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**REGULAR ARTICLE**

## Effect of bee pollen extract on lead-induced toxicity in rat testis

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**ABSTRACT**

Collected by honeybees, Bee pollen is a flower pollen gathered from selected flower species by special pollen traps, therapeutic qualities and reproductive performance of bee pollen are given from biologically active substances. Lead is a heavy metal, which induce reproductive intoxication, comes in the high levels from human activities. The aim of the present study was to evaluate the impact of bee pollen hydro-ethanolic extract on adult *Wistar* rat testis exposed to lead acetate sub-acute intoxication (15 days), highlighted using histological technics *in situ*. The biochemical and biometric measurement were conducted to verify any eventual toxicity of hydro-ethanolic extract *in vivo*. Animals were divided into four equal groups: first control group was treated orally with distilled water (1 ml) by gavage. The second group was administered 100 mg/kg/bw/day of a hydro-ethanolic extract of bee pollen, the third group was administered 30 mg/kg/bw/day lead acetate, the fourth group was given the both treatments in the same levels. The results showed that the relative organs weight indicates that only left kidney ( $p=0.001$ ) and spleen ( $p=0.001$ ) were attenuated without a considerable difference of the liver ( $p=0.07$ ). the same was shown for relative weight of sexual organs. In addition, no significant difference, was marked, in serum biochemical parameters except for: Blood urea nitrogen, Alanine aminotransferase, Total bilirubin and Direct bilirubin. furthermore, ameliorative effect was shown after lead-induced alteration after histological stain of seminiferous tubules, indicating low lumen of seminiferous tubules with presence of spermatogonia and wide height of seminiferous epithelium. Biologic activities of bee pollen extract, alleviate the lead induced toxicity on rat testis, marked by a complete spermatogenesis and prevention of Sertoli cells destruction.

**1. Introduction**

Lead intoxication occurs mainly by ingestion of contaminated food or water. It has an effect on a number of body functions such as central nervous (Todd et al., 1996; Bellinger, 2004), hematopoietic (Vij, 2009), hepatic (Haouas et al., 2014), renal (Lin

et al., 2006), cardiovascular and reproductive system (Navas-Acien et al., 2007; Bergeson, 2008). At the blood lead levels between 30 and 45  $\mu\text{g}/\text{dl}$ , males sperm count is reduced (Navas-Acien et al., 2007). the high dose reduced percentage of motile sperm and increased the percentage of abnormal sperm within the epididymis in CF1 mice (Wadi et

Ahmad, 1999). Certain species and strains were quite resistant to the reproductive toxicity of lead, and endocrine profile was not affected (Apostoli et al., 1998). After recovery period, Serum testosterone and sperm parameters normalised in the prepubertal animals but not in the pubertal animals (Sokol, 1989), these results were shown after 14 days (Sokol, 1990). An increase in GnRH mRNA indicate that disrupted signals within and between the hypothalamus and pituitary gland, by long-term, low-dose lead exposure (Sokol et al., 2002). This stimulatory action of the gonadotropins is more sensitive in rat testis, as the animal matures (Sokol et Berman, 1991).

Nutrition also plays an important role in prevention of lead induced toxicity (Hsu et Guo, 2002). beta-carotene has antioxidant activity and exert some beneficial effects in lead intoxication, independent of chelation (Dobrakowski et al., 2014).

Used as apitherapeutic, Bee pollen, has potential medical and nutritional applications, such as antiallergic (Ishikawa et al., 2009), antifungal (Ozcan, 2004), antimicrobial (Pascoal et al., 2014), antiviral, anti-inflammatory (Maruyama et al., 2010), immunostimulating (Aliyazicioglu et al., 2005), local analgesic, lipid lowering (Samochowiec et Wójcicki, 1981; Samochowiec et Wójcicki, 1983), antioxidant (Campos et al., 2003; Morais et al., 2011), anticarcinogenic (Furusawa et al., 1995), and antithrombotic activities (Bogdanov, 2004; Eraslan et al., 2009; Eraslan et al., 2010; Hegazi, 2012) Bee pollen at dose of 100 mg/kg/bw/day, alleviate negative changes in most of the biochemical parameters in female Wistar rat exposed to propoxur by gavage (Eraslan et al., 2009).

Chemical composition of bee pollen was identified by chromatographic or spectrometric technics, including: Proteins, essential amino acids, essential fatty acids, Sterols, sugar, crude fiber, nucleic acids, vitamins and minerals (Roulston et Cane, 2000; Cogan et al., 2005; Almeida-Muradian et al., 2005; Campos et al., 2008; Campos et al., 2010; Denisow et Denisow-Pietrzyk, 2016). Composed of cellulose and pectin and other difficult or impossible to digest (Roulston et Cane, 2000). Ethanol or water extraction seems to be more efficient for use to obtain therapeutic effects (Kroyer et Hegedus, 2001).

To our knowledge no studies were published on the preventive effect of hydro-ethanolic extract of bee pollen on male fertility in rats. The aim of this

study was to examine possible effects of bee pollen ethanolic extract on rat testicular functions after lead-induced toxicity. Eventual bee pollen ethanolic extract toxicity was taken in consideration, so biochemical and biometric assessment were released.

## **2. Material and methods**

### **2.1. Botanical evaluation of bee pollen**

2g of pollen pellets, was used for pollen analysis. One pollen microscope slide was prepared by washing the pollen in 50% ethanol and prepared using glycerin jelly and paraffin (Barth, 1989).

