Journal of Saudi Chemical Society (2012) **16**, 13–21

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Evaluation of the safety and antioxidant activities of *Crocus sativus* and *Propolis* ethanolic extracts

A. Ramadan [a,*], G. Soliman [b], Sawsan S. Mahmoud [c], Salwa M. Nofal [c], Rehab F. Abdel-Rahman [c]

[a] *Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt*
[b] *Department of Pharmacology, Faculty of Pharmacy, Al-Kharj University, Saudi Arabia*
[c] *Department of Pharmacology, National Research Centre, Cairo, Egypt*

Received 13 September 2010; accepted 12 October 2010
Available online 20 October 2010

KEYWORDS

Crocus sativus;
Propolis;
Acute toxicity;
Sub-chronic toxicity;
Antioxidant;
Rats

Abstract The possible toxicological effects and *in vitro* antioxidant activity of the ethanolic extracts of *Crocus sativus* and *Propolis* were investigated. Both extracts did not cause any mortalities or signs of toxicity in mice when administered orally at doses up to 5 g/kg b.wt. In the sub-chronic study; the tested extracts did not produce any significant change in liver and kidney functions of rats, following oral administration for 8 successive weeks at doses of 500 mg/kg b.wt. of each. *Propolis* showed remarkable *in vitro* antioxidant activity at concentrations of (40–100 mg/ml). In contrast, the ethanolic extract of *C. sativus* ethanolic extract showed weak antioxidant activity in concentrations of (1–10 mg/ml) while at concentrations of (20–100 mg/ml) failed to exhibit any antioxidant activity. It was concluded that: both extracts were non-toxic, as they did not cause any mortalities or signs of toxicity in mice when administered orally at doses up to 5 g/kg b.wt. Daily oral administration of *C. sativus, Propolis* ethanolic extracts alone or in combination for 8 successive weeks to rats was quiet safe and didn't cause any toxic changes in liver and kidney.

* Corresponding author. Address: Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211-Giza, Egypt. Tel.: +20 10 5285350; fax: +202 35725240.
E-mail address: amer-ramadan@hotmail.com (A. Ramadan).

Peer review under responsibility of King Saud University.
doi:10.1016/j.jscs.2010.10.012

Production and hosting by Elsevier

Antioxidant study showed that *Propolis* ethanolic extract was a more potent antioxidant than *C. sativus* extract.

1. Introduction

Crocus sativus L., commonly known as saffron, is a perennial stemless herb of the *Iridaceae* family. It is widely cultivated in Spain and Iran but also cultivated on a lower scale in other countries, such as Italy, India, France and Egypt. The name saffron comes from the Arabic za'faran, which means yellow (Ríos et al., 1996). Saffron was used in folk medicine as an anodyne, antispasmodic, eupeptic, gingival sedative, anticatarrhal, nerve sedative, carminative, diaphoteric, expectorant, stomachic, aphrodisiac and emmenagogue (Ríos et al., 1996). Saffron can also be used topically to help clear up conquer sores and to reduce the discomfort of teething infants (Abdullaev and Espinosa-Aguirre, 2004; Schmidt et al., 2007). Furthermore, modern pharmacological studies have demonstrated that saffron extract or its active constituents have anticonvulsant (Hosseinzadeh and Khosravan, 2002), antidepressant (Hosseinzadeh et al., 2004), anti-inflammatory (Hosseinzadeh and Younesi, 2002) and antitumour effects (Escribano et al., 1996).

Propolis is a resinous material collected by bees from bud and exudates of the plants and is transformed by bee enzymes. Bees use the propolis (bee glue) along with bees wax to construct their hives. It originates as a gum secretion gathered by bees from a variety of plants, and can vary in color depending on the plant species of origin. Etymologically, the Greek word propolis means pro, for or in defence, and polis, the city, that is "defence of the hive". Bees use it to seal holes in their honeycombs, smooth out internal walls as well as to cover carcasses of intruders who died inside the hive in order to avoid their decomposition (Burdock, 1998; Salatino et al., 2005; Najafi et al., 2007). Propolis has remarkable therapeutic qualities. General medicinal uses of propolis include treatment of the cardiovascular and blood systems disorder (anemia), respiratory system (for various infections), dental care, dermatology (tissue regeneration, ulcers, eczema, wound healing; particularly burns, wounds, mycosis, mucous membrane infections and lesions), cancer treatment, immune system support and improvement, digestive tract disorders (ulcers and infections), liver protection and support and many others (Najafi et al., 2007; Sforcin, 2007).

In this study, ethanol extracts of *C. sativus* and *Propolis* were administered orally to mice and rats for determination of their acute and sub-chronic toxicities, respectively. Moreover; *in vitro* antioxidant activity using di-phenyl picryl hydrazyl method (DPPH) was used to investigate their possible free radical scavenging activity.

2. Methods

2.1. Natural remedies

C. sativus (saffron) and *Propolis* were purchased from the local market (Agricultural Seeds, Spices and Medicinal Plants Co., Abd El-Rahman M. Harraz) in April, 2008, stored at 2–4 °C and were identified by Dr. Salwa Ali Kawashty, Prof. of Phytochemistry and Plant Systematic Department, National Research Centre (NRC), Egypt.

2.2. Preparation of ethanolic extracts

2.2.1. Preparation of ethanolic extract of C. sativus

One hundred g of *C. sativus* stigmas (saffron stigmas) were macerated for 3 days in one litre of 80% ethanol at room temperature. The ethanolic extract was filtered using filter paper. The filtrate was concentrated under vacuum using the rotatory evaporator (40 °C), then percolated several times till exhaustion to yield 38 g of dark red residues. The ethanolic extract was chilled in refrigerator until use (Akhondzadeh et al., 2005).

2.2.2. Preparation of ethanolic extract of Propolis

Dried milled, crude *Propolis* (100 g) was extracted in a dark place at 4 °C for 3 days using one litre of 80% ethanol. The extract was filtered using Büchner apparatus then concentrated under vacuum using the rotatory evaporator (40 °C) to yield 40 g of dried *Propolis* ethanolic extract. The extract was chilled in refrigerator until use (Moreno et al., 2000).

