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The activity of three different propolis extracts and their antioxidant activity induced by CCL4 in adult rats

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Abstract
The goal of the current study was to examine the impact of extracting propolis in various solvents in light of the antioxidant activity induced by CCL4. The biological study was conducted using 25 adult Wistar male rats. Five rats served as normal control group which fed on basal diet with drinking tap water. While, other 20 rats received intra-peritoneal single dose of 0.5 ml/kg CCl4 to induce renal and hepatic oxidative stress. Then, rats reclassified into 4 groups (n=5), positive control and the other three groups were treated by watery, ethanolic and ethyl acetate extracts of propolis instead of water till the end of experimental period (9 days). *In vivo* extracts evaluation includes serum Catalase, SOD, GSH, TAOC, and MDA were measured at the end of the experiment. The *In vitro* results showed all extracts exhibited increasing radical scavenging activities with increased concentrations of extract with variation in antioxidant activity according to the type of extract. The CCL4 group rats showed significant ($P<0.05$) decline in the catalase, SOD, GSH and TAOC and significant ($P<0.05$) evaluation in the MDA compared with control. All prepared extracts corrected the deviation occurring in the parameters as a result of administering the CCL4, with variation depending on the type of extract. Watery extract showed best results in enhance catalase level, while ethanolic extract showed best results in enhance GSH activity, no difference in the SOD, T-AOC and MDA between watery and ethanolic extracts, in the same time the ethyl acetate extract was less effective. The *In vitro* and *In vivo* antioxidant activity was varied according to the type of extract; ethanol gave best activity.
Keywords: Propolis, extracts, antioxidants, CCL4

Introduction

Many substances obtained from living things, including bacteria, plants, and insects, have shown to be effective, safe, and reasonably priced treatments for illnesses in both humans and animals (1). Bee products, and particularly propolis, have drawn attention in the different countries since it has been demonstrated that they display a wide range of bioactivities (2). Bees often produce propolis for a variety of purposes, including as nest construction, chemical defense, and protecting honey from microbial deterioration (3). Propolis is made up of a wide variety of complicated chemicals. Propolis has more than 300 different chemicals that have been discovered, including flavonoids, phenolic acids, terpenoids, steroids, and amino acids (4) Propolis requires extraction with solvents to purify it; it cannot be utilized as a raw material. Because the polyphenolic fraction and other active ingredients are thought to have a greater role in the documented therapeutic effects than the other propolis constituents, this method should eliminate the inert material while preserving the polyphenolic fraction (5). Major chemical constituents of propolis, including flavonoids, phenolic acids, and their esters, are thought to be responsible for its primary activities. Recent research and uses on propolis have mostly concentrated on ethanol extracts of propolis, as these lipophilic chemicals are easily removed by alcohol. Additionally, there has been a lot of research done on propolis' volatile oils and water extracts. Propolis is analyzed and differentiated using a variety of techniques, such as HPLC; HPLC-ESI-MS; GC-MS; LC-MS; and DHS-GC-O-MS (6). The widely varied chemical makeup of propolis, which is dependent on the botanical origin and extraction techniques, poses several limitations to its application. Therefore, the biological activity of various propolis extracts very vary (7) Therefore, it is necessary to find ways to improve the chemical compounds content of propolis extracts and thus improve its therapeutic effectiveness. Under the light of these facts, the present work aimed to the evaluate the *In vitro* and *In vivo* antioxidant activity of three different extracts of propolis.

Materials and Methods

Propolis collection and preparation.

The raw propolis materials used in this study was collected from various sites located in Al-Diwaniya city at the end of the spring to early summer. The propolis samples were carefully cleaned with fresh water. The samples were cleaned, then allowed to air dry for a while before being further dried for two weeks at room temperature in the shade. The dried propolis samples were frozen for a full day, after which the prepared samples were chopped into tiny pieces and processed in a grinder to a fine powder. The components that were powdered were stored in an airtight container at room temperature (8).

Propolis extraction

The watery extract of propolis (WPE), the ethanolic extract of propolis (EEP), and Ethyl acetate extract of propolis (EAPE) were obtained by cold maceration of propolis samples in water, absolute ethanol, and absolute ethyl acetate solvents respectively via placed in the dark glass containers at room temperature for 15 days with strong shaker for 10 minutes daily, followed by filtration by Whatman filter paper

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No.1 with diameter 185 mm to remove waxes and insoluble constituents and evaporation of the solvents to dryness under reduced pressure at 60° C. (9,10)

***In vitro* antioxidant evaluation**

The In vitro antioxidant activity of different prepared propolis extracts was investigated by using the following methods.