### **2.2. Bee pollen collection and Extract preparation**

Bee Pollen samples were obtained from the expert beekeepers of Sidi Bel Abbes in April 2018 (West of Algeria). One hundred of dried and powdered were homogenized in mixture (ethanol/water) (280/80ml: V/V) for 72h at room temperature and with replacement of solvent every 24h. The extract was filtered with Whatman No 1 filter paper and evaporated separately to remove the solvent in a rotary evaporator at 40°C.

### **2.3. Biochemical and antioxidant proprieties of bee pollen extract**

#### **2.3.1. Estimation of total phenols content**

The estimation of total phenols (TPC) content was carried out as described earlier (Benhammou et al., 2006). The total phenolic content was expressed as mg of gallic acid equivalents/100 g of dry matter.

#### **2.3.2. Estimation of total flavonoids content**

Total flavonoids (TFC) assessment was carried out as described earlier (Zhishen et al., 1999). The total flavonoids content was expressed as catechine (mg CE/100g dry weight).

#### **2.3.3. Estimation of condensed tannins content**

Condensed tannins content (CTC) was carried out as described earlier (Schofield et al., 2001). condensed tannins content was expressed as catechine (mg CE/100g dry weight).

#### **2.3.4. DPPH radical scavenging activity assay**

Total antioxidant activity was carried out as described earlier, according to free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Molyneux, 2004), radical scavenging activity of DPPH was expressed as SC<sub>50</sub>.

## 2.4. Blood and tissue samples

After animal sacrifice, blood samples were collected from carotid artery. Brain, Liver, kidneys, spleen and testes tissues were removed. Each organ was quickly weighed and fixed in neutral buffered formalin for histopathological studies.

## 2.5. Experimental design

Twenty male Wistar rats (280–350 g) were used. The animals were selected and divided into four equal groups. The first group (C) was maintained as control and received 1 ml of distilled water, whereas Group 2 (P) was administered 100 mg/kg/bw/day of a hydro-alcoholic extract of bee pollen in a volume of 1 ml distilled water. Group 3 (Pb) was administered 30 mg/kg/bw/day lead acetate in a volume of 1 ml distilled water and Group 4 (Pb + P) was given 30 mg/kg/bw/day lead acetate in association with 100 mg/kg/bw/day of a hydro-ethanolic extract of bee pollen in a volume of 1 ml for a period of 15 days by gavage. The animals are provided with *ad libitum* drinking water and rat pellet feed (containing 7% crude cellulose and 18% crude). The animals are housed at a temperature of 25 °C, and were exposed to a 12 h light/12 h dark cycle. Experimental protocol was approved by the institutional review board of Biology Department, Faculty of Life and Natural Science, University of Djillali Liabes, Sidi-Bel-Abbes, Algeria.

## 2.6. Serum biochemical parameters determination

Serum was separated from the whole blood samples by centrifugation at 5000 rpm for 10 min. and stored at -20°C until analysis with Erba Mannheim reagent (Erba Lachema s.r.o., Karásek 1d, 621 00 Brno, CZ) of: Glucose (Glu); Blood urea nitrogen (BUN); Creatinine (Crea); uric acid (UA), C-Reactive Protein (CRP); Total protein (Prot); Albumin (Alb), Aspartate aminotransferase (AST); Alanine aminotransferase (ALT); Lactate dehydrogenase (LDH); Total bilirubin (Bil T); Direct bilirubin (Bil D); Indirect bilirubin (Bil I); Glucose (Glu); Total cholesterol (Chol); Triglyceride (TG).

Calcium (Ca<sup>++</sup>), Sodium (Na<sup>+</sup>) and Potassium (K<sup>+</sup>); were assessed using EasyLyte Na/K analyser (Medica corporation, 5 Oak Park Drive Bedford, Ma 01730-1413 USA).

## 2.7. Histological study

Tissue samples were fixed immediately in 10% buffered formaldehyde, dehydrated with ethanol series, cleaned with xylene (Leica), embedded in

parafn (Leica EG 1160), and sectioned as 5 µm (Leica). Tissue sections were stained with hematoxylin and eosin (H&E) (Leica ST 4040) then examined under a light microscope (Optika B 350). a second coloration of toluidine blue was used.

## 2.8. Statistical analysis

Collected data are expressed as mean ± standard deviation. statistical analysis was performed using IBM SPSS v 23, The comparison between groups was established using ANOVA, Brown Forsyth, Welch or Kruskal Wallis tests, sustained if significant with Tukey post hoc test or U Mann Whitney test. the significance level is 0.05.

## 3. Results and discussion

### 3.1. Results

#### 3.1.1. Botanical evaluation of bee pollen

Four plant taxa were identified: Asteracées: *Calendula arvensis* L.; Papaveracées: *Papaver rhoeas* L.; Asteracées: *Calendula bicolor* Raf.; Brassicaceae: *Sinapis arvensis* L.

#### 3.1.2. Phytochemical proprieties of bee pollen extract

Phytochemical and antioxidant proprieties of bee pollen extract are represented in Table 1.

