, 1.62±0.09, 0.40±0.03 g L^{-1}, 19.44±2.14 mg L^{-1}, 3.94±0.24, 1.17±0.11 ng mL^{-1} and 1.23±0.02 µg L^{-1}, respectively) in the same experimental conditions such as (Table 1). Hematological results displayed low red cells and hemoglobin in animals administrated with cadmium (2.7±0.3×10^6 IU and 5.1±0.8 g dL^{-1}, respectively) compared to controls (6.7±0.4×106 IU and 15.6±1.8 g dL^{-1}, respectively) and animals treated with propolis in group 3 (4.8±2.2×106 IU and 8.1±0.8 g dL^{-1}) and group 4 (5.2±0.0×10^6 IU and 9.9±0.7 g dL^{-1}) (Table 1).

Treatment with $CdSO_4$ induced significant ($p<0.01$) decrease in the activities of testes catalase (CAT) and glutathione S-transferase (GST) and reduced glutathione (GSH) compared to control animals. While, animals treated with propolis showed significant increase in CAT, GST and GSH ($p<0.01$) (Table 1). In groups 3 and 4, propolis was able of recovering the activities of CAT and GST and the level of GSH compared to controls (Table 1).

Table 1: Variation of biochemical parameters in rats treated with $CdSO_4$ and propolis

Variables	Group 1 (controls)	Group 2 ($CdSO_4$)	Group 3 ($CdSO_4$/propolis)	Group 4 (propolis/$CdSO_4$)
Body weight (g)	223.10±9.8	167.20±4.4	190.30±2.8	176.60±2.7
Gain body weight (g day^{-1})	1.09	-0.48*	0.17*	0.30*
Testis weight (g)	2.82±0.56	2.28±0.1	2.64±2.01	2.40±0.96
Blood glucose (g L^{-1})	1.03±0.11	1.83±0.1*	1.37±0.1*	1.36±0.06*
Triglyceride (g L^{-1})	0.96±0.05	1.76±0.1*	1.37±0.04*	1.27±0.04*
T-cholesterol (g L^{-1})	0.74±0.06	4.11±0.18	2.26±0.22	1.62±0.09
Urea (g L^{-1})	0.17±0.02	0.84±0.07	0.55±0.06	0.40±0.03
Creatinine (mg L^{-1})	10.84±1.21	62.89±9.36	31.65±6.00	19.44±2.14
Red cells (×10^6 IU)	6.70±0.4	2.70±0.3	4.80±2.2	5.20±0.0
Hemoglobin (g dL^{-1})	15.60±1.8	5.10±0.8	8.10±0.8	9.90±0.7
PSA (ng mL^{-1})	2.27±0.28	9.15±3.62*	4.69±0.48*	3.94±0.24*
Testosterone (ng mL^{-1})	1.31±0.24	0.95±0.11*	1.06±0.10	1.17±0.11*
Cadmium (µg L^{-1})	0.03±0.001	8.80±0.89*	1.40±0.90*	1.23±0.02*
CAT (mol h^{-1} g^{-1} tissue)	6.50±1.2	3.20±0.60	5.70±0.8	8.12±0.92
GST (µmol min^{-1} g^{-1} tissue)	1.09±0.10	0.60±0.15	0.97±0.12	1.46±0.30
GSH (mM g^{-1} tissue)	5.80±0.7	4.10±0.7	5.62±1.9	8.13±0.9

*Results significantly different ($p<0.01$), T-cholesterol: Total cholesterol, CAT: Catalase, GST: Glutathione S-transferase, GSH: Reduced glutathione