2.3. Phytochemical investigation

C. sativus and *Propolis* ethanolic extracts were analyzed for the presence of carbohydrates and/or glycosides using Molisch's reagent, (Molisch, 1963). Flavonoids were detected using Neu's reagent (Naturstoff reagent), (Neu, 1956). Sterols and/or triterpenes and volatile oils were detected according to (Liebermann and Burchard, 1890). Tannins were detected using Ferric chloride reagent, (Smith, 1960). Coumarins, Alkaloids and nitrogenous bases were detected according to the method of Stahl (1969).

2.4. Experimental animals

Mice and rats used in this investigation were obtained from The Animal House, NRC, Cairo. Mature albino mice of 20–25 g b.wt. each, of both sexes were used. Mature Sprague Dawley rats of both sexes, 150–200 g b.wt. were used. All animals were housed in hygienic cages in well ventilated rooms with exhaust fans; received standard pellet diet and water were provided *ad libitum*.

All animal procedures were performed after approval from the Ethics Committee of The National Research Centre- Egypt and in accordance with the recommendations of the proper care and use of laboratory animals.

2.5. Acute toxicity and median lethal dose (LD_{50}) test

The acute oral toxicity and median lethal dose (LD_{50}) of the ethanolic extracts of *C. sativus* and *Propolis* were estimated in mice (Lorke, 1983). In a pilot experiment, three groups each of five mice received the tested extracts suspended in a vehicle

(1% v/v Tween 80) at doses of 10, 100, and 1000 mg/kg b.wt., respectively. Animals were observed for 24 h for signs of toxicity and number of deaths. From the results of the preliminary test, doses of 2000, 3000, 4000 and 5000 mg/kg b.wt. of both extracts were administered to new animal groups, each of 10 mice. Control animals were received the vehicle and kept under the same conditions without any treatments. Signs of toxicity and number of deaths per dose in 24 h were recorded.

2.6. Sub-chronic toxicity

The effect of prolonged oral administration of the ethanolic extracts of *C. sativus, Propolis* and their combination on liver and kidney functions, body weight gain, food consumption and food conversion ratio (FCR) was evaluated in rats. Histopathological changes of liver and kidney were also assessed.

Forty rats were randomly divided into 4 equal groups. Rats of the 1st group were given the vehicle (1% v/v Tween 80) in a dose of 5 ml/kg b.wt and left as normal control. The 2nd and 3rd groups of the rats were administered the ethanolic extract of *C. sativus* and *Propolis,* respectively, in a dose of 500 mg/kg b.wt. The 4th group was medicated with a combination of both *C. sativus* (250 mg/kg b.wt) and *Propolis* (250 mg/kg b.wt) extracts. All medications were administered orally via the aid of an oral tube for 8 consecutive weeks. The animals were observed for signs of abnormalities throughout the experiment. Blood samples (2 ml) were collected from the retro-orbital venous plexus of each rat into clean centrifuge tubes at day 0, then 4 and 8 weeks post-extracts administration. Blood samples that were taken for biochemical examinations were taken without anticoagulant, from which clear sera were obtained by centrifugation at 3000 rpm for 10 min. Sera were separate with Pasteur pipette into sterile serum sample tubes and used for biochemical assay. The position, shape, size and color of the internal organs of the treated rats were visually observed for any signs of gross lesions.

2.6.1. Measurement of liver and kidney function markers

Liver function was evaluated by measuring the serum activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) following the method of Reitman and Frankel (1957). Serum concentrations of creatinine and urea were determined colormetrically as measures of kidney function according to Kroll et al. (1987) and Wills and Savory (1981), respectively.

2.6.2. Effect on body weight gain and relative organs weight

Body weight of all rats was recorded at the beginning of the study and then weekly for 8 weeks. Livers and kidneys were collected from each rat and weighed to determine the relative organ weight (ROW) as described by Chavalittumrong et al. (2004).

$$\mathrm{ROW(kg)} = [\text{organ weight(g)}/\text{body weight(g)}] \times 1000$$

2.6.3. Effect on food consumption and food conversion ratio

A known weighed amount of food was supplied to animals of each group daily in the morning, then the remaining amount after 24 h was weighed and subtracted from the original amount of food. The average amount of food consumed per group was calculated and recorded weekly. Food conversion ratio (FCR) was calculated according to Brady (1968) using the following formula:

FCR = Food consumption in a given period/body weight at the same period.

2.6.4. Histopathological examination

At the end of the experiment, animals were decapitated and tissue samples including liver and kidney were taken to investigate the histopathological changes. Tissues were then fixed in 10% buffered formalin solution (formol saline 10%). The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4–6 μ thickness and stained with hematoxylin and eosin as a routine stain (Carleton, 1976). Tissue slides were examined by a pathologist.

2.7. Evaluation of the antioxidant effect (in vitro)

The activity of (1,1-Diphenyl, 2-picryl hydrazyl) DPPH radical scavenging activity was investigated according to the method of Peiwu et al. (1999). The DPPH shows maximum absorbency at 517 nm, which decreases in the presence of H-donor molecules, so the test compound with antioxidant activity reduces DPPH to yellow colored product, di-phenylpicrylhydrazine, and the absorbance at 517 nm declined (Burits et al., 2001).

A methanolic solution of DPPH (2.95 ml) was added to 50 μl sample (the tested extracts were dissolved in methanol at different concentrations; 1–100 mg/ml for *C. sativus* and 20–100 mg/ml for *Propolis*) in a disposable cuvette. The absorbance was measured at 517 nm at regular intervals of 15 s for 5 min. Ascorbic acid was used as a standard (at 0.1 M concentration) as described by Govindarajan et al. (2003).

$$\text{Radical scavenging activity}\% = \frac{A_c - A_t}{A_c} \times 100$$

Where A_c and A_t are the absorbance of control (DPPH) and the test extract, respectively.

2.8. Statistical analysis

Statistical analysis of results, were done using analytical software named SPSS statistics 17.0, Release (Aug. 23, 2008), Chicago, USA. Values were expressed as means ± S.E. Quantitative differences between values were statistically analyzed by least significant difference test (LSD) followed by Dunnett's multiple comparisons test. P values < 0.05 were considered to be significant.

