EVALUATION OF RADIOPROTECTIVE EFFECTS OF SOME BEE PRODUCTS AND THEIR FLAVONOID CONSTITUENTS: *IN VIVO* STUDY ON MALE RATS

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Abstract. Radioprotection with natural products may be relevant to the mitigation of ionizing radiation-induced damage in mammalian systems. This study was designed to investigate antioxidant activity of honey and propolis *in vitro* through determination of total phenol (*TP*), total flavonoid (*TF*) and free radical scavenging activity (*RSA*). In addition to, in an *in vivo* study, male rats were exposed to fractionated dose gamma irradiation (1 Gy every day up to 5 Gy total doses). Honey and propolis were administered at dose 250 and 90 $mg \cdot kg^{-1} \cdot day^{-1}$. The serum levels of alanine transaminase (ALT) and aspartate transaminase (AST), urea, creatinine and total antioxidants capacity were estimated. Also hemoglobin of rats was investigated through UV absorption spectrum and dielectric measurements. The results indicated that total flavonoid, total phenol and free radical scavenging activity of propolis were greater than honey. AST, ALT, creatinine and urea significantly increased while total antioxidants significantly decreased after irradiation. Moreover, the absolute values of permittivity ε', dielectric loss ε'' and ac-conductivity σ_{ac} increased in addition to a pronounced decrease in the absorbance at Soret band after irradiation compared to control group. Administration of propolis induced a significant recovery of antioxidant balance in rats exposed to ionizing radiation. Indeed, decrease of AST, ALT, creatinine and urea levels decreased in these animals while total antioxidants significantly increased. Also, the values of ε', ε'' and σ_{ac} were nearly close to those of the control group compared to those treated with honey. Finally, the average value of peak height of Soret band was significantly increased compared to irradiated rat. It can be concluded that propolis can be more effective than honey in the protection against oxidative damage induced by ionizing radiation. Further investigations are required to elucidate the mechanisms of propolis and honey actions.

Key words: Gamma irradiation, honey, propolis, total phenol, total flavonoid, antioxidants.

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INTRODUCTION

Gamma irradiation treatment increases the annual dose received by both the patients and physicians because of regular exposure to radiation. Therefore, studying the biological effects induced by ionizing radiation is necessary in the assessment of maximum absorbed dose during radiotherapy or diagnosis. Moreover, development of protective agents presented new solutions for recovery of undesired tissue damage induced by ionizing radiation [10]. Normal cellular function depends on a balance between the reactive oxygen species (ROS) produced and the antioxidant defense mechanisms available for the cell. This equilibrium is hampered by the ROS upsurge that culminates in oxidative stress [20]. Oxidative stress refers to disrupted redox equilibrium between the production of free radicals and the ability of cells to protect against damage caused by these species. Defense against oxidative stress is maintained by using several mechanisms which include antioxidant machinery [27]. ROS arise as by-products of normal cellular metabolism [33] or as a consequence of exposure to some chemicals and/or ionizing radiation [26]. Consequently, the cellular antioxidant capacity is decreased and organs become more susceptible to deleterious effects of ROS [16]. A great deal of research has been carried out on the radioprotective action of some chemical substances. These substances have shown to reduce mortality when administered to animals prior to exposure to a lethal dose of radiation. Most of these chemical radioprotectors have shown toxic side effects that limit their use in medical practice [7]. Radioprotection with natural products has several advantages since they are non-toxic with proven therapeutic benefits. Body endogenous protective system can be supported by natural antioxidant compounds provided from food [55]. Recently, identification and isolation of new antioxidants from natural sources has become an active area of research, as a number of natural products, such as flavonoids, phenolics or terpenes, isolated from plants and food have shown potent antioxidant activity [39].

Many biological properties have been attributed to various types of bee products such as honey and propolis, including anti-inflammatory, antimicrobial, antioxidant, antitumor, wound healing, and immunomodulatory activities [41].

Honey is a sweet, viscous fluid, elaborated by bees from the nectar of plants and stored in their combs as food. Honey contains about 0.5% proteins, mainly enzymes and amino acids [9]. Honey is readily available, affordable and well accepted by patients making it useful for improving the quality of life in irradiated patients [30]. It is widely available in most communities, although its mechanism of action of several of its properties remains obscure and needs further investigation.

Propolis is a resinous substance collected from various plants by bees. It is used in the construction of, and to seal the cracks in, the bee hive. Chemical properties of propolis are not only beneficial to bees but have general pharmacological value as a natural mixture [42]. It is a mixture of resins, essential oils and waxes, and also contains amino acids, minerals, ethanol, vitamins A, B complex, E, and flavonoids [7]. More than 200 constituents have been identified so far from propolis: phenolic acids and their esters, caffeic acid and their esters, phenolic aldehydes and ketones; moreover, proteins, amino acids, vitamins (A, B_1, B_2, B_3 and biotin), minerals (calcium, phosphorus, magnesium, manganese, iron, zinc, silicon, potassium, cobalt and copper) [3]. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. Their antioxidant effectiveness depends on the stability in different systems, as well as the number and location of hydroxyl groups. In many *in vitro* studies, phenolic compounds demonstrated higher antioxidant activity than antioxidant vitamins and carotenoids [46]. Flavones are able to interact with free radicals and substances produced by oxidative stress [26, 36]. Flavonoids (including flavones, flavonols, flavanones and dihydroflavonols) and other phenolics (mainly substituted cinnamic acids and their esters) are the main active constituents of propolis and possess potent antioxidant activities [24].

This study was planned to evaluate modulatory effect propolis and honey on ionizing radiation mediated oxidative stress leading to normal tissues damage during radiotherapy and other radiation exposures.