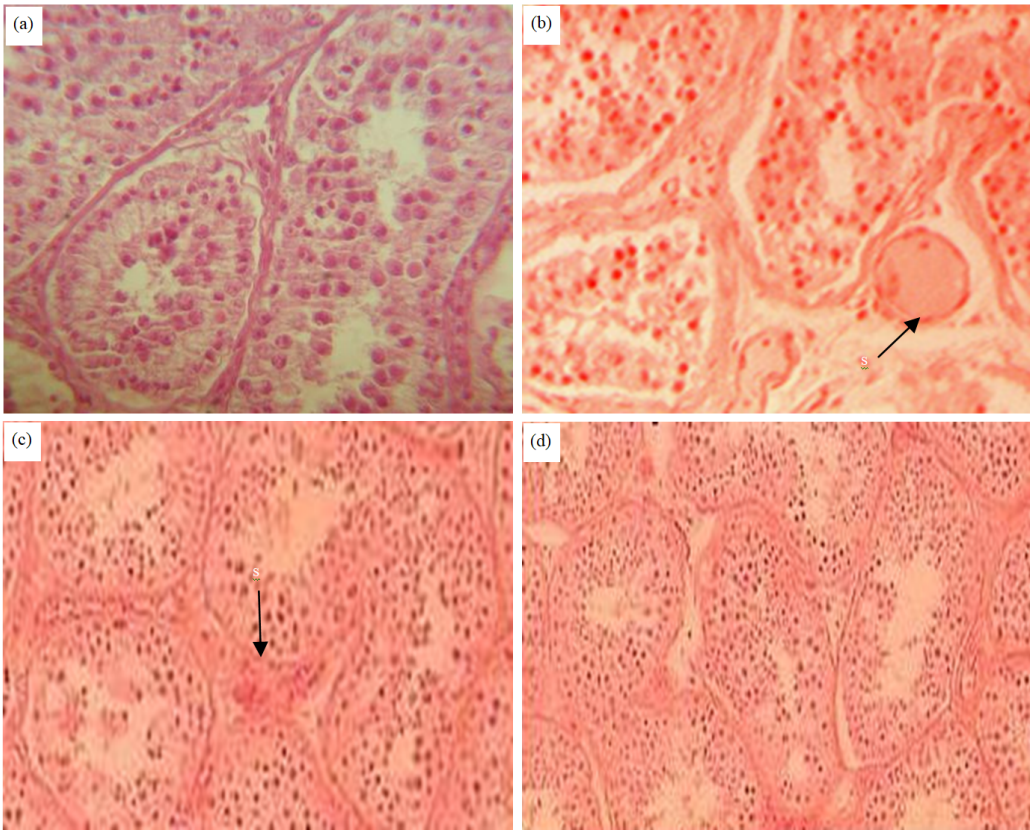

Fig. 1(a-d): Photomicrograph of testis exposed to $CdSO_4$ and treated with propolis extract
S with arrow indicates interstitial edema with congestion of blood vessels

The histological study showed, in control, testis are surrounded by a fibrous tissue. The testis are divided into lobules with several seminiferous tubules, which are surrounded by the interstitial tissue. Each tubule contained germ cells with various stages of spermatogonia, primary and secondary spermatocytes, spermatids and mature spermatozoa located in the centre of the tubule. In the interstitial tissue, Leydig cells were between the seminiferous tubules (Fig. 1a). Observation of treated testes with $CdSO_4$ showed necrosis and alteration. The architecture of seminiferous tubules was confused. Some germ cells had small and darkly stained nuclei. Dilation and congestion of blood vessels were observed in the interstitial spaces. Large Leydig cells were found in the interstitial tissue (Fig. 1b). Animals, initially treated with cadmium then with propolis, revealed that testis recovered more or less their normal structure reaching consistent seminiferous tubules (Fig. 1c). Rats, initially treated with propolis then administrated with cadmium, displayed sections of testes less or more similar to the control sections. The germ cells appeared having regular shape with absence of cytoplasmic vacuolation (Fig. 1d).

