

## 1 **Biophysical Studies of Some Bee Products as Radioprotectors**

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8 **Keywords:** Gamma irradiation, Honey, Propolis, UV, FTIR, Minerals, Serum total protein,  
9 Albumin and uric acid, Osmotic fragility.

### 10 **Summary Statement**

11 Assessment of some antioxidant ingredients in honey and propolis. Also, a study on ionizing  
12 gamma irradiated rats was done to assess these antioxidants as radioprotectors.

### 13 **Abstract**

14 The study had been planned to evaluate some antioxidant ingredients in honey and propolis.  
15 Also, a study on ionizing gamma irradiated rats was done to assess these antioxidants as  
16 radioprotectors. Bioactive ingredients, such as phenols, flavonoids and trace elements, were  
17 explored using FTIR, UV-Vis and AAS spectroscopic techniques. Animals were exposed to  
18 fractionated gamma radiation doses. Honey, propolis and their combination were administrated

19 before and during the irradiation period. Serum levels of total protein, albumin and uric acid  
20 were estimated. Also, the osmotic fragility of Red Blood Corpuscles (RBCs) membranes and a  
21 microscopic examination of blood films were investigated. The analysis demonstrated that the  
22 level of phenolic, flavonoid and trace elements are higher in propolis than honey. The levels of  
23 total protein and albumin decreased post irradiation while the level of uric acid increased.  
24 Likewise, osmotic fragility of RBCs membranes was increased with a sticking forming RBCs  
25 aggregation. It had been found that administration of the natural antioxidants induced  
26 amelioration in most of the studied parameters. It can be concluded that natural antioxidants  
27 produced a modulation against oxidative stress induced by ionizing radiation.

28 **Key words:** *Gamma irradiation, Honey, Propolis, UV, FTIR, Minerals, serum total protein,*  
29 *albumin and uric acid, Osmotic fragility.*

## 30 **Introduction**

31 Exposure to high doses of ionizing radiation is a rare event. Normally, occupational  
32 workers in different fields such as radiologists, industry, mining or research workers have  
33 restricted safety precautions. Although in some cases, such as radiation accidents high dose  
34 exposure may take place. Radio-protective agents have been widely examined to diminish the  
35 oxidative stress initiated by ionizing radiation (Saaya et al. 2017; Smith et al. 2017).  
36 Antioxidants are various types of molecules which in low levels fundamentally delays or  
37 prevents the oxidation effect of free radicals. (Poljsak et al. 2013). Reactive Oxygen Species  
38 (ROS) are produced from normal cellular metabolism (Khan et al. 2018) or as a consequence  
39 exposure to some chemicals and/or ionizing radiation (Hosseinimehr, 2010). Oxidative stresses  
40 can incentive to disturb redox balance between the production of ROS and the ability of cells to

41 protect against them. Defense against oxidative stress kept up by utilizing several mechanisms  
42 which include antioxidant machinery (El-Missiry et al. 2007). The balanced cellular function is  
43 considered as a net result between the produced ROS and the available antioxidant defense  
44 mechanisms of the cell (Vit et al. 2010). Consequently, the decrease of cellular antioxidant  
45 capacity makes the biological system more susceptible to the malicious impacts of ROS (Birben  
46 et al. 2012). A great deal of research has been carried out on the radioprotective action of some  
47 synthetic chemical substances as antioxidants. These substances reduce mortality when  
48 administered pre exposure to lethal doses of ionizing radiation. But most of them have unwanted  
49 side effects that limited their use in medical practice (Cebolla et al. 2017). Using natural products  
50 as radioprotectors has several benefits since they are safe with proven therapeutic advantages.  
51 The body endogenous defensive system is supported by natural antioxidant compounds provided  
52 from nourishment (Nunes et al. 2013). Identification and isolation of antioxidants from natural  
53 sources has become an active field of research. Natural antioxidants can be micronutrients  
54 (vitamins and trace elements), phenolic compounds (flavonoids, and phenolic acids), nitrogen  
55 components (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids.

56 Various types of bees' products, such as honey, pollen, royal gel, honey wax and propolis,  
57 have many therapeutic effects including anti-inflammatory, antimicrobial, antioxidant, antitumor,  
58 wound healing, and immunomodulatory activities (Boorn et al. 2010).

59 Honey is a sweet, viscous fluid, elaborated by bees from the nectar of plants and stored in  
60 their combs as food. Honey contains about 0.5% protein, mainly enzymes and amino acids  
61 (Khanal et al. 2010). Honey is readily available, affordable and well accepted by irradiated  
62 patients and useful for improving their lives (Orsolich et al. 2010). It is broadly accessible in many  
63 communities, although its mechanism of action remains unclear and requires more investigation.