TPC (mg GAE/g DW)	TFC (mg CE/g DW)	CTC (mg/CE/g DW)	DPPH SC <sub>50</sub> (mg/ml)
9.977±0.079	5.156±0.144	1.398±0.603	5.53±0.14

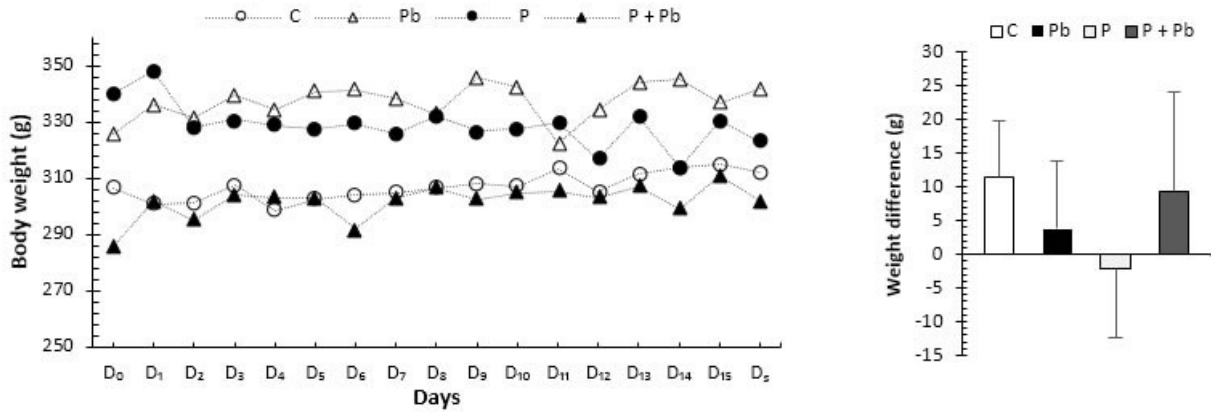
Table 1: Phytochemical and antioxidant proprieties of bee pollen extract.

TPC: Total phenolic content; TFC: Total flavonoid content; DPPH: free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl; GAE = Gallic acid equivalent; CE = Catechin equivalent; DW = Dry Weight

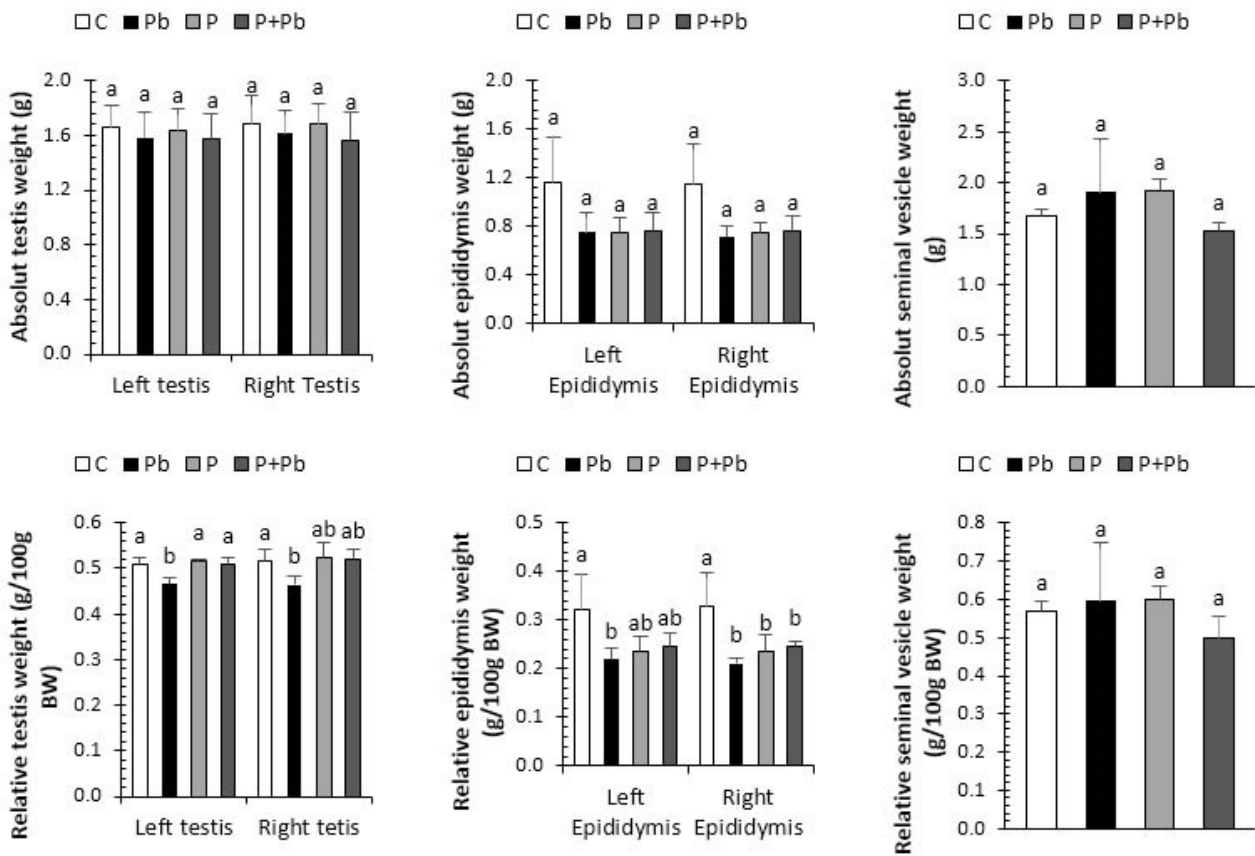
#### 3.1.3. Biometric parameters

body weight difference measurement showed an increase of 3.99% for (C) group, similar to (P + Pb) group (4.05%). In opposite a 1.56% decrease was marked in (Pb) group. In addition (P) treatment indicate a slight decrease (about -0.40% from initial body weight), observed from the first day (Figure 1). Indeed, no considerable difference was shown. furthermore, the comparison between initial and the final body weight indicate no significant difference for all groups except for (Pb) group (p=0.015).