DISCUSSION

The main objective of this present study was to explore whether the propolis effects enabled animals contaminated with cadmium-sulfate recovering their normal biochemical and histological status. Heavy metals are known as toxic and belonged to the class of the carcinogenic products. Several studies suggested that men exposed, at long time, to Cd toxicological environment developed infertility, a decreased sperm count and poor semen quality[19,20]. Chronic exposure to cadmium induced harmful and deleterious effects on the human and animal health. The decrease in body weight gain of animals treated with cadmium were in concordance with recent studies[20]. Cadmium slowed intestinal absorption of nutrients and strongly decreased the appetite of animals. No significant changes, in testes weight, were recorded after cadmium sulfate ingestion. This finding was in contrast with those of Haouem *et al.*[21]. The association between Cd toxicity and hyperglycemia, hypercholesterolemia and hyperlipidemia has been more extensively studied[22]. In this study, it was noticed in animals contaminated with Cd increased blood

glucose, cholesterol triglycerides, urea and creatinine levels. These outcomes were endorsed by the recent studies performed by Lei *et al.*[23] and Son *et al.*[24]. Interesting results were obtained in Cd-exposed workers during 20 years. It was suggested they were characterized by high blood glucose levels and decreased serum insulin. In the same way, studies revealed an association between urinary cadmium levels and the prevalence of diabetes[22]. This present study displayed low red cells account and a decreased hemoglobin rate. This finding also showed an induced anemia cadmium toxicity. Cd induced damage to the erythrocyte membrane resulting in hemolysis which may be the cause of decreased of haemoglobin level[3]. This study also revealed an inverse association between prostate specific antigen (PSA) and blood cadmium level. In prostate cancer diagnosis, blood PSA was used as a tumoral marker for the early detection of prostate cancer[25]. In the present study, accumulation of Cd in testes decreased serum testosterone. This result was in disagreement with the finding of Zeng *et al.*[26] and Haouem *et al.*[21]. $CdSO_4$ intoxicated animals showed an oxidative stress including in CAT, GST and GSH. These finding were similar to that found in the previous studies, which revealed cadmium induced oxidative stress inducing free radical generation[21].

Propolis, natural product elaborated by honeybee, has pharmacological properties such as antibiotic activities, anticancer, antioxidant and anti-inflammatory[27]. Propolis contained bioactive compounds including terpenes, sterols, polyphenols, phenolic acid and flavonoid[28]. Propolis antioxidant activities were illustrated by scavenging reactive oxygen species (ROS) or free radicals and prevention against lipid peroxidation[10]. Propolis also stimulated antioxidant enzymes such as catalase and glutathione S-transferase against oxidative stress caused by ROS[29]. The treatment animals with propolis significantly increased serum testosterone and also the anti-oxidative enzymatic activities of CAT and GST compared to controls and animals intoxicated with Cd. Furthermore, these results revealed treatment of rats with propolis plus $CdSO_4$ decreased glutathione (GSH) level at nearly normal. These results joined other studies that had already shown propolis protected against cadmium chloride toxicity[30]. Several scientific research supported that treatment with propolis enhances damaged testes tissues and limited functional disruption induced by cadmium in testis tissue[31]. The histological changes in testes of animals treated with $CdSO_4$ were in agreement with the study conducted by Cilenk *et al.*[8]. The treatment animals with propolis improved less or more the histopathological profile of testis. The propolis protective effects against Cd-induced testicular injuries was reported by Messaoudi *et al.*[32].

These findings are debatable for different reasons; to change the extraction method and to use different organic solvent instead of ethanol, to explore the toxicity of the propolis , the alcohol propolis extract could be insufficient to promote relevant preventive effects as expected and finally to investigate synergy molecular reactions of bioactive compounds elucidating how do magical molecules acting in cells and fighting oxidative stress resulting from Cadmium's toxicity.

CONCLUSION

This study, first of its kind in Algeria, showed that exposure to Cadmium sulfate induced necrosis in testicular tissue, increased serum PSA and decreased blood testosterone. This study confirmed obvious results in the literature. The treatment with propolis had beneficial effects improving all the biochemical parameters. Therefore, the present study endorsed the preventive effects of propolis to minimize the Cadmium toxicity.

SIGNIFICANCE STATEMENT

This study discovers the high preventive effects of local Algerian propolis against the toxicity of cadmium sulfate in male rats. This study will help researchers to uncover the critical areas of the honeybee product therapy that many researchers were not able to explore. Thus, the new theory on honeybee derived products and food supplements may be arrived at the replacement, the synthetic chemical drugs by natural substances.

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