DPPH scavenging assay

This assay was performed according to the method explained by (11) with slight modifications. The activity of the different prepared propolis extracts for free radical scavenging capacity was done via measuring the intensity of the purple color from the DPPH (2,2-Diphenyl-1-picrylhydrazyl) methanol solution. Each Propolis extract was made by dissolving and diluting it in DMSO at different quantities (20, 40, 60, 80, and 100 µg/mL). Ascorbic acid and blank solution served as the positive and negative controls, respectively. To measure the antioxidant activity, a micro pipette was used to pipette 0.2 ml of each concentrate into tubes, and then 3.8 ml of a 50 µM DPPH solution was added. After thoroughly mixing the mixture and letting it sit in the dark for 30 minutes, the absorbance at 517 nm was measured using a UV-Vis spectrophotometer. By calculating the percentage of DPPH absorption inhibition using the following formula, the quantity of DPPH radical absorption resistance was used to estimate the antioxidant activity of the samples:

DPPH scavenging activity (%) =((A0-A1)/A0) ×100

Where, A0: DPPH radical absorption of 50 µM at 517 nm wavelength; A1: Samples absorptions in DPPH radical of 50 µM at 517 nm wavelength. IC_{50} values indicating the concentration of extract or standard that can inhibit 50% of the DPPH were calculated using the linear regression formula. The lower IC50 value indicated higher radical scavenging capacity.

ABTs radical cation decolorization assay

The assay was conducted, with a few minor modifications, in compliance with the methodology outlined by (12). 5 mL of 4.9 mM potassium persulfate and 5 mL of 14 mM ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) reacted to create the ABTs cation radicals, which were then incubated for 16 hours at room temperature in the dark. Before being used, the mixture was diluted with ethanol to get an absorbance at 734 nm of 0.700 ± 0.06. Subsequently, in a final volume of 2 mL, 40 µL of varying propolis extract strengths (20-100µg/mL) were added to 1960 µL of the reaction mixture, mixed thoroughly, and allowed to sit at room temperature for 7 minutes in the dark. An UV-visible spectrophotometer was used to record the absorbance at 734 nm. The ABTS scavenging activity of the extracts was compared with ascorbic acid, and the percentage inhibition was calculated as. ABTS radical scavenging activity (%) = [(Abs control − Abs sample)]/ (Abs control) ×100.

Where Abs control is the absorbance of ABTS radical; Abs sample is the absorbance of ABTS radical + propolis extract/standard. ABTS radical scavenging activity of extracts was determined by IC50 value as mentioned above in DPPH assay.

Chelating effect on ferrous ions assay

The method described by (13) was followed in order to assess the ferrous ion chelating activity of the various produced propolis extracts. After adding one milliliter of propolis extracts at different concentrations (20-100 µg/mL) to 50 milliliters of FeCl2 (4H2O) solution (2 mM), the mixture was allowed to incubate for five minutes at room temperature. Following the addition of 0.1 mL of ferrozine (5 mM) to start the reaction, the mixture was adjusted to 3 mL with distilled water, given a good shake, and allowed to stand at room temperature for ten minutes. Next, spectrophotometric measurement of the solution's absorbance at 562 nm was conducted. The ferrozine-Fe2+ complex formation inhibition % was computed as follows:

Metal chelating effect (%) = (A0-A1/A0) × 100

where A0 is the absorbance of the control and A1 is the absorbance of the sample. The concentration of extract or standard that can inhibit 50% of the ferrous chelating capacity (IC50) in µg/mL of each extract was determined.

Hydrogen peroxide scavenging assay

The various produced propolis extracts were tested for their ability to scavenge H2O2 using the methodology described in (14). A prepared phosphate buffer (pH = 7.4) was mixed with 0.6 mL H2O2 (40 mM) and 0.4 mL of propolis extracts at varying concentrations (20-100 µg/mL). Ten minutes were spent incubating the reaction mixtures at room temperature. The reaction mixture was measured at 230 nm against a blank solution containing phosphate buffer following the incubation period. The standard used was ascorbic acid.

Percentage (%) of inhibition = (A0-A1/A0) × 100

where A0 is absorbance of H2O2 and A1 is the absorbance of the sample.

Total reduction capacity assay

The total reduction capacity activity of the different prepared propolis extracts was performed accordance to the method explained by (14) A volume of 2.5 mL of 0.2 M of phosphate buffer (pH= 6.6) and 2.5 mL of 1% potassium ferric cyanide were added to the 1 mL of samples of various concentration (20-100 µg/mL) then gently mixing. The reactive mixture was incubated at 50° C in a water bath for 20 minutes. After that 2.5 mL of 10% TCA was added to the mixture and centrifuged at 5000 rpm for 10 minutes. After that 2.5 mL of mixture was taken and putting in to the clean tube containing 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride mixed thoroughly and left for 5 minutes then measured spectrophotometrically at 700 nm. against blank solution. The total reduction capacity of the extracts as compared with ascorbic acid was calculated.

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Percentage (%) of inhibition = (A0-A1/A0) × 100
where A0 is absorbance of H2O2 and A1 is the absorbance of the sample.

***In vivo* antioxidant evaluation**

Experimental animals

Using lab-bred male Wistar albino rats (11-12 weeks) that were acquired from the animal house of the Veterinary Medicine College at Al-Qadisiyah University, the *In vivo* experiment was carried out. All of the test animals were kept in housing with controlled temperatures (24 ± 2°C), relative humidity (60 ± 10%), and a 12-hour light-dark cycle. They also had unlimited access to food and water. Wister rat's male weighing between 200 to 220 gm were utilized for animal experiment. After randomization into different experimental groups, they were allowed to acclimatize to the laboratory conditions for two weeks before beginning the experiment.