The comparison between different treatments, revealed no difference in absolute organs weight. In



**Figure 1: Body weight evolution.**  
**C: Control; Pb: Lead acetate; P: Bee pollen; P+Pb: Pollen and Lead acetate**



**Figure 2: Absolute and relative weight of sexual organs.**  
**C: Control; Pb: Lead acetate; P: Bee pollen; P+Pb: Pollen and Lead acetate. Bars marked with the same letter are not significantly different**

opposite, a considerable increase in relative left kidney weight was shown after Pb administration ( $p < 0.001$ ). and a significant drop of spleen relative weight ( $p < 0.001$ ) (Table 2).

Absolute weight of sexual organs didn't differ significantly (Figure 2). Both left and right relative testis weight decreased in (Pb) group, this drop wasn't shown in other groups. The same as the preceding results, a relative weight drop were observed in epididymis in (Pb) group. the (P) treatment ameliorate testis and epididymis weight. Absolute and

relative weights of seminal vesicle were identical ( $p = 0.237$ ;  $p = 0.342$ ) respectively.

**3.1.4. Biochemical parameters**

Glucose (Glu), Total cholesterol (Chol) and Triglyceride (TG) are summarized in (Figure 3). Even (Glu), (Chol) and (TG) was higher in (P) treated group, it stayed in physiological value. In addition, a diminution was marked in (Pb) treated group. Indeed, no amelioration was shown.

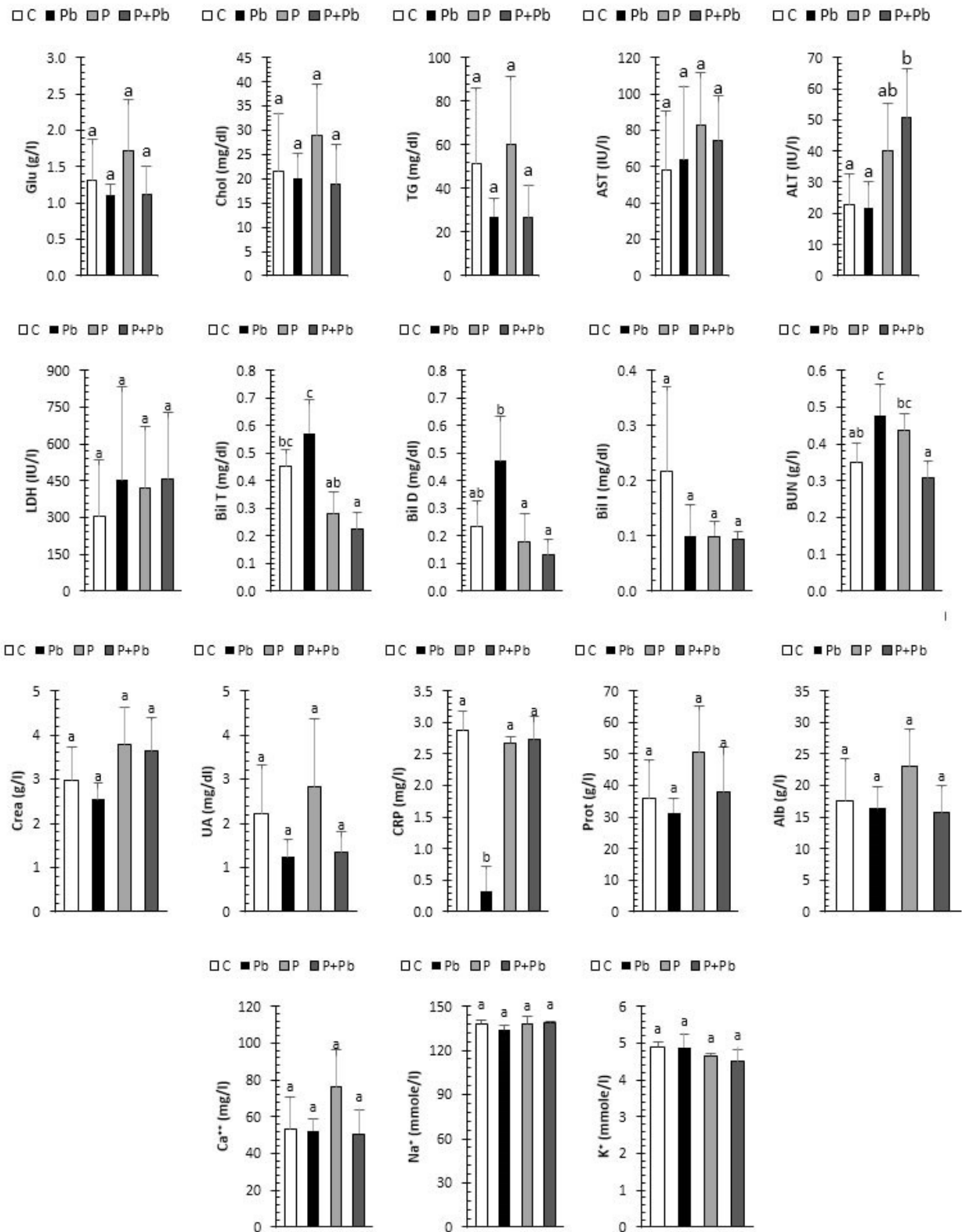


Figure 3: Biochemical parameters.