3. Results

3.1. Phytochemical investigation

Phytochemical analysis of *C. sativus* and *Propolis* ethanolic extracts has shown that both extracts contain flavonoids, carbohydrates &/or glycosides, sterols and/or terpenes. They do not contain volatiles, coumarins, alkaloids &/or nitrogenous compounds, tannins or saponins.

3.2. Acute toxicity and median lethal dose (LD_{50}) test

The results revealed that all the examined doses of *C. sativus* and *Propolis* (up to 5000 mg/kg b.wt.) did not produce any demonstrable acute toxic effects or deaths in all groups of mice.

3.3. Sub-chronic toxicity in rats

The effect of prolonged oral administration of the tested extracts (8 successive weeks) on serum transaminases activity, serum creatinine and urea was recorded in rats (Tables 1 and 2). Oral administration of the ethanolic extract of *C. sativus* and *Propolis* in a dose of 500 mg/kg b.wt. and their combination in their half dose levels for 8 weeks did not affect the serum activity of AST and ALT as compared to those of the normal control rats or their basal values. The obtained results showed no significant effect on serum levels of creatinine and urea in all groups when compared to control group and when compared to their basal values.

The effect of oral administration of the ethanolic extracts of *C. sativus*, *Propolis* and their combination for 8 successive weeks on body weight gain and relative organs weight of rats was determined (Table 3).

Ethanolic extracts of *C. sativus*, *Propolis* and their combination showed no significant effect on body weight gain of rats all over the experimental period compared to the control group. They significantly increased the relative weights of kidneys. The relative weights of liver were significantly increased in rats that were medicated with either *C. sativus* alone or the combination of *C. sativus* and *Propolis*. *Propolis* alone did not alter the relative weight of liver to any significant extent at the different times of measurement.

Daily oral administration of ethanolic extracts of *C. sativus*; *Propolis* or their combination for 8 consecutive weeks to rats showed insignificant effect on food consumption and food

Table 1 Effect of prolonged oral administration of ethanolic extracts of *Crocus sativus* (CS); *Propolis* (PP) and their combination (CS + PP) for 8 successive weeks; on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum levels of rats.

Groups	Dose (mg/kg b.wt.)	Basal		4 weeks		8 weeks	
		AST (U/ml)	ALT (U/ml)	AST (U/ml)	ALT (U/ml)	AST (U/ml)	ALT (U/ml)
Control	0	118.1 ± 4.08	71.0 ± 4.21	118.0 ± 6.25	73.9 ± 5.75	117.8 ± 6.00	75.2 ± 3.78
CS	500	122.5 ± 6.21	69.4 ± 5.15	119.7 ± 6.41	78.5 ± 4.30	120.6 ± 5.34	78.6 ± 2.49
PP	500	119.5 ± 5.87	72.9 ± 2.70	116.8 ± 7.22	72.3 ± 5.82	118.2 ± 1.56	77.7 ± 2.39
CS + PP	250 + 250	117.3 ± 3.21	68.7 ± 5.06	125.8 ± 8.52	79.0 ± 4.67	124.9 ± 4.32	73.4 ± 3.52

Values represent the mean ± S.E. ($n = 10$).
No significant difference from control (LSD).
No significant difference from basal value of each group (Paired *t*-test).

Table 2 Effect of prolonged oral administration of ethanolic extracts of *Crocus sativus* (CS); *Propolis* (PP) and their combination (CS + PP) for 8 successive weeks; on creatinine and urea serum levels of rats.

Groups	Dose (mg/kg b.wt.)	Basal		4 weeks		8 weeks	
		Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Control	0	0.43 ± 0.02	19.8 ± 0.75	0.44 ± 0.03	22.4 ± 0.55	0.42 ± 0.02	20.2 ± 1.04
CS	500	0.44 ± 0.02	19.3 ± 0.91	0.44 ± 0.02	20.7 ± 0.33	0.43 ± 0.02	21.1 ± 0.61
PP	500	0.40 ± 0.02	19.0 ± 0.75	0.43 ± 0.03	21.1 ± 1.08	0.41 ± 0.02	20.3 ± 0.31
CS + PP	250 + 250	0.40 ± 0.01	19.5 ± 1.17	0.42 ± 0.02	21.8 ± 0.37	0.46 ± 0.04	20.5 ± 0.47

Values represent the mean ± S.E. ($n = 10$).
No significant difference from control (LSD).
No significant difference from basal value of each group (Paired *t*-test).

Table 3 Effect of prolonged oral administration of ethanolic extracts of *Crocus sativus* (CS); *Propolis* (PP) and their combination (CS + PP) for 8 successive weeks; on relative organs weight of rats.

Groups	Dose (mg/kg b.wt.)	Body weight gain (g)	Relative organs weight (g)	
			Liver	Kidneys
Control	0	108 ± 4.2	33.6 ± 1.18	5.0 ± 0.10
CS	500	106 ± 6.7	38.3 ± 1.01[a]	5.5 ± 0.07[a]
PP	500	100 ± 5.2	36.3 ± 0.81	5.5 ± 0.13[a]
CS + PP	250 + 250	104 ± 8.8	37.6 ± 1.02[a]	5.7 ± 0.17[a]

Values represent the mean ± S.E. ($n = 10$).
[a] $P < 0.05$: Statistically significant from control (LSD followed by Dunnett's test).

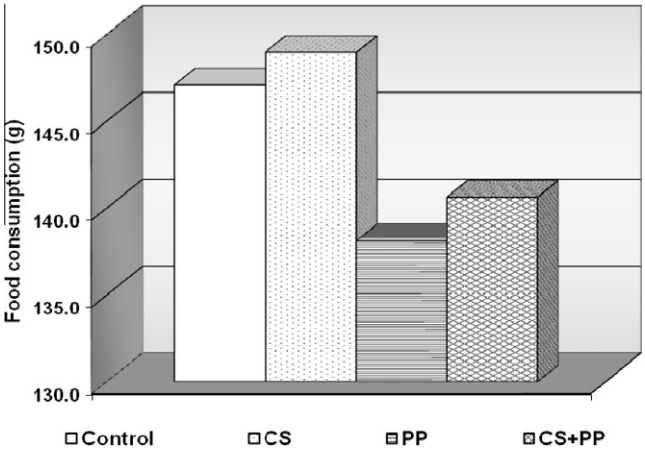

Figure 1 Effect of prolonged oral administration of ethanolic extracts of *Crocus sativus* (CS); *Propolis* (PP) and their combination (CS + PP) for 8 successive weeks; on food consumption (g) of rats, (n = 10).