Induction of oxidative stress

Acute oxidative stress was induced in male rats via intra-peritoneal (IP) administration of a single dose of 0.5 ml/kg CCl4 (15)

Animal grouping and treatment.

Four equal groups of five male rats each were created by random selection. As was already mentioned in the previous paragraph, oxidative stress was caused. For nine days, the rats were given the appropriate treatments every day. Every day before the experiment, the dosed solutions were freshly made. A 2-milliliter oral gavage was used to deliver the dosed amounts orally. Rats that were subjected to oxidative stress were divided into three groups based on the dosage formulation. The rats in each group received the same treatment, with dualistic control groups being negative and positive. Negative control group:(G1) rats' treatment by vehicle (PBS) which are used for preparation of selected drug (n= 5 rats)
Positive control group (G2) Rats induced oxidative stress via receive CCL4 and left without treatment (n= 5 rats)
Watery propolis extract (G3) Rats received CCl4 and treated with watery propolis extract (WPE) (50mg/kg BW for 9 days) (n= 5 rats)
Ethanolic propolis extract (G4) Rats received CCL4 and treated with ethanolic propolis extract (EPE) (50mg/kg BW for 9 days) (n= 5 rats)
Ethyl acetate propolis extract (G5) Rats received CCL4 and treated with ethyl acetate propolis extract (EAPE) (50mg/kg BW for 9 days) (n= 5 rats)

Rat scarification and blood collection.

At the end of the experiment, All the food was removed for 12 hours before anesthesia but were allowed free access to water. Blood samples were obtained using an intracardiac puncture technique. The serum was then separated from the whole blood by centrifuging the mixture at 3,000 rpm for 20 minutes at 4°C in a chilled centrifuge. The serum was then maintained at -20°C until analysis was completed.

Determination of serum catalase

Following the manufacturer's instructions, an enzyme linked immunosorbent assay kit was used to detect the catalase activity in the serum. Using a microplate ELISA, the apparatus's wavelength was set to 450 nm (16).

Determination of serum SOD

Based on the manufacturer's instructions, the concentration of superoxidase dismutase (SOD) in the serum was determined using an enzyme linked immunosorbent test kit. A microplate ELISA was used to set the wavelength of the instrument to 450 nm.

Determination of serum T-AOC

The spectrophotometer method was used to test the serum's total antioxidant capacity (T-AOC) in accordance with the manufacturer's instructions. The device's wavelength was set to 593 nm, and distilled water was used to reset the zero point.

Determination of serum reduced glutathione

Following the manufacturer's directions, the spectrophotometer technique was used to measure the reduced glutathione activity in the serum. At 412 nm, the equipment was modified.

Determination of serum MDA

Following the manufacturer's instructions, the spectrophotometer method was used to measure the serum's level of malondialdehyde (MDA). Three separate wavelengths—532, 450, and 600 nm—were used to tune the device.

Statistical analysis

The SPSS software application, version 32, was used to conduct the statistical evaluations. The One-way ANOVA test was used to assess the differences between the groups, and the least significant difference was used to determine the results (17).

Results

***In vitro* antioxidant activity**

DPPH scavenging activity

The concentration-dependence of the inhibitory activity of different propolis extracts on the DPPH formation is shown at table 1 Fig. 1 The concentration of ascorbic acid and propolis extracts which inhibited DPPH by 50% was 49.43, 73.31, 54.87, 84.89 µg/ml for vitamin C, watery, ethanolic, ethyl acetate propolis extracts respectively with maximum DPPH scavenging inhibitory activity recorded at 100 µg/mL of 85.3±0.93, 73.4±0.18, 84.2±0.36, and 59.8±0.42 % respectively. In the same time the mean inhibitory activity was 57.03±9.46, 46.92±9.53, 53.7±10.2, 37.74±7.02 for ascorbic acid, watery, ethanolic, and ethyl acetate propolis extracts

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respectively and statistical analysis reveals significant (P<0.05) difference in the mean activity among different propolis extracts.

Table 1: DPPH scavenging activity of different propolis extract and ascorbic acid

Concentrations (µg/mL)	Ascorbic acid	Watery propolis extract	Ethanolic propolis extract	Ethyl acetate propolis extract
20	32.2±1.01^{A}	22.4±0.21^{A}	26.2±0.56^{A}	18.6±0.14^{A}
40	41.16±0.9^{B}	29.5±0.73^{B}	39±1.12^{B}	29.1±0.38^{B}
60	58.34±0.56^{C}	47.8±0.65^{C}	51.6±0.94^{C}	36±0.15^{C}
80	68.17±0.72^{D}	61.5±0.66^{D}	67.5±0.68^{D}	45.2±0.23^{D}
100	85.3±0.93^{E}	73.4±0.18^{E}	84.2±0.36^{E}	59.8±0.42^{E}
Mean ± SE	57.03±9.46a	46.92±9.53b	53.7±10.2c	37.74±7.02d
LSD (P<0.05)	**1.08**	**0.56**	**0.43**	**0.51**

Data presented as mean ± SEM for 3 batches. The different superscript letters denoted to significant differences (*p*<0.05)