Glu: Glucose; Chol: Total cholesterol; TG: Triglyceride; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; Bil T: Total bilirubin; Bil D: Direct bilirubin; Bil I: Indirect bilirubin; BUN: blood urea nitrogen; Crea: Creatinine; UA: uric acid; CRP: C-Reactive Protein; Prot: Total protein; Alb: Albumin; Ca<sup>++</sup>: Calcium; Na<sup>+</sup>: Sodium; K<sup>+</sup>: Potassium. C: Control; Pb: Lead acetate; P: Bee pollen; P+Pb: Pollen and Lead acetate.

Bars marked with the same letter are not significantly different

	Organs	C		Pb		P		P + Pb		p	
Absolute weight (g)	Brain	1.88±0.14		1.99±0.16		1.97±0.14		1.86±0.18		0.495	
	Liver	12.06±1.11		11.90±1.87		11.19±2.23		11.98±1.51		0.846	
	Left kidney	1.04±0.18		1.24±0.23		1.06±0.17		1.20±0.29		0.425	
	Right kidney	1.14±0.22		1.12±0.02		1.13±0.17		1.19±0.19		0.940	
	Spleen	1.13±0.20		1.22±0.14		0.86±0.27		1.16±0.15		0.064	
Relative weight (g/100g/bw)	Brain	0.61±0.06		0.59±0.07		0.62±0.08		0.61±0.06		0.966	
	Liver	3.86±0.17		3.51±0.26		3.44±0.35		3.88±0.40		0.070	
	Left kidney	0.32±0.01	a	0.36±0.01	b	0.34±0.01	ab	0.37±0.02	b	0.001	**
	Right kidney	0.35±0.01		0.35±0.02		0.35±0.01		0.38±0.03		0.539	
	Spleen	0.36±0.05	a	0.38±0.05	a	0.23±0.03	ab	0.35±0.03	a	0.001	**

**Table 2: Absolute and relative organs weights.**

bw: body weight; p: significance; \*: significant, \*\*: highly significant, \*\*\*: strongly significant. C: Control; Pb: Lead acetate; P: Bee pollen; P+Pb: Pollen and Lead acetate. a,b,c,: mean values in same rows with no common superscripts differ significantly ( $p < 0.05$ )

In order to determine whether lead acetate attenuated the liver damage in the Pb-treated rats, LDH, ALT and AST enzyme activity in plasma was measured. No significant increase was shown in groups treated with (pb), (P) or (Pb +P). ALT enzyme activity of (Pb) and (PB + P) groups was significantly higher than in the control group, but stay in physiological levels. Furthermore, LDH enzyme activity increase in (Pb) group, similarly to (Pb) and (Pb + P) groups but not significantly different from (C) group. While Total Bilirubin (Bil T) and direct Bilirubin (Bil D) was increased in (Pb) group, (P) and (Pb + P) treated animals showed a significant decrease, with similar levels of indirect bilirubin (Bil I).

Even highly significant difference for blood urea nitrogen (BUN) ( $p=0.004$ ) was shown, then no significant difference for creatinine (Crea) ( $p=0.126$ ) and uric acid (UA) ( $p=0.202$ ) was marked between overall groups. The multiple comparison between groups indicate a considerable increase of BUN in (Pb) group *versus* a significant drop in (P + Pb) group.

Lead treatment reduced plasma protein levels compared to controls. Pollen extract increase serum proteins, to get control level for (Pb + P) group. (P) group reached higher levels than overall groups. No physiopathological values were revealed.

Results showed no considerable difference in electrolytes:  $\text{Na}^+$  ( $p=0.296$ ),  $\text{K}^+$  ( $p=0.152$ ), or  $\text{Ca}^{++}$  ( $p=0.110$ ). It was marked that  $\text{Ca}^{++}$  was higher in (P) treated group.

### 3.1.5. Histological study

Photomicrographs of the seminiferous tubules of groups of rats are shown in (Figure 4).

Histological study of (H & E) stain testis revealed a

remarkable difference in all groups. the (C) group appeared normal, with full tightened seminiferous tubules (ST), low lumen of seminiferous tubules and wide height of seminiferous epithelium (SE), and complete spermatogenesis. Same results were shown in (P) group. the (Pb) group exhibited an alteration in the last stages of spermatogenesis marked by the abnormal basement membrane (BM) of ST, reduction of SE thickness, with the empty spaces between germ cells, indicating a Sertoli cells desperation, spermiogenesis perturbation with a diminution of spermatozoa in the lumen. In addition, vascular congestion and Leydig cells proliferation. These results differ from a rat to another, and intensive alteration was observed approximately to the blood vessels (BV). Finally, (P + Pb) group showed a partial ameliorative effect with a larger diameter of SE, presence of spermatozoa, normal BM of ST, a large diameter of the SE, reduced lumen of ST, spermatogonia adjacent intimately to the BM, Indeed, BV congestion persist.

## 3.2. Discussion

### 3.2.1. Phytochemical proprieties of bee pollen extract

Phytochemical assessment of total phenols, total flavonoids and condensed tannins showed an important content. Various study revealed a variable levels of polyphenols (Yildiz et al., 2013; Saral et al., 2016). This content differ according to the floral origin, area or season (Ng et al., 2000; Campos et al., 2008). Antioxidant activity of dietary flavonoids considered as oxidative stress modulator (Terao, 2009). this activity depends on their polarity, metabolism, absorption and their resulting structural forms in the circulation (Rice-Evans, 2001; Heim et al., 2002).