Comparative study of the antioxidant properties of some bee products, honey and propolis, was designed in an *in vitro* and *in vivo* study. In the *in vitro* experiments, the total phenolic, total flavonoid and free radical scavenging activity of both products have been done. The *in vivo* study was performed on the irradiated rats through biophysical measurements that include UV absorption spectrum and dielectric measurements of hemoglobin of rat and biochemical measurements which include: the serum levels of ALT, AST, blood urea nitrogen, creatinine and total antioxidants.

MATERIALS AND METHODS

MATERIALS

Honey samples

Three honey samples were collected. The first (H_1) from El Fayoum area while the other two samples (H_2, H_3) from Agriculture Research Center, Giza,

Egypt. Honey diluted with water and administered orally to animals at a dose of 250 mg/kg in a volume of about 1 mL/rat.

Propolis extraction

Two propolis samples were collected. The first one from the Agriculture Research Center – Giza, Egypt (P_1) while the second sample from local supermarket (P_2). Propolis was extracted with ethanol about 10 g of propolis added to 100 mL of 70% ethanol solution. The resultant solution was filtered through Whatman No: 1 filter paper. The extract was completely evaporated under reduced pressure. Propolis was freshly prepared and administered to animals orally at a dose of 90 mg/kg.

In vivo experimental design

Sixty male albino rats weighing 150–180 g were used in this study. Animals were obtained from the National Research Center (Giza, Egypt). All the procedures used in handling the animals and the entire *in vivo* experimental protocol have been designed according to the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt. Animals were maintained under standard conditions of water and diet supply. After two weeks of acclimatization, animals were divided into two groups divided into 6 subgroups (n = 10 each) according to the treatment and requirements of the experiment. The period of the experiment was 29 days.

Group 1: control group which was divided into:

N: Normal control sub group, rats in this group were neither treated nor irradiated.

H: Honey control subgroup; rats in this group were administered honey orally at a dose (250 mg·kg^{-1}) for two weeks.

P: propolis control subgroup; rats in this group were administered propolis orally at a dose (90 mg·kg^{-1}) for two weeks.

Group 2: Irradiated group which was divided into:

R: Control irradiated subgroup; the whole body of rats was exposed to gamma radiation with a fractionated dose (1 Gy every day up to 5 Gy total doses).

HR: Honey treated-irradiated subgroup; irradiated rats were administered honey at dose (250 mg·kg^{-1}) for 10 days before irradiation exposure and 5 day during irradiation.

PR: Propolis treated-irradiated subgroup; irradiated rats were administered propolis at a dose (90 mg·kg^{-1}) for 10 days before irradiation exposure and 5 day during irradiation.

Irradiation source

The irradiation source used was cobalt-60 (gamma-cell 220), Atomic Energy of Canada Limited, installed at the Middle Eastern Regional Radioisotopes Center for the Arab Countries, Dokki, Cairo. This source provided an average exposure rate of 3.1 Gy per minute in the center of the cage of the machine of irradiation.

METHODS

Determination of total polyphenols

Phenolic compounds (*TP*) were estimated using a modified Folin-Ciocalteu method [56]. Folin-Ciocalteu's phenol reagent (Alpha Chemical, India) was used. Gallic acid (Sigma Aldrich, Egypt) was used to generate the standard curve. Samples were analyzed in triplicate; the results were expressed as mean ± standard deviation and presented in milligrams of gallic acid equivalents (GAEs).

Determination of total flavonoids

The *TF* content of honey and propolis was determined according to the colorimetric assay developed by Zhishen *et al*. [64]. Aluminum chloride in ethanol solution 20% (S D Fine-Chem Limited, India) was used. A calibration curve was prepared using standard solutions of catechin (Sigma Aldrich, Egypt). The results were expressed as mg catechin equivalents (CEQ).

Free radical-scavenging activity

The antioxidant activity of honey and propolis was studied by evaluating the free radical-scavenging effects on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Sigma Aldrich, Egypt). The assay was based on the method proposed by Ferreira *et al*. [18]. The reduction of the DPPH radical was determined by measuring the absorbance at 517 nm by UV-160-IPC (Shimadzu, North America) spectrophotometer [23].

The *RSA* was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A_{DPPH} - A_S) / A_{DPPH}] \times 100 \quad (1)$$

where A_S is the absorbance of the sample solution and A_{DPPH} is the absorbance of the DPPH solution.

Blood collection

Each experimental rat was anaesthetized with diethyl ether and then blood samples will be withdrawn from orbital venous plexuses at different time intervals

1st, 7th and 14th days post last dose of irradiation exposure. For serum separation, blood samples were collected, left to clot and then centrifuged at 3000 rpm for 15 minutes. Serum was stored at –80 °C for evaluating the biochemical parameters.

For hemoglobin extraction, blood samples were collected in heparinized tube to prevent blood clotting. The hemolysate from the washed erythrocytes was prepared by a modification of the method of Travelled and colleagues [58]. Heparinized blood samples taken from rats were centrifuged at 3000 rpm for 20 min, then the supernatant plasma were removed and packed cells were washed three times with two volumes of physiological saline (0.9% NaCl) and the washing saline was removed after each washing. Packed cells were lysed with de-ionized water and then the mixture was centrifuged at 6000 rpm at 4 ºC for 45 min in order to obtain hemoglobin solution in the supernatant.

Biochemical analysis

ALT and AST activities in the serum were measured by colorimetric assay according to Reitman and Frankel [47]. Urea and creatinine in the serum were determined according to Kaplan [25] and Murray [43]. Total antioxidant capacity was determined according to Koracevic *et al*. [32] using a commercial kit (Biodiagnostic Company, Egypt).