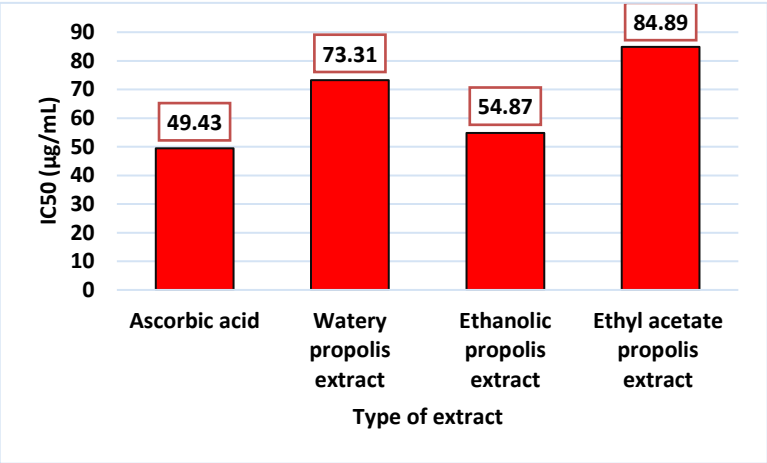

Figure 1: IC_{50} values of different propolis extracts and ascorbic acid on DPPH scavenging activity.

ABTs radical cation decolorization assay

As shown in Table 2 and Figure 2 there was significant (P<0.05) differences in the ABTs radical reduction capacity among different propolis prepared extracts. The concentration of ascorbic acid and propolis extracts which scavenging ABTs radicals by 50% was 44.78, 53.13, 46.02, and 69.78 µg/ml for ascorbic acid, watery, ethanolic, ethyl acetate propolis extracts respectively with maximum ABTs decolorization activity at 100 µg/mL of 96.4±0.21, 80.1±1.11, 89±0.4 and 68.4±0.56% respectively. In the same time the mean decolorization activity was 62.64±11.7, 54.22±8.74, 60.22±10.3 and 44.54±7.92% for ascorbic acid, watery, ethanolic, and ethyl acetate propolis extracts respectively.

Table 2: ABTs radical cation decolorization activity of different propolis extracts and ascorbic acid

Concentrations (µg/mL)	Ascorbic acid	Watery propolis extract	Ethanolic propolis extract	Ethyl acetate propolis extract
20	29.5±0.94^{A}	28.6±0.19^{A}	30.1±0.28^{A}	23.1±0.12^{A}
40	46.4±0.6^{B}	45±0.81^{B}	46.6±0.32^{B}	34±0.18^{B}
60	62.1±0.71^{C}	52.4±0.38^{C}	60.3±0.18^{C}	42.2±0.14^{C}
80	78.8±0.54^{D}	65±0.32^{D}	75.1±0.74^{D}	55±0.22^{D}
100	96.4±0.21^{E}	80.1±1.11^{E}	89±0.4^{E}	68.4±0.56^{E}
Mean ± SE	62.64±11.7a	54.22±8.74b	60.22±10.3c	44.54±7.92d
LSD (P<0.05)	**0.68**	**0.29**	**0.34**	**0.23**

Data presented as mean ± SEM for 3 batches. The different superscript letters denoted to significant differences (*p*<0.05)

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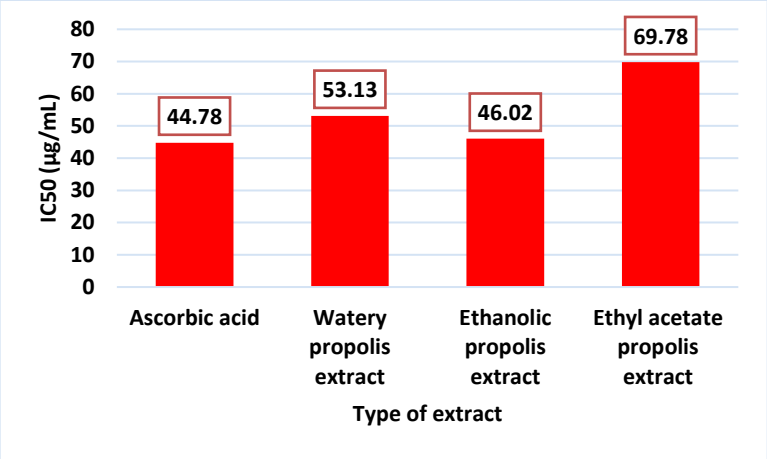

Figure 2: IC50 values of different propolis extracts and ascorbic acid on DPPH scavenging activity

Chelating effect on ferrous ions assay

As shown in Table 3 and Figure 3 the scavenging ability of the propolis extracts and EDTA for chelating effect on ferrous ions gradually increased with the increase in the tested concentration. The concentration of EDTA and propolis extracts which chelating ferrous ions by 50% was 49.63, 69.86, 47.78, and 73.31 µg/ml for EDTA, watery, ethanolic, ethyl acetate propolis extracts respectively with maximum chelating activity at 100 µg/mL 98.2±0.93, 70.2±0.55, 84.2±0.36 and 68.8±0.24% respectively. In the same time the mean chelating activity was 68.74±10.7, 44.32±8.22, 58.08±9.36and 41.6±8.99% for EDTA, watery, ethanolic, and ethyl acetate propolis extracts respectively.

