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Antispasmodic and nephroprotective potentials of native Algerian propolis and bee pollen: An experimental study in mice

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Abstract

The present work examined the *in vivo* antispasmodic and nephroprotective potentials of methanolic extracts obtained from Algerian native propolis and bee pollen. The *in vivo* antispasmodic activity was assessed by the intraperitoneal injection of acetic acid (1%) which induced long-lasting visceral pain in mice. The renal damage was modelled by intraperitoneal injection of a cisplatin (CP; 10 mg/kg) followed by histopathological changes in kidneys. In addition, the beehive by-products were screened for their bioactive content and *in vitro* antioxidant activities. The propolis and bee pollen are rich sources of bioactive compounds. The propolis showed the highest antioxidant potencies as evaluated by β-carotene bleaching system (87.16 ± 3.69%), DPPH (176.05 ± 0.20 µg/mL), and FRAP (0.61 ± 0.002 µmol Fe(II)/g) assays. The antispasmodic test revealed that propolis extract (250 mg/kg) significantly inhibited the number of spasms (61.04 ± 3.92%) induced by acetic acid. Based on histopathology examinations, bee pollen extract at 250 mg/kg significantly reduced nephrotoxic effects induced by CP injection. These results provided a good scientific basis for future research on antispasmodic and nephroprotective effects and/or mechanisms of propolis and bee pollen, which confer them a real application in drug discovery.

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Introduction

Cisplatin (CP) is an antitumour drug used against various tumours. Unfortunately, the clinical use of CP is frequently correlated with adverse effects, including kidney dysfunction (Yao *et al*., 2007). Inflammation, DNA damage, and lipid peroxidation have been reported in CP-induced nephrotoxicity (Oh *et al*., 2014). Numerous studies have demonstrated that the administration of natural antioxidants reduces the renal damage induced by CP in various animal models (Tilyek *et al*., 2016).

Increasing incidences of some chronic diseases have raised awareness regarding the importance of diet. Several publications have confirmed that the consumption of fruits and vegetables prevents the risk of various diseases (Volpe, 2019).

Bees manufacture several natural products to produce their hive and honey such as beeswax, royal jelly, pollen, and propolis. Beehive by-products were used for treating various ailments such as stomach and intestinal disorders (Gonçalves *et al*., 2013). Propolis is natural resinous substance gathered by diverse honeybee species from plants (Ruttner, 1988). The importance of propolis comes from its rich and complex composition, with more than 150 constituents identified including vitamins (A, B_1, B_2, B_3, and B_7), minerals (copper, manganese, and iron) (Dubero *et al*., 2015), aliphatic fatty acids (oleic and stearic acids), esters (Afrouzan *et al*., 2018), amino acids (arginine and proline) (Eroglu *et al*., 2016), phenolic acids (caffeic, cinnamic, gallic, syringic, ferulic, and *o*-coumaric acids) (Popova *et al*., 2014; Mohdaly *et al*., 2015), flavonoids (luteolin, quercetin,

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rutin, formononetin, and liquiritin) (Salatino, 2018), and terpenes (Dubero *et al*., 2015). Bee pollen contains valuable compounds such as essential amino acid, vitamins (C, E, B_1, B_2, B_3, B_5, B_6, B_9, and H), mineral salts (iron, calcium, manganese, potassium, phosphorus, selenium, magnesium, and sodium), and phenolic compounds known for their multitude of biological potentials (Campos *et al*., 2010).

Previous studies have researched the biological potentials of beehive by-products. However, there is no data on the nephroprotective effect on CP-induced renal dysfunction of propolis and bee pollen. Therefore, the main aim of the present work was to examine the antispasmodic and nephroprotective potencies of Algerian native propolis and bee pollen on CP-induced acute kidney injury, as well as their antioxidant properties to unravel possible new applications in medicine as nephroprotective agents.