Biophysical analysis

Hemoglobin spectrum. Hemoglobin spectrum was recorded by the UV/visible spectrophotometer Jasco V-570 (Jasco, Germany) in the 250 nm to 700 nm range.

Dielectric measurement. Dielectric measurements were done in the frequency range of 100 Hz to 100 kHz using LCR meter type AG-411 B (Ando Electric, Japan). The measuring cell has two squared platinum black electrodes of area (1×1) cm^2.

The relative permittivity ε', loss tangent $\tan\delta$, dielectric loss ε", electrical conductivity σ_{ac} of the hemoglobin sample was calculated at each frequency with the following formulas:

$$\varepsilon' = \frac{C\,d}{\varepsilon_0\,A} \tag{2}$$

$$\tan\delta = \frac{1}{2\pi f R C} \tag{3}$$

$$\varepsilon'' = \varepsilon' \tan\delta \tag{4}$$

$$\sigma_{ac} = 2\pi f \varepsilon'' \cdot \varepsilon_0 \quad (5)$$

where C is the capacitance of the specimen, d is the interelectrode distance, A is the area of electrode, f is the applied frequency in Hz, R is the resistance of the specimen, and ε_0 is the permittivity of free space, which equals 8.85×10^{-12} F/m.

To eliminate the contribution of electrode polarization and direct current conductivity occurring in the lower frequency, the electric modulus (M*) has been taken into consideration [21]. This is defined as

$$M^* = M' + M'' \quad (6)$$

$$M' = \varepsilon' \Big/ \left(\varepsilon'^2 + \varepsilon''^2\right) \quad (7)$$

$$M'' = \varepsilon'' \Big/ \left(\varepsilon''^2 + \varepsilon'^2\right), \quad (8)$$

where M', M'' are the real and imaginary parts of the electric modulus.

STATISTICAL ANALYSIS

In *in vivo* study the data were expressed as mean ± standard error (SE) of ten replicate determinations. Statistical analysis was performed using two-way analysis of variance (ANOVA) to assess significant differences among different groups [57]. The results are considered to be significant when $P < 0.05$. All statistical analyses were performed using SPSS software program version 17 (SPSS® Inc, USA).

RESULTS AND DISCUSSION

TOTAL PHENOL, TOTAL FLAVONOID AND ANTIOXIDANT ACTIVITY

The *TP* and *TF* values found for various honey samples given in Table 1 indicated that *TP* ranged between 69.15–128.5 mg GAE/100 g honey while *TF* ranged between 3.03–4.67 mg (CEQ) / 100 g honey. *TP* content of various propolis studied samples ranged between 113.7–121.6 mg GAE/g propolis while *TF* of propolis ranged between 118.3–124.5 mg (CEQ)/g propolis. Polyphenols, especially flavonoids and phenolic acids, are known to play an important role as antioxidants and honey or propolis are regarded as an important source of these compounds [60]. The presence and concentrations of these compounds in honeys and propolis can vary depending upon the floral source, the geographical and climatic conditions [12, 62]. Table 1 also summarizes the percentage of DPPH

degradation with 60 µg/mL of different samples of honey and propolis. Figure 1 shows the dose response curve for the free radical scavenging activity of different diluted honey and propolis samples that increased with the increase in the concentration of the sample. This result indicated that the percentage of antioxidant of propolis is higher than honey. This finding also demonstrated that *TP* and *TF* correlated very well with antioxidant activity. This is similar to the previously reported data [49, 63]. Flavonoids have been reported to be the most abundant and the most effective antioxidant in propolis [2], antioxidant activity of flavonoid is attributed to the presence of phenolic hydroxyl groups in flavonoid structure [53, 62].

Table 1

Total phenol, total flavonoid contents and antioxidant activity of honey and propolis. The values of *TP* and *TF* expressed as mg/100 g for honey and mg/g for propolis. The results are expressed as mean ± SD

Sample	Total phenol	Total flavonoid	%*RSA*
Honey			
H_1	128.5±0.16	4.67±0.23	42.94±0.21
H_2	111.89±0.1	4.58±0.65	48.47±0.21
H_3	69.15±0.17	3.03±1.09	52.55±0.28
Propolis			
P1	121.6±0.31	124.5±0.28	87.2±0.31
P2	113.7±0.29	118±0.41	83.6±0.17

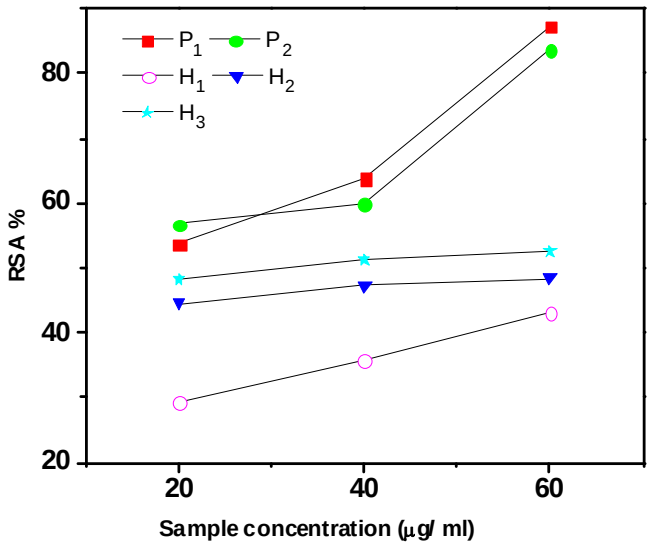

Fig. 1. Dose response curve for the free radical scavenging activity of different honey and propolis samples.