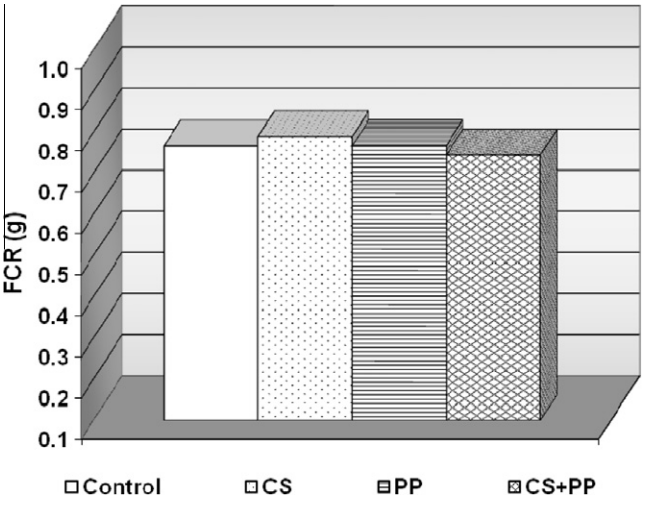

Figure 2 Effect of prolonged oral administration of ethanolic extracts of *Crocus sativus* (CS); *Propolis* (PP) and their combination (CS + PP) for 8 successive weeks; on food conversion ratio of rats, (n = 10).

conversion ratio along the experimental period when compared to control (Figs. 1–3).

3.4. Evaluation of antioxidant activity (in vitro)

The antioxidant activity of the ethanolic extract of *C. sativus, Propolis* and their combination was studied *in vitro* using the DPPH method. Results of the kinetics of DPPH scavenging reaction of the tested extracts and L-ascorbic acid were demonstrated in Figs. 4–6. The ethanolic extract of *C. sativus* in different concentrations (20, 40, 60, 80, and 100 mg/ml) showed no antioxidant activity *in vitro*. While in concentrations of (1, 2, 4, 6, 8, and 10 mg/ml) showed weak antioxidant activity, with the maximum reactive reaction rates of (2.4%, 0.9%, 0.9%, 4.8%, 6.9% and 10.9%), respectively.

Different concentrations of the ethanolic extract of *Propolis* (40, 60, 80, and 100 mg/ml) showed marked significant scavenging activity (antioxidant activity) in a concentration-dependent manner. After 5 min, the maximum reactive reaction rates for the used concentrations were 9.0%, 26.8%, 34.7% and 68.5%, respectively. The reactive reaction rate (inhibition%) of L-ascorbic acid was 95.8%. L-ascorbic acid was the only compound reaching a steady state in about 60 s. On the other hand, *Propolis* extract at a concentration of 20 mg/ml showed no antioxidant activity.

4. Discussion

The acute toxicological evaluation revealed that oral administration of the ethanolic extract of both *C. sativus* and *Propolis* in doses up to 5000 mg/kg b.wt. did not produce any demonstrable acute toxic effect or death in all the groups of mice. Accordingly, it suggested that oral LD_{50} of the extract was higher than 5000 mg/kg b.wt. Therefore, the tested extracts can be categorized as highly safe since substances possessing LD_{50} higher than 50 mg/kg b.wt are non toxic (Buck et al., 1976). According to guidelines of the Organization for Economic Cooperation and Development (OECD, 2001); substances possessing LD_{50} dose of 2000 mg/kg b.wt. or higher are categorized as unclassified and hence our extracts are found to be non-toxic.

Concerning the acute toxicity and lethality study of *C. sativus* ethanolic extract our result is in agreement with Abdullaev et al. (2003). They found that oral administration of saffron extract at concentrations from 0.1 to 5 g/kg b.wt. was non-toxic in mice. Moreover, Khare (2007) set the recommended dose of saffron stigma and styles in human as 0.5–1.5 g/day, as the dose at 1.5–5 g is toxic. On the other hand, the present results of acute toxicity of *Propolis* are in agreement with Arvouet-Grand et al. (1993). They reported that oral LD_{50} of *Propolis* extract in mice was greater than 7.34 g/kg b.wt. in addition; Mohammadzadeh et al. (2007) showed that oral administration of hydroalcoholic solution of *Propolis* extract in rats at doses of (4.5, 9, 13 and 20 g/kg b.wt) has no toxic effects.

Administration of ethanolic extracts of *C. sativus* and *Propolis* and their combination in rats for 8 successive weeks showed no significant changes in the activity of serum transaminases, creatinine and blood urea nitrogen levels in all medicated groups, compared to the control. These data were supported by the absence of any abnormal histopathological findings.

In addition, the body weight gain throughout the experimental period showed insignificant difference in all extracts groups and their combination when compared to the control one. Our results are not in agreement with those obtained by Mohajeri et al. (2007). They reported that intraperitoneal administration of ethanolic extract of saffron to rats in doses of 0.35, 0.70 and 1.05 g/kg b.wt. for 2 weeks, significantly increased serum ALT, AST, urea and creatinine levels. They also recorded significant decrease in the body weight gain in a dose-dependent manner. Moreover, Modaghegh et al. (2008) reported that, no significant changes in serum urea or creatinine levels following administration of saffron stigmas tablets for 7 days in healthy volunteers.