64 Propolis is a resinous substance that bees collect from different plants. It is utilized in the  
65 construction of, and to seal the cracks in, the beehive. Chemical properties of propolis are not  
66 only advantageous to bees but have also pharmacological incentive as a natural mixture (Ali et  
67 al. 2010). It is a mixture of resin, basic oils and waxes (Cebolla et al. 2017). More than 200  
68 constituents have been distinguished so far from propolis such as phenolic acids and their esters,  
69 caffeic acid and their esters, phenolic aldehydes, flavonoids and ketones; moreover, amino acids,  
70 proteins, vitamins (A, C, biotin, B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>), minerals (calcium, cobalt, copper, iron,  
71 magnesium, manganese, phosphorous, potassium, silicon and zinc) (Re et al. 1999). Phenolic  
72 compounds are able to scavenge ROS due to their electron donating properties. Also, their  
73 antioxidant adequacy relies on the dependability of various systems, besides the number and  
74 location of hydroxyl groups. Phenolic compounds exhibited higher antioxidant activity than  
75 carotenoids and vitamins (Moreira et al. 2011). Flavones are able to interact with free radicals  
76 and with the products of oxidative stress (Moreira et al. 2011; Treml and Smejkal, 2016).  
77 Flavonoids (including flavones, flavonols, flavanones and dihydroflavonols) and other phenolics  
78 (mainly substituted cinnamic acids and their esters) are the main active constituents of propolis  
79 and possess potent antioxidant activities (Almeida et al. 2011). Nutrient antioxidants may act  
80 together to reduce free radical's levels (Stan et al. 2012).

81 Mineral elements are essential regulators of physiological processes. Calcium, zinc and  
82 magnesium are important as cofactors in enzymatic processes, mainly in the structure of the  
83 DNA repair system. Some minerals are components of important enzymes such as Zn for  
84 superoxide dismutase and Fe for catalase. Both enzymes protect the cell membranes from  
85 oxidative damage. Also Iron is a part of the heme of hemoglobin (Hb), myoglobin and  
86 cytochromes (Wołonciej et al. 2016). The high concentration of these mineral elements in

87 propolis must promote the formation of these enzymes, and as a consequence provide its potent  
88 antioxidant capacity ([Kocot et al. 2018](#)).

89 The main goal of this work is to assess the level of some antioxidants constituents in honey  
90 and propolis such as phenols and trace elements using different spectroscopic techniques (*in*  
91 *vitro* study). Also it aimed to examine the extent of the healing ability of honey, propolis and  
92 their combination as radioprotectors against ionizing gamma radiation induced oxidative stress  
93 damages in male rats (*in vivo* study).

#### 94 **Materials and methods**

95 For **the in vitro studies**, three honey samples (H<sub>1</sub>, H<sub>2</sub> and H<sub>3</sub>) and two propolis samples (P<sub>1</sub>  
96 and P<sub>2</sub>) were used. H<sub>1</sub> was from El-Fayoum area while the other two samples H<sub>2</sub> and H<sub>3</sub> were  
97 from the agricultural research center (Giza-Egypt). P<sub>1</sub> was from agriculture research center -  
98 Giza-Egypt while P<sub>2</sub> sample was from a local supermarket.

99 **UV-Visible (UV-Vis) spectroscopic techniques** type V-570 (Jasco, Germany) was used for the  
100 identification and quantification of phenolic compounds ([Stan, 2012](#)). Solutions of honey and  
101 propolis samples at a concentration of 0.1 mg/ml, and 10 mg/ml for honey only, were prepared  
102 as follows: 100 mg of honey and propolis are dissolved in 10 ml of distilled water and ethyl  
103 alcohol respectively in a ratio 1:1, then 1 ml of each solution diluted up to 100 ml of the same  
104 solvent. The absorbance between 200-600 nm was measured using the UV-visible  
105 spectrophotometer.

106 **Fourier Transform Infrared (FTIR) spectroscopic analysis** (Jasco FTIR 300 E, Japan) was  
107 chosen to explore the chemical composition of both honey and propolis samples for its  
108 straightforwardness and capacity to give fingerprints of the measured samples. Honey and

109 propolis were dried at 150°C then grinded to fine powder, 2 mg of the powder sample was mixed  
110 with 200 mg KBr (FTIR grade) and pressed to make a pellet. The pellet was placed into the  
111 sample holder of FTIR to record the spectrum at the range of 4000-400 cm<sup>-1</sup>.