BLOOD PARAMETERS

The data of ALT, AST, creatinine and urea were summarized in Figures 2–5. The results indicated that these parameters were significantly increased in rats exposed to γ irradiation compared with the normal subgroup. However, the same parameters in rats receiving honey or propolis extract alone (subgroups H and P respectively) were significantly lower than the irradiated subgroup. Pretreatment with honey or propolis for 10 days before and 5 days during exposure to γ irradiation, induced a significant decrease in these parameters when compared to irradiated rats at all interval times on 1st, 7th and 14th day. A two way ANOVA analysis on these data reveals in the case of ALT that the treatment produced significantly different responses in the investigated variants ($F = 7.149$, $P < 0.05$) since the time after the irradiation has not influenced the responses ($F = 0.053$, $P = 0.948$), no significant interaction between factors was detected ($F = 0.10$, $P = 0.534$) (Fig. 2). Also for AST the treatment produced significantly different responses in the investigated variants ($F = 23.87$, $P < 0.05$) and the time after irradiation produced significantly different responses ($F = 5.525$, $P < 0.05$) and no significant interaction between factors was detected ($F = 0.712$, $P = 1.0$) (Fig. 3). As indicated in Figure 4, two-way ANOVA demonstrated a significant effect of treatment responses on creatinine levels ($F = 8.455$, $P < 0.05$), but not on the time after irradiation ($F = 0.952$, $P = 0.389$). In addition, no interaction between factors was detected ($F = 0.661$, $P = 0.758$). As well as, the data indicated that the treatment produced significantly different responses on urea levels ($F = 28.633$, $P < 0.05$) but no significant time after irradiation ($F = 0.118$, $P = 0.889$) (Fig. 5). Whereas, the data revealed that a significant interaction between treatments and time in the case of urea produced significantly different responses ($F = 5.534$, $P < 0.05$). Also as shown in Figure 6, γ irradiation markedly decreased total antioxidant capacity to 36.73%, 72 % and 55.84 % of the normal value after the 1st, 7th and 14th days of the last exposure. These percentage changes were calculated by the following equation:

$$\% = M_2 / M_1 \times 100, \tag{9}$$

where M_1 means of control rats, M_2 means of irradiated rats.

This means that after irradiation total antioxidant decreases with time interval. On the other hand, pretreatment with honey or propolis maintained the antioxidants close to the control level (the honey a little bit under the control and the propolis above the control) and provided significant protection against the damaging effects of radiation on the antioxidants activities. Indeed, the ANOVA analysis proves a significant influence of treatment ($F = 5.341$, $P < 0.05$), but not of the time after irradiation ($F = 0.271$, $P = 0.763$) and of the interaction between these two factors ($F = 0.369$, $P = 0.957$).

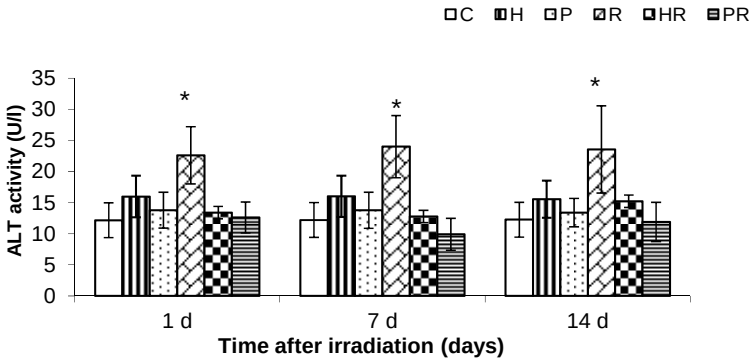

Fig. 2. Serum ALT activity (U/L) of irradiated rats treated with honey or propolis extract compared to normal one. Values are expressed as means ± SE for each treatment group. *Significantly different with respect to control group ($P < 0.05$).

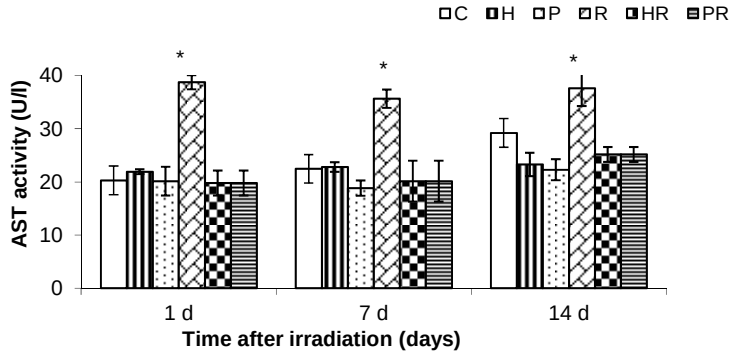

Fig. 3. Serum AST activity (U/l) of irradiated rats treated with honey or propolis extract compared to normal one. Values are expressed as means ± SE for each treatment group *Significantly different with respect to control group ($P < 0.05$).

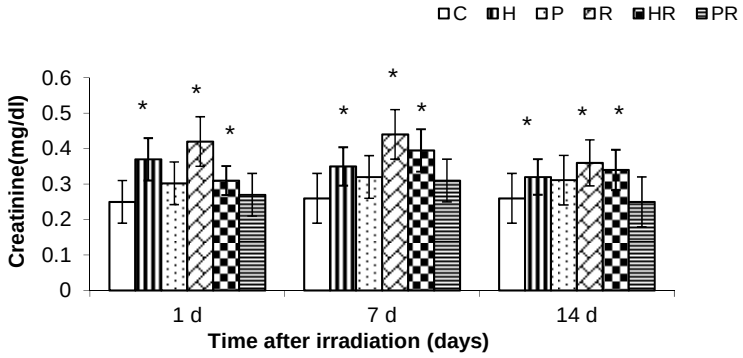

Fig. 4. Serum creatinine level (mg/dL) of irradiated rats treated with honey or propolis extract compared to normal one. Values are expressed as means ± SE for each treatment group. *significantly different with respect to control group ($P < 0.05$).