Results of the present study are not in accordance with the findings of Newairy et al. (2009). They reported that oral administration of *Propolis* for 70 days; decreased the activities

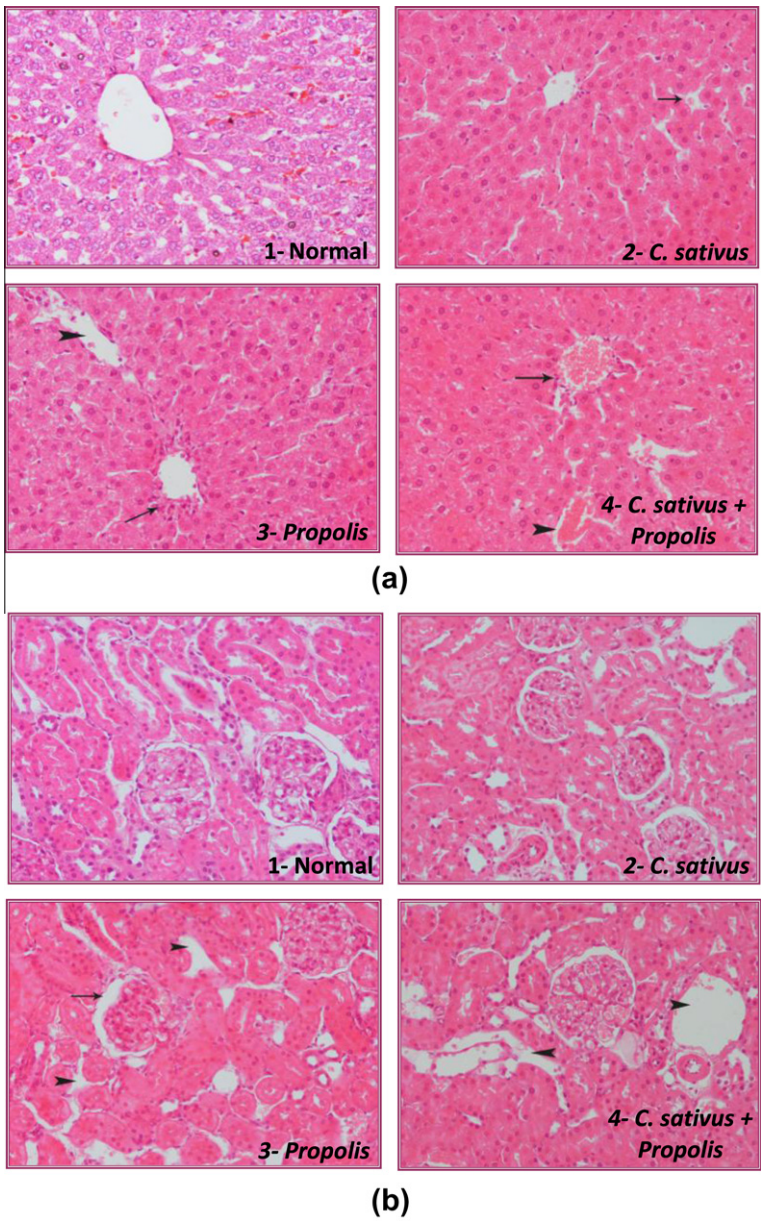

Figure 3 Histopathological examination of (a) liver and (b) kidney, (Hx.&E. X 200): (a-1) Photomicrograph of a section of liver tissue from a control rat showing normal structure. (a-2) Administration of *Crocus sativus*; showed mild dilatation and congestion of blood sinusoids (arrow). The central vein as well as hepatocytes appear normal in size and shape. (a-3) Administration of *Propolis*; showed noticeable dilatation of blood sinusoids (arrow head) denoting edema, together with cellular infiltrate around the central vein (arrow). The central vein itself shows no dilatation. (a-4) Administration of *Crocus sativus* and *Propolis* ethanolic extracts; revealed observable dilatation and congestion of blood sinusoids (arrow head). Also congestion of central vein is seen with mild cellular infiltrate around (arrow). (b-1) Photomicrograph of a section of renal tissue of a control rat showing showing normal structure. (b-2) Administration of *Crocus sativus*; showed normal tissue architecture, but with little widening in some tubules' lumen. (b-3) Administration of *Propolis*; showed widening in the urinary space of the Bowman's capsule (arrow) and in the interstitial tissue between the tubules (arrow head). (b-4) Administration of *Crocus sativus* and *Propolis* ethanolic extracts; revealed noticeable widening in the interstitial tissue in between the tubules (arrow head).

of AST and ALT in plasma but did not cause any significant change in urea and creatinine levels. Also, Sforcin et al. (2002) reported that treatment of rats with *Propolis* does not induce any alteration in AST level. Moreover, (Mani et al., 2006) found no alteration in AST value in the serum of *Propolis* treated rats for (30 or 90 or 150 days) at doses of (1, 3 and 6 mg/kg/day). There was significant increase in the relative weights of liver, in the *C. sativus* group and the combination group. Also there was significant increase in the relative weights of kidneys in all groups when compared to that of control group. Mohammadzadeh et al. (2007) recorded slight significant increase in liver weight as a ratio of body weight in rats

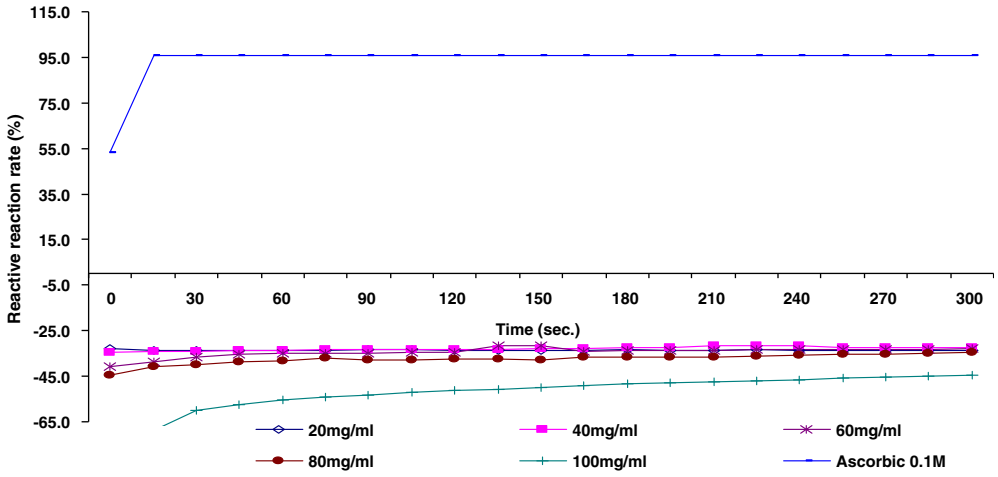

Figure 4 Antioxidant activity of ethanolic extracts of *Crocus sativus* and ascorbic acid (0.1 M concentration) in vitro, using DPPH radical scavenging activity method.