112 **Atomic absorption spectroscopy (AAS)** was used to study the mineral composition of honey  
113 and propolis. Six trace elements, Calcium (Ca), Sodium (Na), Iron (Fe), Magnesium (Mg),  
114 Potassium (K), and Zinc (Zn), were determined in three honey and two propolis samples.  
115 Samples were dried at 105°C and then mineralized by wet digestion method using (HNO<sub>3</sub> -  
116 H<sub>2</sub>SO<sub>4</sub>). About 0.5 g of each sample was pre-digested in 4 mL of 65% HNO<sub>3</sub> (Sigma- Aldrich,  
117 Germany) for 24 hours at room temperature, then 4 mL of 98% H<sub>2</sub>SO<sub>4</sub> were added. After  
118 cooling, the solution was diluted to 20 mL with deionized water. Atomic absorption  
119 spectrophotometer (Agilent Technologies 200 series AA, USA) was used.

120 For **in vivo studies** Cobalt-60 Radiation source (gamma- cell 220), Atomic Energy of  
121 Canada Limited, installed at the Middle Eastern Regional Radioisotopes Center for the Arab  
122 Countries, Dokki, Cairo was used with an average exposure rate of 3.1 Gy per minute.  
123 Experimental rats were subjected to whole body irradiation with gamma fractionated doses of  
124 (1Gy / day for 5 continues days) i.e. 5 Gy total dose.

125 Eighty male albino rats weighing 150 –180 g from the National Research Center (Giza,  
126 Egypt) were used. The roles of the Medical Ethical Committee of the National Research Centre  
127 were taken in place. Animals were maintained for two weeks under acclimatization conditions of  
128 water and diet. They were divided into two main groups, control and irradiated ones. Each group  
129 was subdivided into four subgroups n=10 each. The four controls were the untreated one, the  
130 honey, propolis and their combination treated subgroups. The four irradiated subgroups were the

131 irradiated one, and the subgroups which irradiated and protected with honey, propolis and their  
132 combination.

133 **Honey** was diluted with water and administered orally to animals at a dose of 250 mg/kg/day in  
134 a volume of 1 ml/rat for 15 continuous days of the honey sub-control groups. The irradiated  
135 subgroups received honey 10 days before irradiation and 5 days during the fractionated  
136 irradiation doses.

137 **Propolis** was extracted using 70% ethanol; about 10 g of propolis was dissolved in 100 ml  
138 ethanol. The supernatant was separated using filter paper Whatman No (1). The extract was  
139 completely evaporated under reduced pressure. Propolis was freshly prepared pre- oral  
140 administration in saline at a dose of 90 mg/kg/day for 15 continuous days of the sub-propolis  
141 control group. The irradiated subgroups received propolis 10 days before irradiation and 5 days  
142 during the fractionated irradiation doses.

143 **Honey and propolis combined subgroups** received 250 mg/kg honey plus 90 mg/kg propolis  
144 /day for continuous 15 days of the combined control sub-group. The irradiated subgroup received  
145 the same dose for 10 days before irradiation and 5 days during the fractionated irradiation doses.

146 **Blood samples** were collected from orbital venous plexuses of the rat eye at different time  
147 intervals 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> days post last dose of irradiation. Serum samples were stored at -20°C  
148 for the biochemical investigations. Heparinized blood samples were collected for osmotic  
149 fragility and blood films were stained for microscopic examination.

150 **Biochemical analysis** of total protein was determined according to Gornal et al. (1949).  
151 Albumin was determined according to Gendler (1984). Uric acid was determined according to  
152 Bahram and Trinder (1972). Commercial kits from Biodiagnostic Company, Egypt were used.

153 **The osmotic fragility of Red Blood Corpuscles test** is used to detect the fragility of RBCs of  
154 different groups. Whole blood was added to varying percent buffered sodium chloride solution of  
155 concentrations 0.900, 0.765, 0.675, 0.585, 0.540, 0.500, 0.450, 0.400, 0.360, 0.315, 0.270, 0.180,  
156 0.090 and 0.000 and allowed to incubate at room temperature. The amount of hemolysis is then  
157 determined by reading the absorbance of the supernatants at 540 nm on the spectrophotometer  
158 Unicam UV-Visible Spectrometry (Helios, United Kingdom). Normal and treated blood samples  
159 had been tested at the same condition ([Brown, 1993](#)).