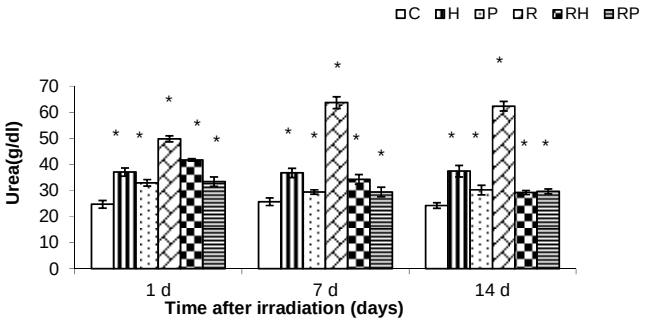

Fig. 5. Serum urea level (g/dL) of irradiated rats treated with honey or propolis extract compared to normal one. Values are expressed as means ± SE for each treatment group. All the variants are *significantly different comparing to the control ($P < 0.05$).

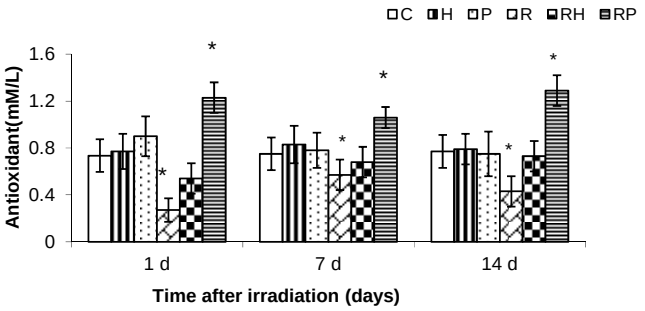

Fig. 6. Serum antioxidant concentration (mM/L) of irradiated rats treated with honey or propolis compared to normal one. Values are expressed as means ± SE for each treatment group. *Significantly different from control group ($P < 0.05$).

The rise in both AST and ALT levels is considered to be one of the most familiar indicators of hepato-cellular damage [48]. On the other hand, increasing antioxidant capacity plays an important role as hepatoprotective [44]. Esters of phenolic acids in propolis have been recognized as hepato protective agents [29]. These observations are similar to the previously reported data [6, 38]. Exposure to radiation decrease level of enzymatic antioxidant in plasma consequently the cellular antioxidant capacity decreased and the organs become more susceptible to deterious effects of free radicals [15, 16]. Our results indicated cytoprotection induced by both honey and propolis. In all propolis-pretreated animals (for all the time of measurements) the antioxidant activity is higher than in the control. So propolis pretreatment not only recovered the antioxidant capacity, but even increases antioxidant enzyme activities. Instead, honey only succeeded to recover it for the 7 and 14 day as shown in Figure 6.

The protective effect of propolis against ionizing radiation could be explained by both the direct scavenging of free radicals [14] and the activation of oxidative repair enzymes [5]. Although honey is not a major source of antioxidants it has also the ability to scavenge free radicals [59]. It has been suggested that therapeutic activities of honey and propolis depend mainly on the presence of flavonoids [4, 14]. These flavonoids may be able to suppress the formation of free radicals by binding to heavy metal ions which are known to catalyze many processes leading to the generation of free radicals [13]. Another study [31] reported that the treatment with propolis significantly prevented the release of transaminases and significantly enhanced protein towards control, suggesting its hepatoprotective potential.

In this study, nephrotoxicity was manifested by inhibition of kidney function as indicated by increased serum creatinine and urea levels in irradiated group compared to normal subgroup. These results are supported by similar findings [19, 52]. Increase in serum urea was due to increase in glutamate dehydrogenase enzyme as a result of irradiation and this may increase carbamoyl phosphate synthetase activity leading to increase in urea concentration [45]. The increased serum creatinine in the irradiated group indicates development of nephritis and renal dysfunction, a result in agreement with [8, 11].

In all honey and propolis pretreated animals (for all the time of measurements) serum creatinine and urea levels remained close to normal. The mechanism by which the natural products honey and propolis prevents renal oxidative stress may include an increasing rate of Glutathione (GSH) or by induction of its synthesis or by a scavenger effect. Instead of the toxic reactive metabolites binding to glutathione and consume, they will be captured by the flavonoids (naringenin, pinostrombin and galangin). So there is great interest in the clinical roles of propolis [40].

HEMOGLOBIN INVESTIGATION

Absorption spectra

Absorption spectra for hemoglobin (Hb) extracted from the animals of different subgroups are illustrated graphically in Figures 7A-D at wavelength range 250–700 nm the obtained bands which characterize hemoglobin are as follows: 578 nm (hem-hem interaction band), 540 nm (Fe-N in porphyrine) nitrogen iron bonds in porphyrine, 414 nm (Soret band), 340 nm (globin-hem interaction band) and 275 nm (protein band). These wavelengths are comparable with those found in literature [22, 37, 54].

The average values of peak height of Soret band and the absorption ratios of A_{578}/A_{540} in the absorption spectra for hemoglobin extracted from the animals of the six subgroups were calculated and given in Table 2.

As shown in Figure 7A, no detectable change was observed in absorption spectra for hemoglobin extracted from the animals of control subgroups.

From Figures 7B-D, great differences were detected in heme parts at visible wavelength for hemoglobin extracted from the animals exposed to γ-irradiation at 1st, 7th and14th day after irradiation. The average value of peak height of Soret band and A_{578}/A_{540} ratio were significantly decreased compared with normal subgroup.