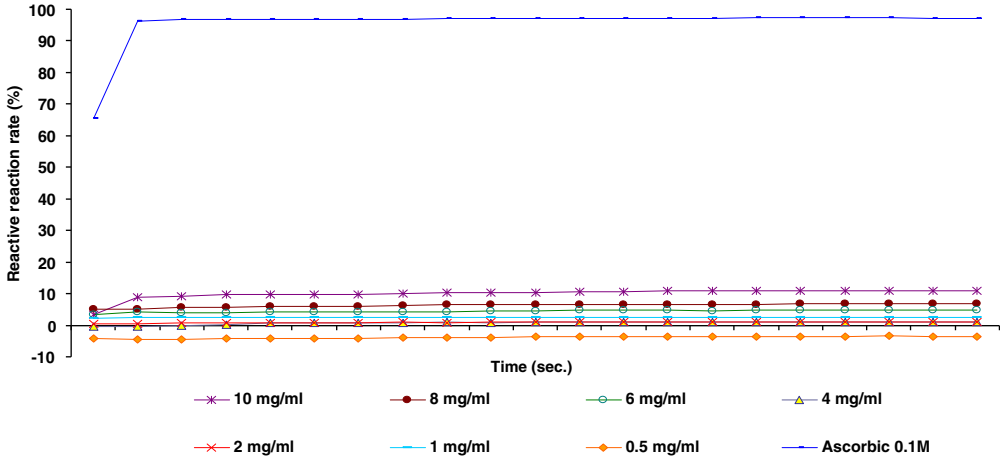

Figure 5 Antioxidant activity of ethanolic extract of *Crocus sativus* and ascorbic acid (0.1 M concentration) in vitro, using DPPH radical scavenging activity method.

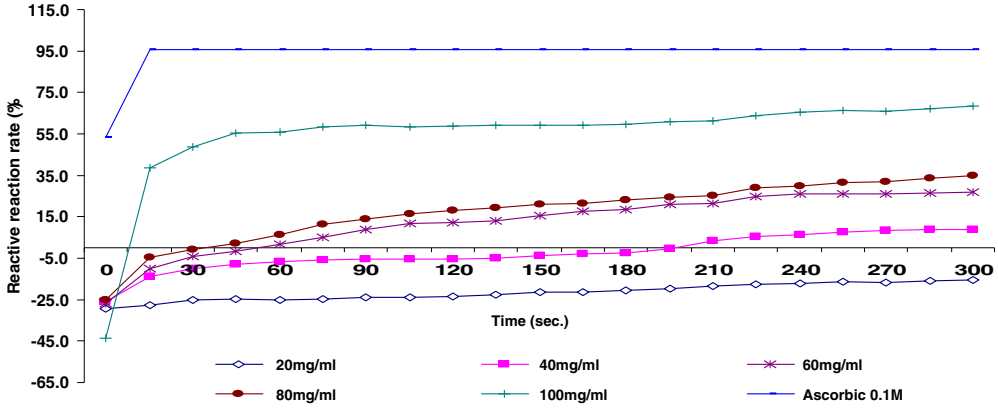

Figure 6 Antioxidant activity of ethanolic extract of *Propolis* and ascorbic acid (0.1 M concentration) in vitro, using DPPH radical scavenging activity method.

receiving *Propolis* hydroalcoholic solution containing 2 g/kg/day; for 9 successive weeks. Those findings were not in accordance to our results, which may be due to the difference in dose level used in their study and ours. In addition, no significant difference was observed in the amount of feed consumed weekly in *Propolis*-treated rats when compared to control

group. This finding was parallel to that of Mohammadzadeh et al. (2007).

Our results revealed that the ethanolic extracts of both *C. sativus* and *Propolis* either alone or in combination induced insignificant effect of on the food conversion ratio (FCR) when compared to control group. On the other hand, the histopathological examination of liver and kidney sections showed normal findings. These results are not in agreement with that obtained by Mohajeri et al. (2007) who reported that there were prominent hepatic and renal tissue injuries in saffron extract treated rats in doses up to 5 g/kg b.wt. intraperitoneally for 2 weeks. The difference between results may be attributed to the difference in dose and route of administration used. While our results are in agreement with those cited by Burdock (1998), no histological changes were observed when alcoholic extract of *Propolis* was administered in drinking water to rats in a 30-days study.

The variability in results from study to another was discussed by Raina et al. (1996) and Maggi et al. (2009) whose assured that the harvest time, processing temperature, storage and packaging of saffron, highly affects the quality of the final product. As during the drying process of saffron stigmas, significant modifications are observed in the color, taste and aroma. Since, different drying processes depending on the country of production result in different characteristics and qualities of saffron. Also Fogden and Neuberger (2003) cited that the active constituents themselves are influenced by a wide range of factors: harvest season, preparation methods, plant species, and location including altitude and climate, and quality control. Other concerns include the misidentification of herbs in a product either accidentally or deliberately, varying quality of herbs and contamination of products by heavy metals, chemicals (e.g. pesticides) or drugs (e.g. digitalis or corticosteroids).

Free radical-scavenging activity of both *C. sativus* and *Propolis* ethanolic extracts were evaluated, using a method based on the reduction of methanolic solution of DPPH (di-phenyl picryl hydrazil). *C. sativus* ethanolic extract in concentrations of (20, 40, 60, 80, and 100 mg/ml), showed no antioxidant activity compared to ascorbic acid. While in concentrations of (1, 2, 4, 6, 8, and 10 mg/ml) showed weak antioxidant activity. In this respect Assimopoulou et al. (2005) and Chen et al. (2008) found that methanolic and ethanolic saffron extracts exhibited appreciable scavenging properties against DPPH radicals, which were concentration dependant. They referred the significant antioxidant activity of the saffron extract to the synergistic action of its bioactive constituents, crocin and safranal. In addition to that Kanakis et al. (2009) tested the antioxidant activity of some saffron derivatives as safranal, crocetin, dimethylcrocetin using the DPPH antioxidant activity assay. The achieved results demonstrated that the free radical scavenging activity of crocetin was higher than that of dimethylcrocetin and by increasing their concentrations, a decrease in the antioxidant activity was observed. They explained this finding by the fact that higher concentrations of dimethylcrocetin or crocins show pro-oxidant effect since theoretically it could generate more radicals than it consumes.