Materials and methods

Chemicals

β-carotene, catechin, sodium hydroxide, gallic acid, Tween 40, linoleic acid, Folin-Ciocalteu (FC) reagent, and CP were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Solvents (methanol and chloroform), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, sodium bicarbonate, vitamin E, butylated hydroxytoluene (BHT), acetic acid, trichloroacetic acid, iron (III) chloride hexahydrate, and iron(II) sulphate heptahydrate were purchased from Sigma-Aldrich GmbH (Stemheim, Germany). Sodium nitrite and aluminium chloride were purchased from Fluka Co. (Bushes, Switzerland).

Propolis and bee pollen origins

Propolis and bee pollen of *Apis mellifera* were gathered by beekeepers from the Chlef region (West of Algeria) in February, 2018. After collection, the samples were stored in a freezer for subsequent analysis.

Extract preparation

The methanolic extracts of propolis and bee pollen were prepared as described by Falleh *et al*. (2008). At laboratory conditions, 2.5 g of propolis and bee pollen were soaked in 25 mL of absolute methanol under continuous shaking (WIS-10, Daihan Scientific Co. Ltd., Korea) for 30 min. After 24 h of contact at room temperature, the mixtures were filtered and concentrated under vacuum (Büchi, Switzerland) at 50°C to obtain crude extracts, which were kept in airtight bottles at 4°C until further use.

Bioactive content

Total phenolic content (TPC)

The TPC was measured by the Folin-Ciocalteu (FC) method according to Kamazawa *et al*. (2002). Briefly, reaction mixture contained 200 µL of each extract (1 mg/mL), 1.5 mL of FC reagent (1:10), and 1.5 mL of sodium bicarbonate (Na_2CO_3) (60 g/L). Mixtures were shaken and left to stand at room temperature for 90 min before measuring spectrophotometrically (Optizen 2120, Mecasys Co. Ltd., Korea) the absorbance at 725 nm. All tests were performed in triplicate. Results were expressed as milligrams per gram of gallic acid equivalent (mg GAE/g of extract).

Total flavonoid content (TFC)

The TFC was measured following the colorimetric method of Biglari *et al*. (2008). Briefly, 1 mL of each extract (1 mg/mL) was mixed with 4 mL of distilled water and 0.3 mL of sodium nitrite ($NaNO_2$) (5%). After 5 min, 0.3 mL of aluminium chloride ($AlCl_3$) (10%) was added and allowed to stand for 1 min, then, 2 mL of sodium hydroxide NaOH (4%) was added to the mixture. Immediately, 2.4 mL of distilled water was added. After incubation period at room temperature for 5 min, absorbance was measured at 510 nm using a spectrophotometer. All tests were performed in triplicate. Results were expressed as catechin equivalents (mg CEQ/g of extract).

In vitro antioxidant potentials

β-carotene-linoleic assay

The bleaching inhibition rate was measured according to Mikami *et al*. (2009), based on the ability of extracts to decrease oxidative losses of β-carotene in a β-carotene/linoleic acid emulsion. Briefly, 3 mg of β-carotene was dissolved in 30 mL of chloroform. Next, 1 mL of this solution was mixed with 40 mg of linoleic acid and 400 mg of Tween 40. After removal of chloroform at 40°C, 100 mL of distilled water saturated with oxygen were added to the mixture with vigorous shaking. Then, 3 mL of the resulting emulsion were added to 50 µL of the methanolic extracts (20 µg/mL). BHT and vitamin E at 10 µg/mL were used for comparison. The absorbance of the mixtures was measured at 470 nm

using a spectrophotometer. The antioxidant activity (AA) of the extracts was calculated using Eq. 1:

$$AA\% = [(A_{t0} - A_{t60}) / A_{t0}] \times 100 \quad \text{(Eq. 1)}$$

where, A_{t0} and A_{t60} = absorbance values measured at time zero and 60 min of the incubation for test samples and controls, respectively.