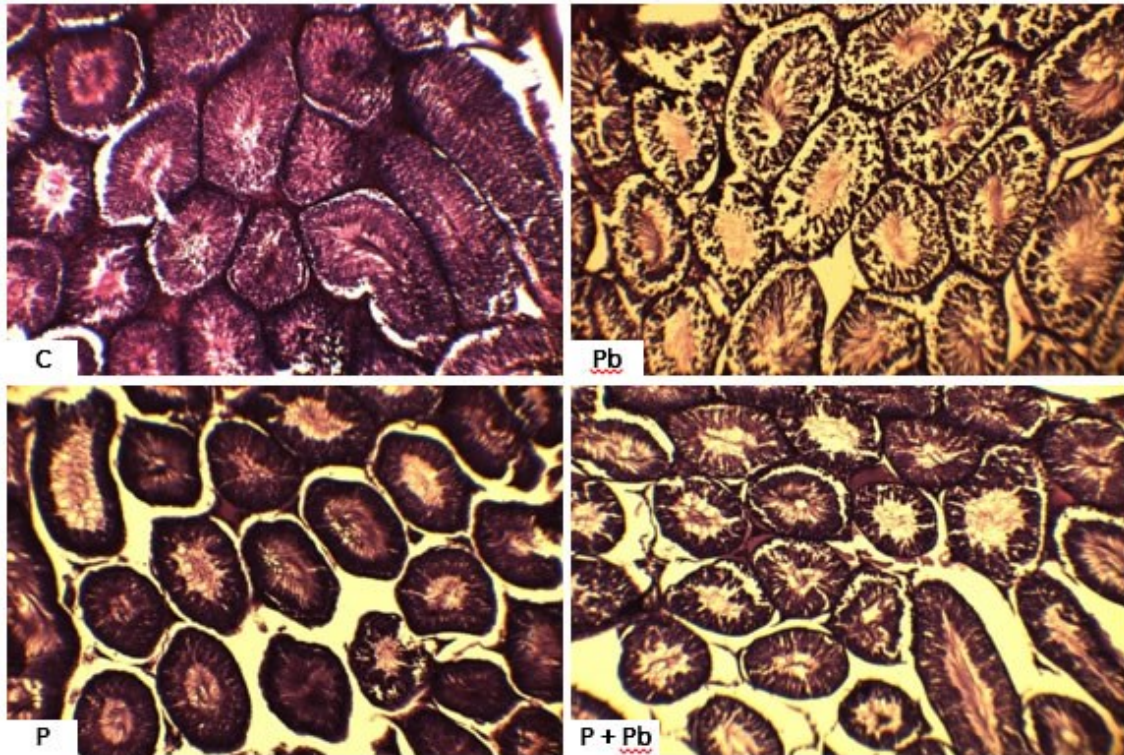


Figure 4: Photomicrographs of the seminiferous tubules of several groups of rats.  
C: Control; Pb: Lead acetate; P: Bee pollen; P+Pb: Pollen and Lead acetate. Stain: H&E; Magnification:  $\times 100$

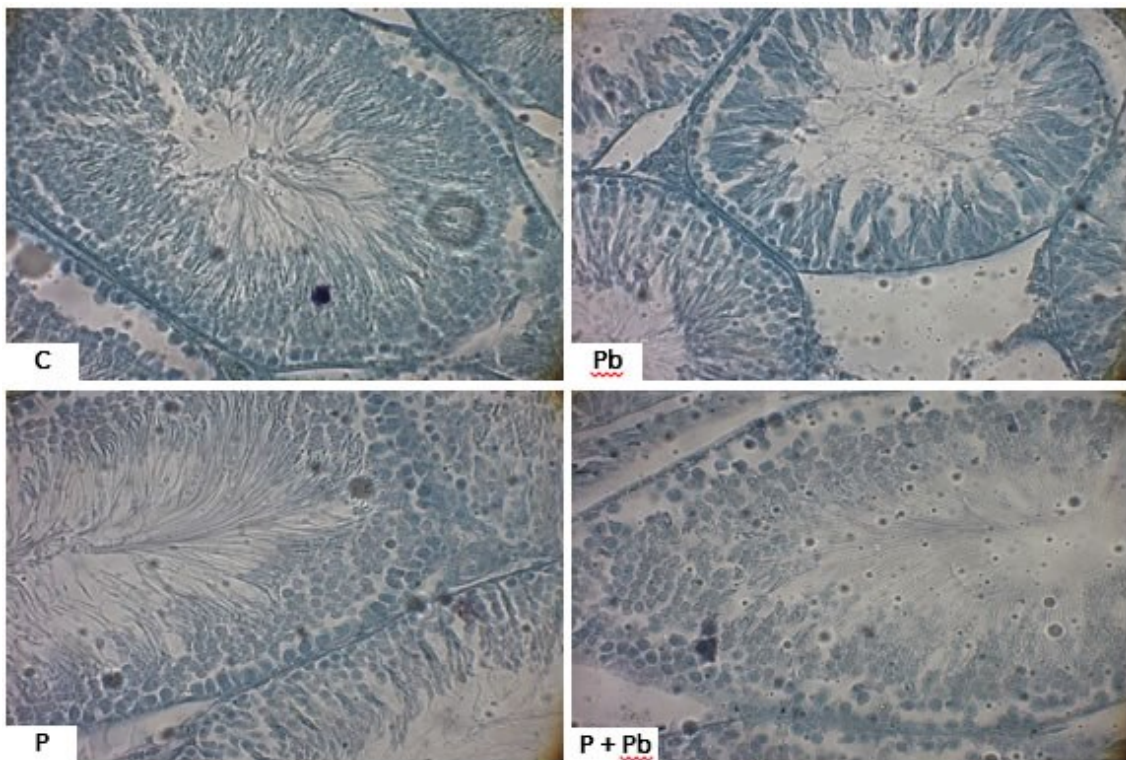


Figure 5: Photomicrographs of the seminiferous tubules of several groups of rats.  
C: Control; Pb: Lead acetate; P: Bee pollen; P+Pb: Pollen and Lead acetate. Stain: Toluidine blue; Magnification:  $\times 400$