These results indicate a partial loss of Hb molecule stability [37]. Irradiation disrupted the heme groups, resulting in decrease of the absorbance at sort band. It causes a slight breakdown of the polypeptide chain break covalent bonds and disrupts the ordered structure of proteins [34, 50] as a result of the increase in the free radical production [17, 35]. These free radicals contribute to hemoglobin denaturation and precipitation, leading to anemia [37]. Also these free radicals deplete levels of known antioxidant [16]. This promoted oxy hemoglobin to meet hemoglobin [22, 50].

Table 2

The average values of peak height and peak position of Soret band and the absorption ratios of A_{578}/A_{540} of animals from the six subgroups. Data expressed as mean ± SE, $n = 3$, *significantly different with respect to control ($P < 0.05$).

Group	**Peak height**	**Peak position (nm)**	**A_{578}/A_{540}**
Control			
N	2.510±0.006	414±2	1.008±0.004
H	2.545±0.022	414±2	1.000±0.018
P	2.568±0.199	415±1*	1.020±0.002
Irradiated group at 1st day			
R_{1d}	2.015±0.040*	417±1*	0.983±0.022*
HR_{1d}	2.255±0.001*	417±2*	1.020±0.011
PR_{1d}	2.347±0.200*	416±2 *	1.035±0.020 *
Irradiated group at 7th day			
R_{7d}	1.959±0.079*	417±1*	0.980±0.008 *
HR_{7d}	2.091±0.105 *	417±0 *	1.004±0.032
PR_{7d}	2.187±0.150 *	415±1*	1.033±0.010*
Irradiated group at 14th day			
R_{14d}	1.700±0.110*	418±0*	0.980±0.018*
HR_{14d}	1.903±0.120 *	418±0*	0.997±0.001
PR_{14d}	2.008±0.150 *	416±2 *	1.014±0.016*

Administration of honey and propolis was determined to alleviate some sort of these effects. This finding was achieved by the studied parameters given in Table 2 for the investigated animals treated with honey and propolis at time intervals 1st, 7th and 14th day after last exposure. A two way ANOVA analysis on these data reveals in the case of peak height that the treatment produced significantly different responses in the investigated variants ($F = 6.564$, $P < 0.05$) since the time after the irradiation has not influenced the responses ($F = 1.684$, $P = 0.196$), no significant interaction between factors was detected ($F = 0.534$, $P = 0.330$). Also in the case of peak position the treatment produced significantly different responses in the investigated variants ($F = 18.055$, $P < 0.05$) while the time after the irradiation has not influenced the responses ($F = 0.163$, $P = 0.851$). As well as, the data indicated the treatment produced significantly different responses on A_{578}/A_{540} ($F = 5.042$, $P < 0.05$) but not significant of time after irradiation ($F = 1.170$, $P = 0.319$). In addition, no interaction between factors was detected ($F = 0.263$, $P = 1.01$). Although honey dose was greater than propolis the results showed that propolis could be more effective than honey. Propolis acts as direct free radical scavenger and detoxifies the highly cytotoxic $OH^{\bullet}$ and other radicals produced by ionizing radiation [24].

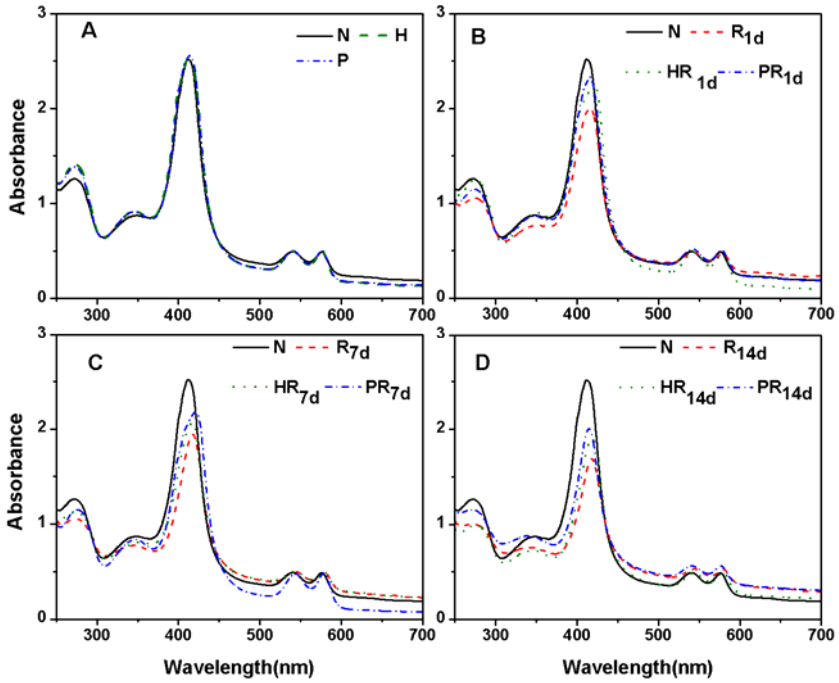

Fig. 7. Absorption spectra of hemoglobin extracted from animals of (A) control group. (B), (C) and (D) irradiated group at 1st, 7th and 14th day post irradiation.

Dielectric measurements

The dielectric permittivity ε', the dielectric loss ε'' as well as the electric conductivity σ_{ac} are studied in the frequency range 102–105 Hz for hemoglobin extracted from the animals of different groups. The data obtained were illustrated graphically in Figures 8–11. The data given in Figure 8 for the hemoglobin extracted from the animals of normal subgroup (N) indicated that the conductivity σ_{ac} is frequency dependent and shows step like increase towards higher frequency and it is a mirror image to dielectric permittivity ε' that shows step like decrease towards higher frequency, then pass into the plateau and shows an anomalous dispersion [28, 37, 61]. On the other hand, dielectric loss ε'' shows a strong decrease with frequency that appears as a loss of peak at a low frequency range due to dc conductivity.