Table 3: The chelating effects of different propolis extracts and EDTA on ferrous ions

Concentrations (µg/mL)	EDTA	Watery propolis extract	Ethanolic propolis extract	Ethyl acetate propolis extract
20	37.1±1.01^{A}	23.1±0.06^{A}	32.2±0.56^{A}	19.4±0.12^{A}
40	55.2±0.9^{B}	33.3±0.45^{B}	44.2±1.12^{B}	25.6±0.88^{B}
60	67.8±0.56^{C}	40.8±0.18^{C}	57.3±0.94^{C}	41.2±0.56^{C}
80	85.4±0.72^{D}	54.2±0.31^{D}	72.5±0.68^{D}	53±0.52^{D}
100	98.2±0.93^{E}	70.2±0.55^{E}	84.2±0.36^{E}	68.8±0.24^{E}
Mean ± SE	68.74±10.7a	44.32±8.22b	58.08±9.36c	41.6±8.99d
LSD (P<0.05)	**1.04**	**0.51**	**0.69**	**0.58**

Data presented as mean ± SEM for 3 batches. The different superscript letters denoted to significant differences (*p*<0.05)

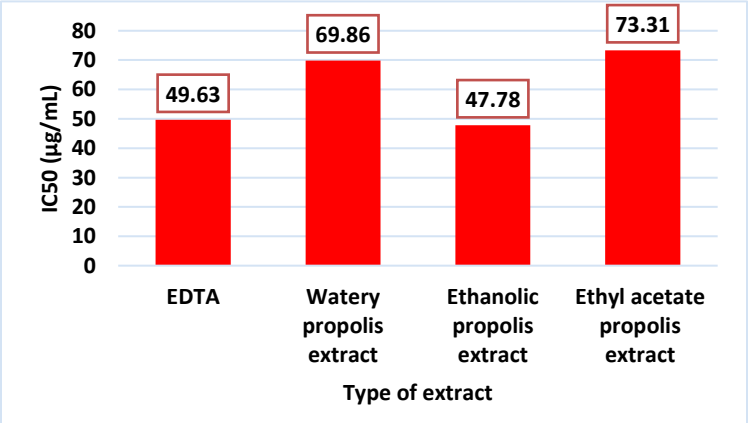

Figure 3: IC_{50} values of the different propolis extracts and EDTA on ferrous ion chelating effect.

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Hydrogen peroxide scavenging assay

As shown in Table 4 and Figure 4 the scavenging ability of the propolis extracts and ascorbic acid for hydrogen peroxide gradually increased with the increase in the tested concentration. The concentration of ascorbic acid and propolis extracts which inhibit H_2O_2 free radicals by 50% was 48.7, 46.44, 48.39, 50.09 µg/ml for ascorbic acid, watery, ethanolic, ethyl acetate propolis extracts respectively with maximum scavenging activity at 100 µg/mL of 95.34±0.91, 90±0.54, 91.2±0.16, 79.5±0.24% respectively. In the same time the mean value activity was 60.51±13.2, 54.1±13.9, 54.66±13.7, and 45.62±12.31% for ascorbic acid, watery, ethanolic, and ethyl acetate propolis extracts respectively. The statistical analysis recorded there was significant (P<0.05) differences in the inhibitory activity among different propolis extracts.

Table 4: The hydrogen peroxide scavenging activity of different propolis extracts and ascorbic acid

Concentrations (µg/mL)	Ascorbic acid	Watery propolis extract	Ethanolic propolis extract	Ethyl acetate propolis extract
20	20.1 ± 0.15^{A}	15.3 ± 0.11^{A}	18 ± 0.22^{A}	12.4 ± 0.16^{A}
40	42.6 ± 0.25^{B}	31.2 ± 0.21^{B}	33.1 ± 1.01^{B}	26.1 ± 0.42^{B}
60	66.2 ± 0.52^{C}	56.6 ± 0.06^{C}	51.2 ± 0.09^{C}	44.5 ± 0.55^{C}
80	78.34 ± 0.4^{D}	77.4 ± 0.74^{D}	79.8 ± 0.48^{D}	65.6 ± 0.18^{D}
100	95.34 ± 0.91^{E}	90 ± 0.54^{E}	91.2 ± 0.16^{E}	79.5 ± 0.24^{E}
Mean ± SE	60.51±13.2a	54.1±13.9b	54.66±13.7b	45.62±12.31d
LSD (P<0.05)	**0.77**	**0.53**	**0.61**	**0.48**

Data presented as mean ± SEM for 3 batches. The different superscript letters denoted to significant differences (*p*<0.05)

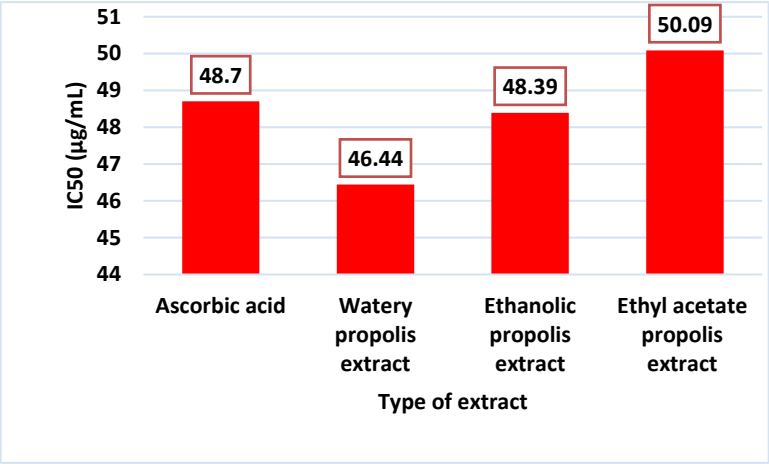

Figure 4: IC_{50} values of the different propolis extracts and ascorbic acid on hydrogen peroxide scavenging activity.