DPPH radical-scavenging

The method proposed by Okada and Okada (1998) was used to measure the radical-scavenging activity. Briefly, 2.7 mL of various concentrations (2.81, 5.62, 11.25, 22.5, 45, and 90 µg/mL) of the methanolic extracts of propolis and bee pollen were added to 0.3 mL of methanol solution of DPPH (0.004%). After incubation period at room temperature (30 min), the absorbance was read against a blank at 517 nm using a spectrophotometer. The same procedure was repeated with BHT and vitamin E as positive control and blank (containing all reagents except for the test compound). The inhibition activity (I %) was calculated using Eq. 2:

$$I\ (\%) = [(A_{blank} - A_{sample}) / A_{blank})] \times 100 \quad \text{(Eq. 2)}$$

where, A_{blank} = absorbance of the control, and A_{sample} = absorbance of the test compound.

The antiradical potency was expressed as IC_{50} (µg/mL) (the scavenging of 50% of DPPH radical). All tests were performed in triplicates.

Ferric-reducing antioxidant power assay (FRAP)

Briefly, 1 mL of each extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide $K_3Fe(CN)_6$ solution at 1%. After incubation period at 50°C for 20 min, 2.5 mL of trichloroacetic acid $C_2HCl_3O_2$ (10%) was added to stop the reaction, and the mixtures were then centrifuged (NF 200, Turkey) at 3,000 rpm for 10 min. Next, 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 0.50 mL of chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) solution (0.1%). The absorbance was spectrophotometrically measured at 700 nm. The standard curve was linear between 100 and 2000 µmol/L of iron (II) sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) solution. Results were expressed as µmol Fe(II)/g of extract (Benzie and Strain, 1996).

Experimental animals

Animals

Forty male Swiss albino mice weighing between 25 and 30 g (8-week old) were obtained from Pasteur Institute (Algiers, Algeria). All the animals were housed in controlled environmental conditions, photoperiod (a 12 h light, 12 h dark), and temperature (24 ± 2°C). The mice were acclimatised to environmental conditions for 2 d, and had free access to food and water. Before experiments, the animals were fasted overnight but had free access to water.

Ethics

All the experiments for animals were approved by the Department of Nutrition and Food Sciences, Faculty of Life and Natural Sciences, Hassiba Benbouali University (Chlef, Algeria). The experiments were conducted following the guidelines and the recommendation of the "Guide for the Care and Use of Laboratory Animals".

In vivo antispasmodic activity

The reduction of spasms of the propolis and bee pollen was performed according to Bhowmick *et al*. (2014). The animals were randomly assigned into three groups, each containing five mice as follow:

i. Control group: fed with saline (3 mL/kg body weight).
ii. Reference group: fed with the ibuprofen (200 mg/kg body weight) (Bhowmick *et al.*, 2014).
iii. Tested group: fed with extracts of propolis and pollen by oral gavage (250 mg/kg body weight) (da Silva *et al.*, 2015).

After 30 min, 200 µL of acetic acid solution (1%) was injected intraperitoneally. After 5 min, the number of abdominal contortions per mouse was counted for 15 min. The percentage reduction of spasms (percentage of protection) was calculated using Eq. 3:

$$\%\ \text{of protection} = [(AV_c - AV_t) / AV_c] \times 100 \quad \text{(Eq. 3)}$$

where, AV_c = average of spasms in the control group, and AV_t = average of spasms in mice that received the extracts and ibuprofen.

Nephroprotective activity

The nephroprotective activity *in vivo* of the extracts was measured according to Domitrovic *et al*.

(2013). A dose of 250 mg/kg methanolic extracts was orally administered to the mice. The mice were divided into three groups as follow:

i. Normal group: orally administered with 9% normal saline solution (1 mL/Kg body wt.)
ii. Control group: injected intraperitoneally with a single dose of CP (10 mg/kg body wt.)
iii. Tested group: intragastrically administrated with the extracts of propolis and pollen (250 mg/kg body wt.) for 3 d, and injected intraperitoneally with a single dose of CP (10 mg/kg body wt.)

At the end of the experimental period, mice were anesthetised with light ether in a desiccator, and rapidly dissected. The kidneys were quickly removed, rinsed with saline, and fixed in 10% formalin solution for histological assessment. The sections were assessed on haematoxylin and eosin (H&E), and examined with an optic microscopy (Carl Zeiss Microlmaging GmbH, Germany).