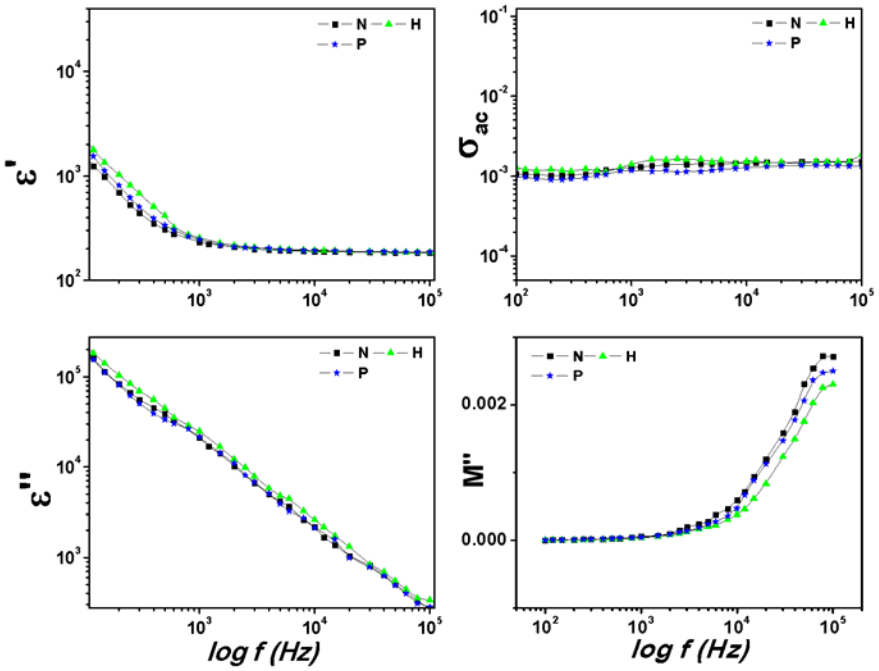

Fig. 8. The variation of dielectric permittivity ε', dielectric loss ε'', electrical conductivity σ_{ac} and imaginary part of electric modulus *M*'' as a function of the frequency for hemoglobin extracted from animals of control group.

The data of ε', ε'' and σ_{ac} given for hemoglobin extracted from animals of control group and γ-irradiated group at time interval 1st, 7th and 14th day after

irradiation were illustrated graphically in Figures 8–11. No detectable changes were noticed in the animals from the control group as shown in Figure 8. On the other hand, it is noticed that the absolute values of ε', ε" and σ_{ac} slightly increased for γ-irradiated group. This can be ascribed to the increase of free radicals which are expected to be formed by exposing to γ-irradiation that leads to an increase in the conductivity [58]. These free radicals cause damage to hemoglobin molecule resulted in hemoglobin viscosity decrease after irradiation [51]. Also the administration of honey and propolis alleviate the toxic effects of these free radicals on 1st, 7th and 14th day. These results indicate that the ε', ε" and σ_{ac} at the whole frequency range increase in the manner R > HR > PR > N. The protective effect of these materials is due to the presence of flavonoid and phenol which act as antiradical.

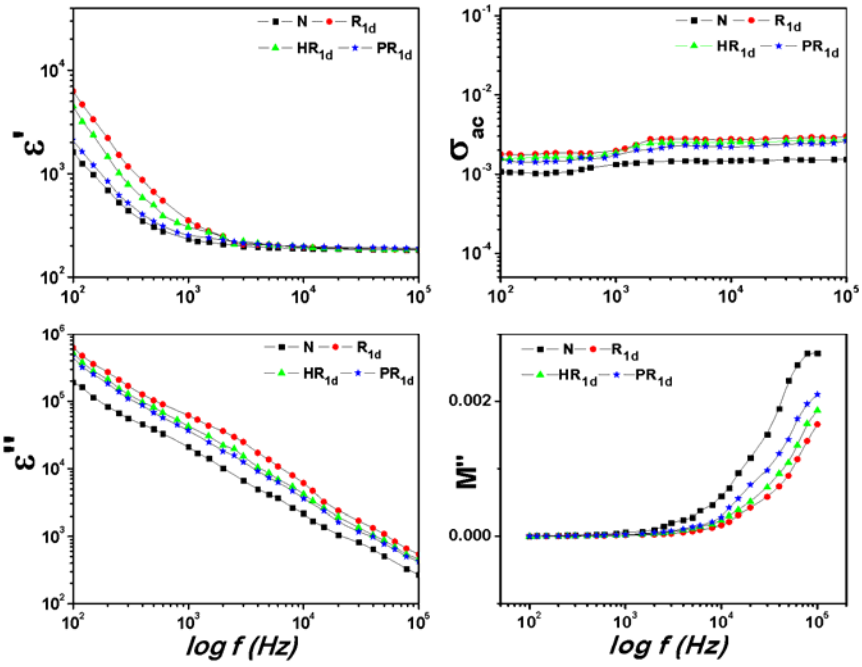

Fig. 9. The variation of dielectric permittivity ε', dielectric loss ε", electrical conductivity σ_{ac} and imaginary part of electric modulus *M*" as a function of the frequency for hemoglobin extracted from animals of irradiated group on 1st day.