Total reduction capacity assay

As shown in Table 5 and Figure 5 the reduction capacity of the propolis extracts and standard drug was increased with the increasing concentration of the tested extracts and ascorbic acid. The concentration of ascorbic acid and propolis extracts which cause ferric ion reduction ability by 50% was 41.83, 55.03, 54.87, 78.66 µg/ml for ascorbic acid, watery, ethanolic, ethyl acetate propolis extracts respectively with maximum activity at 100 µg/mL of 89.45±0.88, 80.98±0.42, 84.2±0.36, 65.4±0.24% respectively. In the same time the mean value of reduction capacity was 62.69±9.89, 53.34±9.53, 53.7±10.2, and 37.94±9.21% for ascorbic acid, watery, ethanolic, and ethyl acetate propolis extracts respectively. The statistical analysis recorded there was significant (P<0.05) differences in the inhibitory activity among different propolis extracts.

Table 5: Total reduction capacity activity of different propolis extract and ascorbic acid

Concentrations (µg/mL)	Ascorbic acid	Watery propolis extract	Ethanolic propolis extract	Ethyl acetate propolis extract
20	34.68 ± 1.26^{A}	25.78 ± 0.14^{A}	26.2 ± 0.56^{A}	15.3 ± 0.12^{A}
40	47.66 ± 0.99^{B}	41.2 ± 0.38^{B}	39 ± 1.12^{B}	21.22 ± 0.88^{B}
60	63.8 ± 0.65^{C}	53.34 ± 0.15^{C}	51.6 ± 0.94^{C}	37.6 ± 0.56^{C}
80	77.89 ± 0.28^{D}	65.42 ± 0.23^{D}	67.5 ± 0.68^{D}	50.2 ± 0.52^{D}

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100	89.45±0.88^{E}	80.98±0.42^{E}	84.2±0.36^{E}	65.4±0.24^{E}
Mean ± SE	62.69±9.89a	53.34±9.53b	53.7±10.2b	37.94±9.21c
LSD (P<0.05)	**1.17**	**0.56**	**1.03**	**0.60**

Data presented as mean ± SEM for 3 batches. The different superscript letters denoted to significant differences (*p*<0.05)

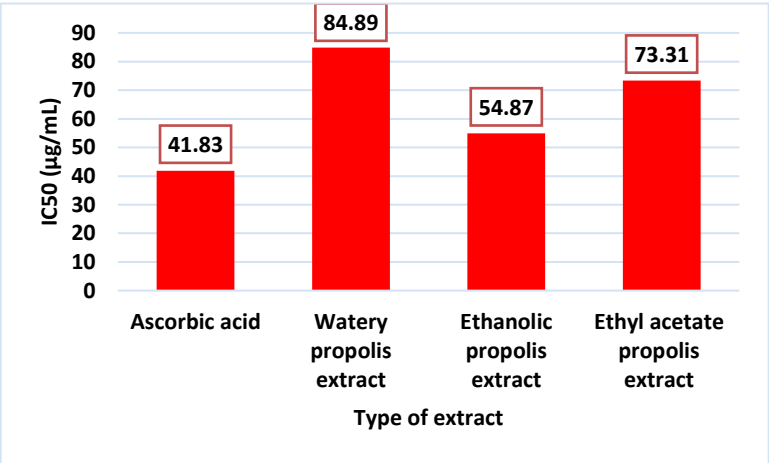

Figure 5: IC_{50} values of the different propolis extracts and ascorbic acid on total reduction capacity activity.

***In vivo* antioxidant activity**

Serum catalase activity

Data illustrated, in Table 6 showed that serum level of the catalase was significantly (P<0.05) declined in the CCL4 induced rats (PC) 228.2±4.12 U/L as compared with G1 group 426.12±1.08 U/L. On the other hand, propolis watery, ethanolic and ethyl acetate extract rats showed a significant (P<0.05) raise in catalase levels 306.2±2.63, 301.1±2.08, and 247.14±1.76 U/L respectively as compared with G2 group. Moreover, watery extract group gave best results as compared with other extracts groups. Conversely, there was significant (p<0.05) alterations in the catalase value between ethanolic and ethyl acetate rats' groups.