160 The percent of hemolysis of different samples was calculated for each supernatant as  
161 follow:

$$162 \quad \% \text{ Hemolysis} = \frac{\text{O.D. of supernatant}}{\text{O.D. supernatant No 14}} \times 100$$

163 O.D. is the Optical Density of supernatant of different NaCl concentrations. O.D.  
164 supernatant No 14 is the O.D. of 0.000 NaCl concentrations which represents 100% hemolysis.

165 **Blood films** were stained with Leishman stain for 5 min then washed with water for  
166 examination. They were scanned and examined using the light microscope (PB362040x) at a  
167 magnification power 400 x. The images were captured using a digital camera (Yashica, EZ8032,  
168 8.2 megapixels).

169 **Statistical analysis** of the biochemical data was performed by two-way analysis of  
170 variance (ANOVA) test and Duncan test using SPSS software program version 17 (SPSS Inc,  
171 USA).

## 172 **Results**

173 **UV - Visible spectroscopic analysis** results are appeared in [figure \(1A &1B\)](#). [Figure](#)



174 (1A) represents a simple spectrophotometric registration of UV-visible spectra of different honey  
175 and propolis samples at a concentration of 0.1 mg/ml. The first propolis sample spectrum has an  
176 absorption band with  $\lambda_{\max}$  at 288 nm. The second propolis sample spectrum has a plateau with  
177  $\lambda_{\max}$  between 270-290 nm. Both propolis samples have an absorption peak at 230, while honey  
178 samples did not show any absorption band in this region. At a concentration of 10 mg/ml, honey  
179 samples showed spectra with  $\lambda_{\max}$  around 280 nm, while the absorption peak at 230 nm only  
180 observed in H<sub>1</sub> sample as appeared in Figure (1B).

181 **FTIR spectroscopic analysis** shows the spectra pattern of honey in Figure (1C) and of  
182 propolis in Figure (1D). The presence of distinct bands in the spectra pattern is considered to be  
183 an indication of the presence of certain functional group and the expected compounds are listed  
184 in Table (1). The variations in the intensity of such bands of different samples were listed in  
185 Table (2). The correlation coefficient ( $R^2$ ) of FTIR band intensity is represented in Table (3).

186 The total flavonoid and phenols in P1 sample is found to be slightly higher than that P2.  
187 H1 sample contains higher content of flavonoid and phenols than H2 and H3 samples. Also, it has  
188 been found that propolis has greater contents of flavonoid and phenols than honey.

189

## 190 **Mineral composition**

191 Honey and propolis mineral composition was measured using atomic absorption  
192 spectroscopy. Six elements (Fe, Mg, Na, K, Ca and Zn) were determined in three honey and two  
193 propolis samples. The concentrations of these minerals in honey and propolis samples are listed  
194 in Table (4). One-way ANOVA test demonstrated a significant difference at  $p < 0.05$  in the

195 content of each mineral amongst propolis and honey samples as well as between different honey  
196 or propolis samples.

## 197 **Biochemical analysis**

198 The data of serum total protein and albumin were summarized in [Tables \(5\) and \(6\)](#). A  
199 two- way ANOVA test and Duncan test indicated a significant decrease in their levels ( $P < 0.05$ )  
200 in irradiated rats contrasted to the normal group. Also, treatments with honey, propolis, and their  
201 combination induced significant ameliorations compared to irradiated rats. ANOVA showed  
202 no significant change between different time intervals post irradiation ( $P > 0.05$ ) of total protein  
203 and albumin and also no significant interaction between factors ( $P > 0.05$ ).

204 The data of serum uric acid are summarized in [Tables \(7\)](#). A two- way ANOVA test and  
205 Duncan test indicated that uric acid significantly increased ( $P < 0.05$ ) in rats exposed to  $\gamma$ -  
206 radiation compared with the normal group. Also, different treatments with honey, propolis, and  
207 their combination induced significant amelioration compared to irradiated group. The propolis  
208 and combination pretreated animal groups showed improvement in serum uric acid levels more  
209 than the honey pretreated group. Also, ANOVA demonstrated no significant difference between  
210 the level of uric acid at various time intervals post irradiation ( $P > 0.05$ ) and furthermore no  
211 significant interaction between factors ( $P > 0.05$ ).

## 212 **Biophysical examinations**

### 213 **Red blood cells membranes studies**

214 **Osmotic fragility:** [Figures \(2A- H\)](#) shows the effect of NaCl percent concentrations on osmotic  
215 fragility of RBCs membrane and its differentiation.

216 Measurements illustrate the variation of the percentage hemolysis as a function of the

217 percentage of NaCl concentration in buffer solution for RBCs of different animal subgroups as  
218 appeared in [Figures \(2 A, C, E and G\)](#).