Statistical analysis

Results were given as mean values ± standard deviation (SD) of three repetitions. Statistical analysis was performed using the SPSS Statistics 16. 0 (Inc., Chicago, IL). Analysis of variance (ANOVA) was used to determine the statistical comparisons among multiple groups. Pearson's correlation coefficients were calculated to reveal the relationship between antioxidant potentials and the bioactive content. The significance level (Tukey's HSD test) was accepted at $p < 0.05$.

Results and discussion

Bioactive content

The levels of bioactive contents in propolis and bee pollen are presented in Table 1. The TPC value of propolis (136.60 ± 0.10 mg GAE/g) was significantly higher ($p < 0.05$) than that of bee pollen (87.28 ± 1.23 mg GAE/g). These results are in agreement with Nieva Moreno *et al*. (2000) who found that the TPC of Brazilian bee pollen extracts ranged from 19.28 - 48.90 mg GAE/g. Mohammadzadeh *et al.* (2007) found that the TPC of Iranian propolis ranged from 3.08 ± 0.02 to 8.46 ± 0.03 mg EAG/g of propolis, which are higher as compared to those in the present work. Popova *et al*. (2014) stated that the syringic, caffeic, ellagic, hydroxybenzoic, vanillic, ferulic and *o*-coumaric acids are the common phenolics in propolis, whereas gallic, benzoic, cinnamic, and phenyl acetic acids are the most dominant phenolic compounds in bee pollen (Rzepecka-Stojko *et al*., 2015). The phytochemical composition of propolis extracts is affected by the geographic origin (local flora, climate) and the season of the resins gathered by the bees (Silva *et al*., 2008).

Table 1. Bioactive contents of propolis and bee pollen methanolic extracts.

Methanolic extract	Total phenolic content (mg GAE/g)	Total flavonoid content (mg CEQ/g)
Propolis	136.60 ± 0.10^{a}	44.96 ± 0.80^{b}
Bee pollen	87.28 ± 1.23^{b}	74.83 ± 0.51^{a}

Means followed by different lowercase superscripts in the same row are significantly different at $p < 0.05$.

As shown in Table 1, the TFC value of bee pollen extract (74.83 ± 0.51 mg CEQ/g) was significantly ($p < 0.05$) higher than that in propolis extract (44.96 ± 0.80 CEQ/g). The TFC of bee pollen from Southern Brazil varied from 2.10 to 28.33 mg CEQ/g (Carpes *et al*., 2009). Ahn *et al*. (2004) reported that the TFC of propolis from Korea ranged between 15.9 and 135.2 mg CEQ/g. A recent study with 13 Anzer pollens from Turkey reported that the TFC ranged between 44.07 and 124.10 mg CEQ/g (Ulusoy and Kolayli, 2014). This variability might be attributed to the origin of each honeybee product; the coloured (flavonoids are pigments) flowers offer more flavonoids to the phenolic mixture of pollen. The phenolics of propolis were dominated by non-flavonoids fraction. Based on the literature, the qualitative features of beehive by-products are variable, and depend on the choice of solvent, the botanical and/or biogeographical origins. Luteolin, quercetin, rutin, formononectin, and liquiritin were mentioned as the major flavonoids in propolis from Brazil (Salatino, 2018). Bee pollen extracts also

contained apigenin, rutin, catechin, epicatechin, luteolin, quercetin, kaempferol, and naringenin (Mohdaly *et al*., 2015; Sousa *et al*., 2015).

Antioxidant activity

β-carotene bleaching assay

The antioxidant activities of the extracts were estimated by the β-carotene bleaching assay. The inhibition potential of extracts in comparison with BHT and vitamin E is shown in Table 2. The bleaching inhibition rate of the extracts and synthetic antioxidants significantly decreased ($p < 0.05$) in the order of BHT > vitamin E > propolis > bee pollen. Carpes *et al*. (2009) found that the antioxidant activity of ethanolic extracts of Brazilian bee pollen with the β-carotene bleaching method ranged from 40 to 90%. Although synthetic antioxidants were more superior, beehive by-products could also be considered excellent antioxidants, especially when the synthetic ones could exert serious adverse effects on human health (Martínez *et al*., 2019).