Table 6: Effect of different propolis extracts on serum catalase level in CCl4-treated rats

Groups	Catalase (U/L)
Negative control(G1)	416.1±3.18^{A}
Positive control (G2)	228.2±4.12^{E}
Watery propolis extract (G3)	306.2±2.63^{B}
Ethanolic propolis extract (G4)	301.1±2.08^{C}
Ethyl acetate propolis extract (G5)	247.14±1.76^{D}
LSD (P<0.05)	**3.88**

Data presented as mean ± SEM for 5 batches. The different superscript letters denoted to significant differences (*p*<0.05)

Serum SOD activity

Data illustrated, in Table 7 revealed that of SOD activity values was significantly (P<0.05) decline in the CCL4 given rats 411.24±4.14 U/L as compared with negative control rats 726.2±3.51 U/L. in the same trend, propolis watery extract, Propolis ethanolic extract, ethyl acetate groups showed significant (P<0.05) elevated in SOD activity 470.54±3.94, 473.1±6.45, 468.2±5.62 U/L respectively as compared with CCL4 induced rats. The statistical analysis also revealed that there were no significant (P>0.05) alterations in the SOD activity in the three propolis extracts treated rats.

Table 7: Effect of different propolis extracts on serum SOD activity in CCl4-treated rats

Groups	SOD activity (U/L)
Negative control(G1)	726.2±3.51^{A}
Positive control (G2)	411.24±4.14^{C}
Watery propolis extract (G3)	470.54±3.94^{B}
Ethanolic propolis extract (G4)	473.1±6.45^{B}
Ethyl acetate propolis extract (G5)	468.2±5.62^{B}
LSD (P<0.05)	**5.63**

Data presented as mean ± SEM for 5 batches. The different superscript letters denoted to significant differences (*p*<0.05)

Serum T-AOC (µmol/mL)

The results in table 8 showed that the T-AOC levels in the serum in the different treated groups. The T-AOC values in the group of DOX rats 7.11±1.26 µmol/ml

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were significantly (p<0.05) lower than basic values of negative control 12.08±1.13 µmol/ml and no significant alteration with ethyl acetate propolis extract treated group. Conversely, there was significant (p<0.05) elevation in the serum T-AOC values in both watery propolis extract treated group 8.73±0.86 µmol/mL and ethanolic extract treated group 8.67±0.73 µmol/mL as compared with the CCL4 treated groups. Additionally, the results revealed no statistical changes in the T-AOC levels between watery and ethanolic extract treated groups.

Table 8: Effect of different propolis extracts on serum T-AOC concentration in CCl4-treated rats

Groups	Serum T-AOC
Negative control(G1)	12.08 ± 1.13^{A}
Positive control (G2)	7.11 ± 1.26^{C}
Watery propolis extract (G3)	8.73 ± 0.86^{B}
Ethanolic propolis extract (G4)	8.67 ± 0.73^{B}
Ethyl acetate propolis extract (G5)	7.66 ± 0.91^{C}
LSD (P<0.05)	**0.901**

Data presented as mean ± SEM for 5 batches. The different superscript letters denoted to significant differences (*p*<0.05)

Serum GSH activity

Table 9 showed the serum level of the GSH activity in the treated and control groups. There was marked significant (P<0.05) decline in the serum GSH activity in the CCL4 given rats 14.12±0.38 µg/ml compared with negative control rats 68.12±1.51 µg/ml. In the same time, three different propolis extracts administrated rats exhibited a significant (P<0.05) elevation in GSH activity 27.22±0.56, 29.13±1.02, 21.27±0.18 µg/ml in the watery, ethanol, ethyl acetate administration rats respectively as compared with CCL4 group. The results also recorded significant (P<0.05) alterations in the GSH among the different propolis extracts groups.

Table 9: Effect of different propolis extracts on serum GSH activity in CCl4-treated rats

Groups	GSH (µg/mL)
Negative control(G1)	68.12 ± 1.51^{A}
Positive control (G2)	14.12 ± 0.38^{E}
Watery propolis extract (G3)	27.22 ± 0.56^{C}
Ethanolic propolis extract (G4)	29.13 ± 1.02^{B}
Ethyl acetate propolis extract (G5)	21.27 ± 0.18^{D}
LSD (P<0.05)	**1.21**

Data presented as mean ± SEM for 5 batches. The different superscript letters denoted to significant differences (*p*<0.05)

Serum MDA

Data illustrated, in Table 10 revealed that the serum concentration of MDA values of positive control rats 0.812±0.12 nmol/mg protein was significantly (P<0.05) higher than basic values of negative control group 0.385±0.06 nmol/mg protein. In the same trend, there was significant (p<0.05) reduction in the serum MDA values in the different propolis extracts (watery, ethanolic and ethyl acetate extracts) as compared with the positive control 0.614±0.16, 0.602±0.08, 0.708±0.1 nmol/mg protein respectively. There was no significant (P>0.05) difference the MDA concentration between watery and ethanolic extract groups whereas there was significant (P<0.05) improvement of two extracts groups as compared with ethyl acetate extract group.