DPPH radical scavenging measurement of bee pollen extract exhibit a potential antioxidant activity. The propolis and pollen samples exhibited higher radical scavenging activity (Saral et al., 2016). This may due to the highest absorption rate of pollen (Alvarez-Suarez et al., 2013).

### 3.2.2. Biometric parameters

The results demonstrate that Lead induced toxicity is marked by a body weight loss which is not significant. Similar results were approved by other works (Allouche et Hamadouche, 2009). It was obvious that the administration of bee pollen treatment showed a considerable decrease of body weight at the end of the experiment. Moreover, it showed an ameliorative increase of body weight, compared to lead treated group.

At the end of the 7<sup>th</sup> day of Carbon Tetrachloride intoxication, the weight loss was the least amount in the pollen-treated groups. The more the amount of pollen fed, the less the weight loss in the rats, suggesting a nutrition supplementation in the CCl<sub>4</sub>-exposed rats (Yildiz et al., 2013).

The findings, indicate that there is no significant difference in organs relative weights except for left kidney and spleen ( $p=0.001$ ) and slight difference of hepatosomatic index which is not statistically considerable ( $p=0.07$ ). intraperitoneal injection (ip) of three different doses of lead acetate (25, 50 and 100 mg/ kg bw/day) carried out on Wistar male rats for 7 days showed an increase in organs relative weight from 25 (mg/ kg bw/day) (Saka et al., 2011).

A study of 0.6% lead induced intoxication for 14 days, doesn't show a significant difference in testis, prostate or seminal vesicles. Furthermore, after 60 days of lead acetate intoxication by gavage at the level of (30 mg/kg/bw/day), epididymis and accessory sex glands was significantly decreased in rats treated with lead compared to the control group (Elgawish et Abdelrazek, 2014).

### 3.2.3. Serum biochemical parameters

The present study indicate that no considerable difference was associated to bee pollen administration alone or with lead acetate. In addition, glucose, triglyceride and cholesterol slightly increase in (P) group, but not significantly, due to chemical bee pollen composition (Svoboda et al., 1980). Many studies were conducted on bee pollen lipid-lowering properties (Samochowicz et Wójcicki, 1983; Wójcicki et Samochowicz, 1948). The investi-

gation of the influence of Cernitin on the development of experimental atherosclerosis in rabbits over a period of 12 weeks. compared to rabbits fed with High Fatty diet, the group treated with Cernitin, serum total lipids, cholesterol and free fatty acids was markedly and significantly diminished (Wójcicki et al., 1986). significant improvement in the elevated blood glucose levels was obtained after administration of bee pollen

Cytochrom P-450 dependent 7 $\alpha$ -hydroxylase is involved in the metabolism of cholesterol (Myant et Mitropoulos, 1979). In addition, the liver microsomal 7 $\alpha$ -hydroxylation of cholesterol may be stimulated when bee pollen extract was administered to rabbits, sustained with conversion of cholesterol to bile acids (Wójcicki et al., 1985).

Our findings, indicate a physiological level of AST and ALT enzymes activity. Only ALT showed a significant increase in groups treated with hydroethanolic extract of bee pollen. a significant increase of total and direct bilirubin was marked in Pb-induced toxicity. bilirubin is converted from heme (Tenhunen et al., 1986). Lead affects the heme synthesis, by inhibiting enzymes involved in the synthesis of heme.  $\delta$ -aminolevulinic acid dehydratase (ALAD), which catalyzes the porphobilinogen transformation from  $\delta$ -aminolevulinic acid (ALA), aminolevulinic acid synthetase (ALAS), a mitochondrial enzyme that catalyzes the formation of aminolevulinic acid (ALA), and ferrochelatase that catalyzes the insertion of iron into protoporphyrin to form heme (Piomelli, 2002; Ahamed et al., 2005).

The study of protective effect of pollen extracts (Cernitin T60 and Cernitin GBX) against allyl alcohol damage of the Liver, showed that the application of Cernitin T60 was associated with a marked drop of AST and ALT activity as well as with a decrease of the bilirubin level and liver weight, as compared with rats which received allyl alcohol. Effectiveness, of Cernitin T60 was found to be closely related to the dose given (Wójcicki et al., 1985). The investigation of hepatoprotective potential of aquatic extracts of chestnut bee pollen (7 days) on CCl<sub>4</sub>-induced hepatic damages in Sprague-Dawley rats (Yildiz et al., 2013; Saral et al., 2016), indicate that pollen at 200 mg/kg and 400 mg/kg once per day by gavage, decreased significantly AST and ALT levels. In addition, the AST and ALT levels correlated with the concentration of the pollen given. Moreover, Pollen extracts reduce the hepatotoxicity of paracetamol in Mice (Juźwiak et al., 1992).