DPPH radical-scavenging

The effect of test extracts to reduce DPPH radical was evaluated on the basis of their IC_{50} values. The IC_{50} of compounds is inversely related to its antiradical capacity. There was a large variation ($p < 0.05$) among the analysed extracts (Table 2). Our results displayed superiority of propolis extract as antiradical agents as compared to bee pollen. However, none of the beehive by-products displayed activity as strong as standard compounds, vitamin E and BHT. These findings are in line with Gulcin *et al*. (2010) who reported an IC_{50} of 31.81 µg/mL for propolis originated from Erzurum province in Turkey.

FRAP assay

The antioxidant potential in FRAP test is calculated on the basis of the ability to chelate Fe^{+2}, and the results are represented in Table 2. Values of this assay were in similar order with DPPH, where propolis exhibited high antioxidant potential than bee pollen ($p < 0.05$). LeBlanc *et al*. (2009) reported that bee pollen collected from USA had the ability to reduce ferric potency from 0.93 ± 0.03 to 3.96 ± 0.18 µmol Fe(II)/g. Our results are in line with these investigations.

As can be seen in Table 3, significant positive correlations ($p < 0.01$) were observed between the

Table 2. Antioxidant activities of propolis and bee pollen methanolic extract.

Methanolic extract	β-carotene bleaching assay (%)	IC_{50} DPPH (µg/mL)	FRAP (µmol Fe(II)/g)
Propolis	87.16 ± 3.69^{b}	176.05 ± 0.20^{b}	0.61 ± 0.002^{a}
Bee pollen	64.48 ± 3.83^{c}	604.52 ± 1.79^{a}	0.57 ± 0.002^{b}
Vitamin E	92.78 ± 0.58^{ab}	16.56 ± 0.87^{d}	/
BHT	96.14 ± 1.46^{a}	41.32 ± 1.27^{c}	/

Means followed by different lowercase superscripts in the same column are significantly different at $p < 0.05$. BHT: butylated hydroxytoluene; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric-reducing antioxidant power; and IC_{50}: the concentration that caused 50% scavenging of DPPH.

Table 3. Correlation between bioactive compounds and antioxidant activities of propolis and bee pollen methanolic extracts.

	TPC	TFC	IC_{50}	β-carotene	FRAP
TPC	1				
TFC	0.999**	1			
IC_{50}	1.000**	0.999**	1		
β-carotene	0.965**	0.963**	0.965**	1	
FRAP	0.965**	0.998**	0.996**	0.962**	1

TPC: total phenolic content; TFC: total flavonoid content; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric-reducing antioxidant power; and IC_{50}: the concentration that caused 50% scavenging of DPPH. **Significant correlation at $p < 0.01$.

TPC and the antioxidant potentials estimated by DPPH, β-carotene-linoleic acid, and FRAP methods (1, 0.965, and 0.965, respectively). There were also significant positive relationships ($p < 0.01$) between TFC and TPC (0.999).

Bioactive constituents such as phenolic acids and flavonoids exhibit potent biological activities which might be attributed to their antioxidant capacity (Kim and Shim, 2019).

In vivo studies

Antispasmodic activity

The antispasmodic activity *in vivo* of beehive by-product extracts was assessed by the intraperitoneal injection of acetic acid which induced long-lasting visceral pain in mice. To date, the antispasmodic effect of propolis and bee pollen has not been reported in the literature.

The results of the percentage reduction of spasms (percentage of protection) are shown in Figure 1. The extract of propolis exhibited a significant ($p < 0.01$) activity (61.04 ± 3.92%), whereas the bee pollen exhibited highly significant ($p < 0.001$) activity (45.38 ± 3.39%) when compared to the treated referenced group.