Table 10: Effect of different propolis extracts on serum reduced glutathione in CCl4-treated rats

Groups	MDA (nmol/mg protein)
Negative control(G1)	0.385 ± 0.06^{D}
Positive control (G2)	0.812 ± 0.12^{A}
Watery propolis extract (G3)	0.614 ± 0.16^{C}
Ethanolic propolis extract (G4)	0.602 ± 0.08^{C}
Ethyl acetate propolis extract (G5)	0.708 ± 0.1^{B}
LSD (P<0.05)	**0.016**

Data presented as mean ± SEM for 5 batches. The different superscript letters denoted to significant

Discussion

Because some bioactive substances don't dissolve in different solvents in the same way. Lower activity may result from incomplete extraction of the intended components using the wrong solvents. The study was undertaken to evaluate the antioxidant activities of different solvent extracted propolis samples in both *In vitro* and *In vivo* experiments. Combining different extraction methods can be a strategy to obtain a broader spectrum of bioactive components and potentially enhance the overall antioxidant activity. Bees gather a set of sticky, balsamic chemicals called propolis, often known as bee glue, from bark and buds of a variety of plants, including birch, poplars, oaks, willows, and conifers, among many others (18) The antioxidant activity of propolis, a bee product rich in phenolic compounds, can be significantly affected by the extraction method used (4). This is well documented in both *In vitro* and *In vivo* studies. According to earlier

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research, propolis's most powerful antioxidant and antibacterial qualities are found in derivatives of pinocembrin and galangin (19). Caffeic acid and its esters, phenols, terpenoids, and chrysin have also revealed remarkable antimicrobial and antioxidant potential (20). According to current study, propolis samples include natural chemicals with antioxidant qualities that might reduce oxidative stress brought on by CCL4 administration. The antioxidant activity of the various propolis solvent extracts was evaluated via using the following assays: DPPH scavenging activity, ABTs radical cation decolorization, chelating of ferrous ions, hydrogen peroxidase scavenging properties, and total reduction capacity characteristic. Age-related illnesses like cancer, diabetes, osteoporosis, inflammatory disorders, dementia, and metabolic syndromes are all significantly influenced by oxidative stress (21; 22). In order to regulate cellular processes including cell survival, stressor responses, and inflammation, reactive oxygen species are often produced inside biological systems (23) Nonetheless, oxidative stress can be brought on by elevated reactive oxygen species as they upset the equilibrium between pro-oxidant and antioxidant concentrations (24). Current research evidences revealed that propolis extracted active compounds in different samples have excellent antioxidant properties *In vitro* and *In vivo* tests. This essential activity is consider as one of the main factors for propolis to have many other uses, such as cardioprotective, renal, neuro and hepatoprotective, anti-inflammatory and immunomodulatory properties (25). One of the most viable applications of the antioxidant power is in the treatment of skin wounds, remembering that the intensified production of free radicals makes it difficult for these inflammatory processes to heal (26). Among the propolis extracts, the ethanolic extract was exhibit highest activity in the assays of DPPH, ABTs scavenging, Ferrous ions chelating effects. In the same time, no significant (P>0.05) difference in the antioxidant activity of ethanolic and watery propolis extracts in the assays of hydrogen peroxide scavenging properties and total reduction capacity characteristic. While the ethyl acetate extract showed less activity as compared with other solvent used for extraction process in all *In vitro* assays. The power antioxidant properties of both watery and ethanolic extracts of propolis often matched with highly content from phenolic and flavonoids contents. Ample evidence indicated that there is a positive correlation between DPPH assay, other assays and contents of phenolic and flavonoids of the plant extracts (27). Propolis extracts, both aqueous and ethanolic, are generally thought to possess high concentrations of flavonoid and other chemicals, which contribute to their antioxidant effect. Numerous investigations have found a beneficial relationship between TPC/TFC and plant extracts' ability to function as antioxidants. Actually, one of the most important steps in utilizing propolis' bioactive ingredients is extraction. Ma and his colleagues (28) found that ethanol and methanol extracts showed higher antioxidant activities compared to water, ethyl acetate, chloroform, and benzene extracts.On the other hand, *In vivo* results indicate that among the different solvents extracts tested, the ethanolic extract was the most potent activity in enhance GSH activity, while watery extract gave best results in improvement catalase antioxidant enzyme. In the same time no significant (P>0.05) differences was recorded in the activity of ethanolic and watery extract in the SOD and T-AOC assays. Antilipid peroxidation activity of three extracts showed closed results. It is important to highlight that although the ethyl acetate extract of propolis was less effective in *In vitro* and *In vivo* antioxidant activity, it was giving significant effectiveness in the both experiments. One explanation for this discovery could be because the observed antioxidant effect is caused by a mixture of certain phenolic compounds, even if these compounds are present in low levels. These particular substances may also work in concert to produce effects that are more potent when combined than when taken alone. Studies have demonstrated that propolis extracts obtained with different methods can offer varying degrees of protection against oxidative stress in vivo. Ethanol extracts of propolis have been shown to reduce oxidative damage in liver tissue after exposure to toxins (29). Other studies have reported that propolis extracts obtained with water or aqueous ethanol exhibited superior anti-inflammatory activity compared to those obtained with pure ethanol (30). In conclusion the choice of extraction method significantly impacts the antioxidant activity of propolis due to its influence on the composition and yield of bioactive compounds.

Ethical Approval:

This study was approved by the ethical and research committee of college of veterinary medicine, University of Al-Qadisiyah.

Conflict of interest

There is no conflict of interest, according to the authors.

Author's contribution

Each author made an equal contribution to every aspect of this manuscript.

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