In the present study, the ethanolic extract of *Propolis* exhibited a remarkable *in vitro* antioxidant activity at the different concentrations used. This finding is in agreement with Banskota et al. (2000) and Moreno et al. (2000) who reported that water and alcoholic *Propolis* extracts have scavenging activity towards DPPH free radical in a similar extent. This antioxidant activity was attributed to the flavonoid content of *Propolis* (Isla et al., 2001; Gardana et al., 2007; Xu et al., 2009). The DPPH test provides information on the reactivity of drugs with a stable free radical, but this test does not provide a clear-cut definition of antioxidant effect (Tseng et al., 1995). Therefore, further *in vivo* tests are required to investigate the possible antioxidant, hepatoprotective and/or hepatocurative effects of both *C. sativus* and *Propolis* ethanolic extracts.

5. Conclusion

Ethanolic extracts of both *C. sativus* and *Propolis* were harmless, as they did not cause any mortalities or signs of toxicity in mice when administered orally at doses up to 5 g/kg b.wt. Daily oral administration of *C. sativus, Propolis* ethanolic extracts and their combination for 8 successive weeks in rats was quiet safe and didn't cause any toxic changes in liver and kidney. Also no changes were recorded in body weight gain, food consumption and food conversion rate when compared to normal rats. *Propolis* showed remarkable *in vitro* antioxidant activity more than *C. sativus* at the concentrations under investigation.

Acknowledgements

Thanks to Dr. Nermeen M. Shaffie, National Research Center, Egypt, for her precious help and carrying out the histopathological investigations. Thanks to Dr. Ahmed H. El-Desoky, National Research Center – Egypt for carrying out the phytochemical investigations.

References

Abdullaev, F.I., Espinosa-Aguirre, J.J., 2004. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detection and Prevention 28, 426–432.

Abdullaev, F.I., Riverón-Negrete, L., Caballero-Ortega, H., Manuel Hernández, J., Pérez-López, I., Pereda-Miranda, R., Espinosa-Aguirre, J.J., 2003. Use of *in vitro* assays to assess the potential antigenotoxic and cytotoxic effects of saffron (*Crocus sativus* L.). Toxicology *in Vitro* 17, 731–736.

Akhondzadeh, S., Tahmacebi-Pour, N., Noorbala, A.A., Amini, H., Fallah-Pour, H., Jamshidi, A.H., Khani, M., 2005. *Crocus sativus* L. in the treatment of mild to moderate depression: a double blind, randomized and placebo-controlled trial. Phytotherapy Research 19, 148–151.

Arvouet-Grand, A., Lejeune, B., Bastide, P., Pourrat, A., Privat, A.M., Legret, P., 1993. Propolis extract. I. Acute toxicity and determination of acute primary cutaneous irritation index. Journal de Pharmacie de Belgique 48 (3), 165–170.

Assimopoulou, N., Sinakos, Z., Papageorgiou, V.P., 2005. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. Phytotherapy Research 19, 997–1000.

Banskota, A.H., Tezuka, Y., Adnyana, I.K., Midorikawa, K., Matsushige, K., Message, D., Huertas, A.A., Kadota, S., 2000. Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. Journal of Ethnopharmacology 72, 239–246.

Brady, W.L., 1968. Measurements of some poultry performance parameters. Veterinary Record 88, 245–260.

Buck, W.B., Osweiter, G.D., Van Gelder, A.G., 1976. Clinical and Diagnostic Veterinary Toxicology, second ed. Kendall/hunt Publishing Co., Iowa, p. 5211.

Burdock, G.A., 1998. Review of the biological properties and toxicity of bee propolis. Food and Chemical Toxicology 36 (4), 347–363.

Burits, M., Asres, K., Bucar, F., 2001. The antioxidant activity of the essential oils of Artemisia afra, Artemisia abyssinica and Juniperus procera. Phytotherapy Research 15, 103–108.

Carleton, H., 1976. Carleton's, Histological Technique, fourth ed. Oxford University Press, London, New York, Toronto.

Chavalittumrong, P., Chivapat, S., Attawish, A., Bansiddhi, J., Phadungpat, S., Chaorai, B., Butraporn, R., 2004. Chronic toxicity study of Portulaca grandiflora Hook. Journal of Ethnopharmacology 90, 375–380.

Chen, Y., Zhang, H., Tian, X., Zhao, C., Cai, L., Liu, Y., Jia, L., Yin, H.X., Chen, C., 2008. Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoids* ELLIS and *Crocus sativus* L.: a relationship investigation between antioxidant activity and crocin contents. Food Chemistry 109, 484–492.

Escribano, J., Alonso, G.L., Coca-Prados, M., Fernandez, J.A., 1996. Crocin, Safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells *in vitro*. Cancer Letters 100, 23–30.

Fogden, E., Neuberger, J., 2003. Alternative medicines and the liver. Liver International 23, 213–220.

Gardana, C., Scaglianti, M., Pietta, P., Simonetti, P., 2007. Analysis of the polyphenolic fraction of propolis from different sources by liquid chromatography–tandem mass spectrometry. Pharmaceutical and Biomedical Analysis 45 (3), 390–399.

Govindarajan, R., Rastogi, S., Vijayakumar, M., Shirwaikar, A., Rawat, A.K.S., Mehrotra, S., Pushpangadan, P., 2003. Studies on the antioxidant activities of Desmodium gangeticum. Biological and Pharmaceutical Bulletin 26 (10), 1424–1427.

Hosseinzadeh, H., Khosravan, V., 2002. Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigmas in mice. Achieve of Iranian Medicine 5, 44–47.