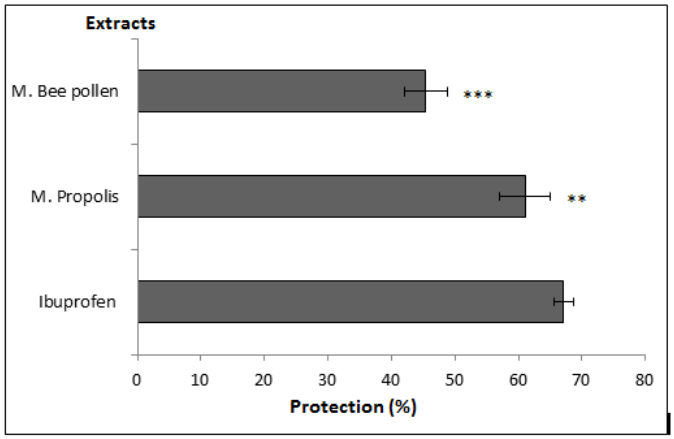

Figure 1. Percentage reduction of spasms (percentage of protection) of propolis and bee pollen methanolic extract. M: methanolic extract. Values are mean ± SD for five mice ($n = 5$). Ibuprofen was used as a reference compound (200 mg/kg). Statistical differences from ibuprofen-treated control as analysed by Dunnett's test (**$p < 0.01$, ***$p < 0.001$).

Several toxicity studies in animal models showed that propolis are generally safe and well tolerated. Studies conducted by da Silva *et al.* (2015) have shown that the oral administration of the hydroalcoholic extract of red propolis at 300 mg/kg presented no death and toxicity in rats throughout the experiment period of 14 days.

The antispasmodic properties may be attributed to diverse mechanisms: (i) the inhibition of the response to the 5-hydroxytryptamine, bradykinin, prostaglandin E2, histamine, and oxytocin by phenolics (McNamara *et al*., 2005); (ii) the ability to block the Na^+ channels, muscarinic receptors, and Ca^{2+} channels can exert an antispasmodic effects (Mehmood *et al*., 2011). Studies on human colon epithelial cell have shown that luteolin effectively suppressed the production of TNF-α, IL-8, histamine, leukotrienes and prostaglandins into human mast cells (Kim *et al*., 2005). Quercetin blocks the adhesion of leukocytes to the endothelial cells of the umbilical veins by inhibiting the expression of ICAM-1 (Intercellular adhesion molecule-1) (Cho *et al*. 2001).

Nephroprotective activity

The mice injected with CP showed loss tubular architecture with vacuolisation, inflammation, and degeneration of morphology of the tubules (Figure 2, B2). The histology of the mice kidney tissues revealed no specific lesions in glomeruli and tubules, with normal architecture in the normal group (Figure 2, A2), and those tested with methanolic extract of bee pollen (Figure 2, D2). Furthermore, it was also clear that methanolic extract of bee pollen provided protection against damage induced by CP in glomeruli, tubules, and architecture of tubular epithelial cells. The methanolic extract of propolis did not show any effect (Figure 2, C2).

The nephroprotective actions of extracts could be attributed to the phenolic contents that act against CP-induced nephrotoxicity by increasing anti-inflammatory and antioxidant activities in renal cells (Surawat *et al*., 2009). Rutin as a member of this phenolic mixture decreases CP-induced elevation in gene expression of tumour necrosis factor alpha, nuclear factor kappa B, mitochondrial cytochrome C, interleukin-1β, caspase-3, and apoptosis-inducing factor in renal cells. This flavonoid decreases renal malondialdehyde (MDA), and increases glutathione secretions (Radwan and Abdel Fattah, 2017).

Several studies evaluated the nephroprotective properties of quercetin against CP-induced renal toxicity in rats. Inflammation, apoptosis, critical

MAPK (mitogen-activated protein kinase) signalling, and oxidative stress in the CP-treated animals were almost normalised by quercetin in the kidneys (Sánchez-González *et al*., 2017).

Gallic acid has been reported as a renoprotective agent in CP-induced nephrotoxicity *in vivo* models. The oral administration of gallic acid showed a significant increase in the levels of serum creatinine, serum urea, blood urea nitrogen, total protein, MDA, glutathione, nitric oxide, catalase, superoxide dismutase, and glutathione peroxidase as compared to the mice receiving gentamicin alone (Ghaznavi *et al*., 2018).