functional disorders of the renal tubules are marked by Changes in serum BUN, creatine, and electrolyte levels. No physiopathological values were marked in all experimental groups. Creatinine and uric acid were similar, whereas, BUN was strongly and significantly different, with high level showed in Pb group and ameliorative affect after (P) treatment, this may due to increased protein catabolism (Yousef et al., 2003). Lower dose of lead acetate (25 mg/kg/bw/day (ip) for 7 days) showed an important increase of creatinine no difference of BUN and uric acid (Saka et al., 2011). So far, studies conducted on populations of workers with lower levels of exposure to Pb have disclosed no renal effect or only infraclinical changes of marginal significance (Cárdenas et al., 1993). Essential cellular processes in rat kidney and brain are affected by bioavailability of lead due to high-affinity lead-binding proteins (Fowler et Du Val, 1991).

This study spots the nutritional potential of bee pollen, which alleviate the alteration caused by lead intoxication. total protein and albumin levels is indicative of the development of a disorder in protein metabolism (Yousef et al., 2003). Total protein and albumin levels and were determined to have decreased but not significantly, Diminished level of CRP was identified in (Pb) group associated to a decrease of total protein and albumin levels. Pb may delay early defence mechanisms through down-regulation of serum IFN $\gamma$  and corticosterone levels and may exacerbate disease by an inappropriate early shift toward an immunosuppressive state by early activation of the hypothalamic-pituitary-adrenal axis induced by IL-6 and possibly other cytokines (Kim et Lawrence, 2000). Certain flavonoids, express their anti-inflammatory activity by modulation of proinflammatory gene expression (Chi et al., 2001; Kim et al., 2004; Tunon et al., 2009). Ethanol extract of bee pollen shows a potent anti-inflammatory activity and its effect acts via the inhibition of NO production (Maruyama et al., 2010).

The present results showed no significant difference in overall groups, indeed, a slight drop of Na<sup>+</sup> was shown in (Pb) group. It has been shown that lead affects the Na<sup>+</sup> ion concentration (Bressler et al., 1999). Ability of lead to substitute for other polyvalent cations and susceptibility of ionic channels to its action (affect different biologically significant processes, including metal transport, energy metabolism, apoptosis, ionic conduction, cell adhesion, inter- and intracellular signalling, diverse en-

zymatic processes, protein maturation, and genetic regulation) (Garza et al., 2006).

It was shown that Ca<sup>++</sup> raised in (P) group this can be explain by mineral Bee pollen composition (Campos et al., 2003).

### 3.2.4. Histological study

The finds demonstrate an alteration of final stage of spermatogenesis, spermatid and spermatozoa, suggesting the development of the spermatozoa was stopped. the empty spaces indicate the Sertoli cells injury, which is shown in Anti-proliferative and Genotoxic Effects of Arsenic and Lead on Human Cells (Aftab et Abdul Rauf, 2014) this results proof that lead alteration doesn't affect cells division, but only maturation. other authors reported that lead exposition didn't influence Sperm concentration in contrast, sperm motility and abnormal sperm morphology, these results are dose dependent (Rubio et al., 2006; Allouche et Hamadouche, 2009). no changeset of serum FSH and LH, indicate no alteration of hypothalamic-pituitary function (Sokol, 1989; Sokol, 1990). A decrease of serum testosterone level indicates lead action on sex hormones (Sokol, 1989; Sokol et al., 2002).

Furthermore, the antioxidant nutrients supplementation protected loss of motility in lead exposed rats (Hsu et al., 1998; Benabbou et al., 2017; Al-Shaikh, 2017). Extract of *Phyllanthus emblica* has a protective effect against lead acetate (Saha et Verma, 2015). In bee pollen treated group, the thickness of germinal epithelium was also improved and recovery of germ cell population, in *Sprague Dawley* rats treated with antituberculous drugs (Bharti et al., 2017). So far, Bee pollen and date palm pollen showed an ameliorative effect on rat testis of diabetic rats. Moreover, the improvement of the spermatogenesis as evident by significant increase in the number of spermatids, spermatogonia, spermatocytes, and Sertoli cells as compared to diabetic rats on one hand and the increase in the number of interstitial Leydig cells in the other hand (Mohamed et al., 2018).

## 4. Conclusion

In the present study, subacute toxicity with 30 mg/kg/day of lead acetate for 15 days, didn't show a biochemical alteration. Whereas, physiopathological alterations were shown, including organic atrophy. The histological study revealed a slight attenuation in rat testis. Furthermore, spermatogenesis alteration marked by absence or abnormal sperma-

tozoa in the lumen, Sertoli cells destruction, Leydig cells proliferation, oedema and blood vessels congestion. These results seem to be intensive nearly to blood vessels.

Nutritional potential of bee pollen extract, lipid lowering activity and anti-inflammatory respond exhibit an ameliorative effect on rat body weight loss induced by lead acetate. Bee pollen supplementation maintains the spermatogenesis progress at the first level with ameliorative proliferation of spermatogonia and spermatocytes, marked by the thickness of germinal epithelium and the normal Sertoli cells. Moreover, the recovery of the development of spermatids into spermatozoa was also approved. The positive effect of bee pollen extract may be also due to the potentiated synthesis of proteins, exhibiting protective properties against the testis cell injury. In the future, studding the mechanism by which the protective activity is brought about should be conducted.

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### Declaration of interest

The authors have declared no conflict of interest.

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