In order to discuss the relaxation mechanisms expected to appear at the higher frequency range, *M*" for the investigated samples was calculated and the data obtained are illustrated graphically in Figures 10–13. These data indicate a strong dispersion in β region at frequency range starting from 104 Hz which is

mainly due to hemoglobin molecule and counter ion molecular relaxation. The expected peak of such relaxation could be obtained at frequency range (~1 MHz) which is higher than the available range. This result is comparable with that found before by evaluation of *Ginkgo biloba* extract on hematological changes affected with hazards of electromagnetic field in which β dispersion due to protein was located between (0.1–5 MHz) [1] and those for human blood as the β-dispersion were found to be at about (1–100 MHz) [61].

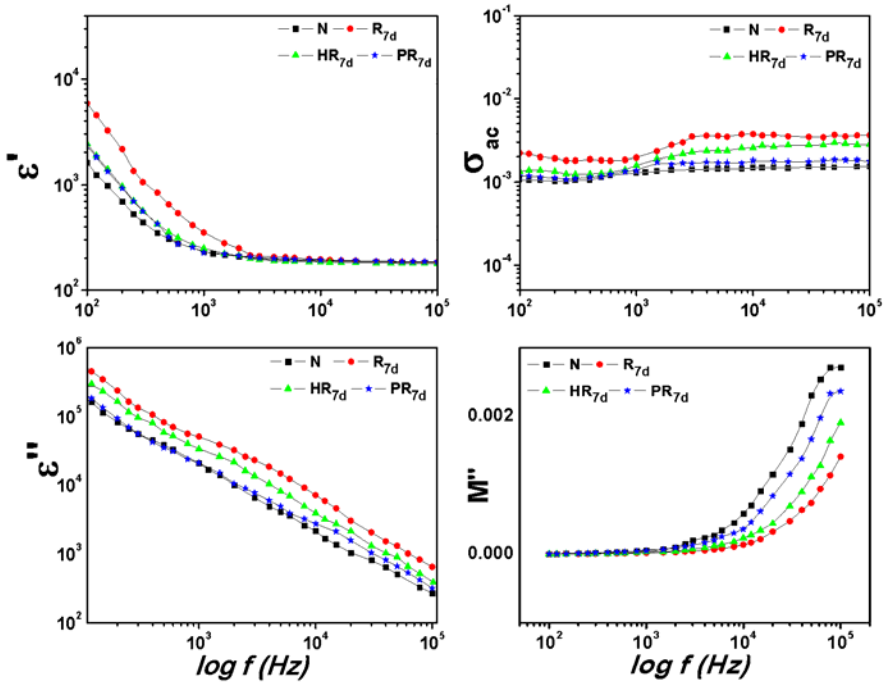

Fig. 10. The variation of dielectric permittivity ε', dielectric loss ε'', electrical conductivity σ_{ac} and imaginary part of electric modulus *M*'' as a function of the frequency for hemoglobin extracted from animals of irradiated group at 7th day.

CONCLUSIONS

The administration of natural antioxidants such as honey and propolis mitigates γ-induced oxidative stress in the rat blood. Phenolic compounds (flavonoids and phenolic acid derivatives) are the most important pharmacologically active constituents in propolis. The propolis sample showed free radical scavenging activity higher than the honey sample. This finding

demonstrated that total phenol and total flavonoid are correlated very well with antioxidant activity, which could be attributed to the presence of phenolic hydroxyl groups in the flavonoid structure. Moreover, the dielectric spectroscopy as well as the absorption spectra in the wave length range 250–700 nm (UV) are proved to be a good physical tool to support the data given by biochemical analysis such as the determination of total antioxidant capacity of serum, creatinine, urea and liver enzymes (ALT, AST).

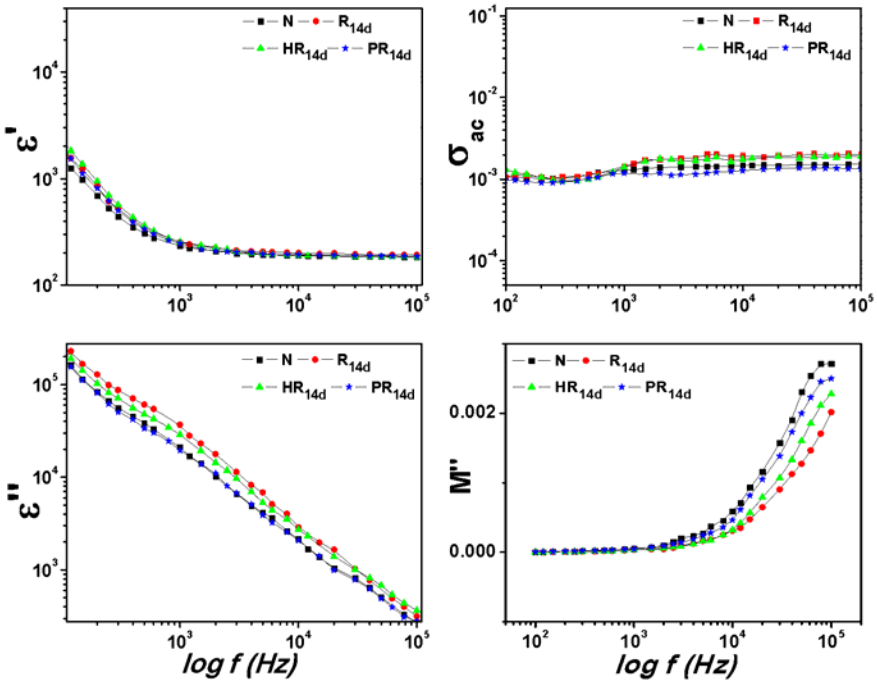

Fig. 11. The variation of dielectric permittivity ε', dielectric loss ε'', electrical conductivity σ_{ac} and imaginary part of electric modulus M'' as a function of the frequency for hemoglobin extracted from animals of irradiated group at 14th day.

Competing interests: The authors declare that they have no competing interests.

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