Aldahmash *et al*. (2016) evaluated the protective potential of propolis against gentamicin-induced mice model of nephrotoxicity. The authors demonstrated that the administration of propolis induced a significant decrease in urea (38 ± 3.00 mg/dL) and creatinine (0.33 ± 0.02 mg/dL) levels as compared to mice receiving gentamicin alone (41 ± 2.0 and 0.36 ± 0.05 mg/dL, respectively).

In addition to polyphenolics, propolis and bee pollen contain vitamins C and E, which could have contributed to the additional prevention against CP nephrotoxicity (Ajith *et al*., 2007). Thus far, there is no data reported about the nephroprotective activity against CP-induced renal damage of the beehive by-products. Results of the present work highlighted the beneficial health properties of these beehive by-products as a possible nephroprotective therapy combined with CP treatment.

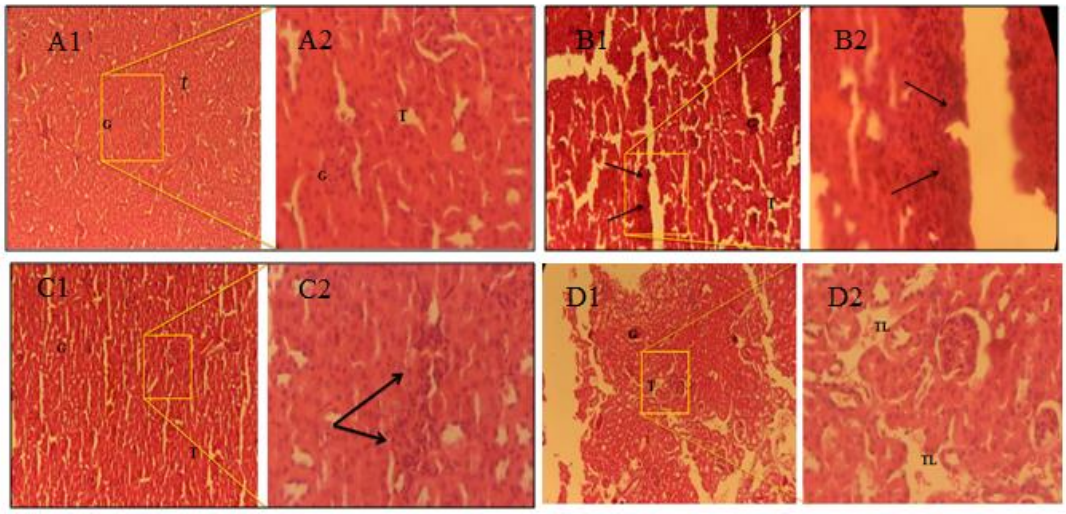

Figure 2. Light microscopy of mice renal tissue stained with H&E. (A1) normal control group at 100× magnification, and (A2) normal control group at 400× magnification: renal tubules showed normal appearance. (B1) cisplatin control group at 100× magnification, and (B2) cisplatin control group at 400× magnification: well defined degenerating tubular structures with vacuolisation and loss of architecture. (C1) methanolic extract of propolis and cisplatin at 100× magnification, and (C2) methanolic extract of propolis and cisplatin at 400× magnification: no protective effect against cisplatin-induced acute kidney injury was observed. (D1) methanolic extract of bee pollen and cisplatin at 100× magnification, and (D2) methanolic extract of bee pollen and cisplatin at 400× magnification: renal histo-architecture was protected, and cisplatin-induced inflammation was countered. G glomerulus, T tubule, TL collecting duct system, → inflammation.

Conclusion

Results of the present work confirmed the richness of propolis and bee pollen in term of phenolic compounds with remarkable superiority observed in propolis as compared to in pollen. These beehive by-products exhibited excellent antioxidant and antispasmodic activities. In addition, the present work proved, for the first time, an appreciable nephroprotective potential of beehive by-products against cisplatin-induced kidney injury; results suggested their possible utilisation as nephroprotective therapy combined with cisplatin for treatment of cancer. The present work also provided a good scientific basis for future research on the nephroprotective action of propolis and bee pollen with potential applications in drug discovery.

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