Hosseinzadeh, H., Younesi, H.M., 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacology 2, 1–8.

Hosseinzadeh, H., Karimi, G.H., Niapoor, M., 2004. Antidepressant effects of *Crocus sativus* stigma extracts and its constituents, crocin and safranal, in mice. Acta Horticulturae (ISHS) 650, 435–445.

Isla, M.I., Nieva, M.I., Sampietro, A.R., Vattuone, M.A., 2001. Antioxidant activity of Argentine propolis extracts. Journal of Ethnopharmacology 76 (2), 165–170.

Kanakis, C.D., Tarantilis, P.A., Pappas, C., Bariyanga, J., Tajmir-Riahi, H.A., Polissiou, M.G., 2009. An overview of structural features of DNA and RNA complexes with saffron compounds: models and antioxidant activity. Journal of Photochemistry and Photobiology B: Biology 95, 204–212.

Khare, C.P., 2007. Indian Medicinal Plants: An Illustrated Dictionary. Springer Science and Business Media edition, p. 178-179.

Kroll, M.H., Roach, N.A., Poe, B., Elin, R.J., 1987. Mechanism of interference with Jaffé reaction for creatinine. Clinical Chemistry 33, 1129–1132.

Liebermann, C., Burchard, H., 1890. Chem. Zent., 16: 1, through El Deeb, K.S. 1985. Chromatographic and thermal analysis of certain volatile oil containing drugs, Ph.D. Thesis; 47, Cairo University, Cairo.

Lorke, D., 1983. A new approach to acute toxicity testing. Archives of Toxicology 54, 275–287.

Maggi, L., Carmona, M., del Campo, C.P., Kanakis, C.D., Anastasaki, E., Tarantilis, P.A., Polissiou, M.G., Alonso, G.L., 2009. Worldwide market screening of saffron volatile composition. Journal of the Science of Food and Agriculture 89, 1950–1954.

Mani, F., Damasceno, H.C.R., Novelli, E.L.B., Martins, E.A.M., Sforcin, J.M., 2006. Propolis: effect of different concentrations, extracts and intake period on seric biochemical variables. Journal of Ethnopharmacology 105, 95–98.

Modaghegh, M.-H., Shahabian, M., Esmaeili, H.-A., Rajbai, O., Hosseinzadeh, H., 2008. Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers. Phytomedicine 15, 1032–1037.

Mohajeri, D., Mousavi, G., Mesgari, M., Doustar, Y., Nouri, M.H.K., 2007. Subacute toxicity of *Crocus sativus* L. (saffron) stigma ethanolic extract in rats. American Journal of Pharmacology and Toxicology 2 (4), 189–193.

Mohammadzadeh, S., Shariatpanahi, M., Hamedi, M., Ahmadkhaniha, R., Samadi, N., Ostad, S.N., 2007. Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. Food Chemistry 103, 1097–1103.

Molisch, H., 1886: Monatsh. Chem., 7, through Stank, J., Gerny, M., Kocourek, J., Pacak, J., 1963. The Monosaccharides, 872, Publishing House of Czechoslovak Academy of Science, Prague.

Moreno, M.I.N., Isla, M.I., Sampietro, A.R., Vattuone, M.A., 2000. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. Journal of Ethnopharmacology 71, 109–114.

Najafi, M.F., Vahedy, F., Seyyedin, M., Jomehzadeh, H.R., Bozary, K., 2007. Effect of the water extracts of propolis on stimulation and inhibition of different cells. Cytotechnology 54, 49–56.

Neu, R., 1956. A new reagent for differentiating and determining flavones on paper chromatograms. Naturwissenschaften 43, 82.

Newairy, A.A., Salama, A.F., Hussien, H.M., Yousef, M.I., 2009. Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food and Chemical Toxicology 47 (6), 1093–1098.

OECD (Organization for Economic Cooperation and Development), 2001. OECD guideline for testing of chemicals. At <http://www.oecd.org>.

Peiwu, L., Hopia, A., Jari S., Yrjönen, T., Vuorela, H., 1999. TLC method for evaluation of free radical scavenging activity of Rapeseed meal by video scanning technology. In: Proceedings of the Tenth International Rapessed Congress, Canberra, Australia.

Raina, B.L., Agarwal, S.G., Bhatia, A.K., Gaur, G.S., 1996. Changes in pigments and volatiles of saffron (*Crocus sativus* L.) during processing and storage. Journal of the Science of Food and Agriculture 71, 27–32.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminase. American Journal of Clinical Pathology 28, 56–63.

Ríos, J.L., Recio, M.C., Giner, R.M., Máñez, S., 1996. An update review of saffron and its active constituents. Phytotherapy Research 10, 189–193.

Salatino, A., Teixeira, E.W., Negri, G., Message, D., 2005. Origin and chemical variation of Brazilian propolis. Evidence-Based Complementary and Alternative Medicine 2 (1), 33–38.

Schmidt, M., Betti, G., Hensel, A., 2007. Saffron in phytotherapy: pharmacology and clinical uses. Wien Med Wochenschr 157, 315–319.

Sforcin, J.M., 2007. Propolis and the immune system: a review. Journal of Ethnopharmacology 113, 1–14.

Sforcin, J.M., Novelli, E.L.B., Funari, S.R.C., 2002. Seasonal effect of Brazilian Propolis on seric biochemical variables. Journal of Venomous Animals and Toxins 8 (2), 244–254.

Smith, I., 1960. Chromatographic and Electrophoretic Techniques, London, Heinemann.

Stahl, E., 1969. Thin Layer Chromatography, second ed. Springer Verlag, Berlin, Heidelberg, New York.

Tseng, T.H., Chu, C.Y., Huang, J.M., Shiow, S.J., Wang, C.J., 1995. Crocetin protects against oxidative damage in rat primary hepatocytes. Cancer Letters 97 (1), 61–67.

Wills, M.R., Savory, J., 1981. Biochemistry of renal failure. Annals of Clinical and Laboratory Science 11 (4), 292–299.

Xu, Y., Luo, L., Chen, B., Fu, Y., 2009. Recent development of chemical components in propolis. Frontiers of Biology in China 4 (4), 385–391.