219 To analyze these data, the graphs were differentiated and plotted as a function of the  
220 percentage of NaCl concentration as shown in [Figures \(2 B, D, F and H\)](#). From osmotic fragility  
221 curves, it is possible to calculate Median Corpuscular Fragility (MCF) which means the NaCl  
222 concentration at which 50% of RBCs are hemolyzed. The points of the differentiation curves  
223 were calculated by subtracting each point from the previous one in the hemolysis curve. The  
224 width at half maximum ( $W_{hmax}$ ) of the differentiation curve represents the relative elastic limit of  
225 RBCs membrane. The increase of  $W_{hmax}$  expresses more elasticity of cell membrane. The MCF  
226 and  $W_{hmax}$  values of RBCs from different subgroups were represented in [Table \(8\)](#).

## 227 **Blood film**

228 Histological examinations of RBCs by light microscope are shown in [Figures \(3 A-P\)](#).  
229 The images of the control samples appear in [Figures \(3 A-D\)](#), for negative control and positive  
230 control administered honey propolis and their combination respectively, have normal structures.  
231 [Figures \(3 E-H, and 3 M-P\)](#) illustrate blood film images of the irradiated and irradiated  
232 pretreated animal subgroups at different time intervals 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> day post- irradiation  
233 respectively. The results showed a remarkable sticking of RBCs forming aggregation, which  
234 persist until the 14<sup>th</sup> day post- irradiation [Figures \(3 E, I and M\)](#). Oral administration of  
235 propolis and the combination diminishes the formation of RBCs aggregation and reduces the  
236 damage of irradiation [Figures \(3 G, K and O\) and \(3 H, I and P\)](#). Instead, honey recovers RBCs  
237 at the 14<sup>th</sup> day post irradiation [Figure \(3 N\)](#) while at 1<sup>st</sup> and 7<sup>th</sup> days [Figures \(3 F and J\)](#) still

238 have some sort of sticking and deformation.

## 239 **Discussion**

240 UV - Vis spectroscopic investigation affirmed the presence of phenolic compounds in  
241 honey and propolis since they exhibit two major absorption bands in UV - Vis region (Moț et al.,  
242 2011). The characteristic bands of phenols are a broad band centered around 280–330 nm and a  
243 second band at 250 nm (Yang, al., 2013).

244 The UV-Vis spectrum in Figure (1a) is comparable with an earlier research work which  
245 isolates and evaluates six antioxidants of propolis at (200-600 nm) (Yang, al., 2013). The spectra  
246 of propolis at a concentration of 0.1 mg/ml are similar in shape and position to the spectra of  
247 honey at a concentration of 10 mg/ml Figure (1b). These results are in coincidence with  
248 beforehand published works (Mihai, et al., 2011).

249 In general, it is thought that the appearance of the absorption band at 270-330 nm is  
250 attributable to flavonoids and phenols (Mărghitaș et al., 2013) while the absorption band at 230  
251 nm is characteristic for flavone/flavonol by-products (Yang, t al., 2013). Also, it is reported that  
252 band with  $\lambda_{\max}$  290 nm, is believed to arise from Hispertin (Makawi et al., 2009). Variation in  
253 intensity and shift in the absorption band might be attributed to the different antioxidant ratios in  
254 flavonoids and phenols (Mărghitaș et al., 2013). A high correlation was found between the UV-  
255 Vis spectra of samples and total phenolic compounds that were determined by the Folin-  
256 Ciocalteu method (Hamieda et al., 2015).

257 FTIR spectroscopic data in Tables (1) and (2) are comparable with those early reported  
258 (Gallardo-Velázquez et al., 2009; Ali, et al., 2012). The bands in Table (2) showed different  
259 correlations to antioxidant activity represented in Table (3) which is in coincidence with Mot

260 and his coworkers' results (Moř et al., 2011). It is clear that the antioxidant activity of  
261 polyphenols does not depend only on the amount of the present compounds but also on their  
262 chemical structures (Omene et al., 2012).

263 The Fe, Mg, Na, K, Ca and Zn are basic elements since they play important roles in the  
264 biological systems one of them is their antioxidant activity (Pohl et al., 2012).

265 The higher minerals concentration in propolis than honey is in coincidence with previously  
266 reported data (Grembecka and Szefer 2013; Formicki et al., 2013). Also, honey and propolis  
267 samples differ in their mineral constituents depending on the resources in the soil and kind of  
268 plants from which the bees took nectar (Shah et al., 2014). These elements do many biological  
269 functions since they act as coenzymes and participate in other processes like Redox one (Kurek-  
270 Górecka et al., 2013).

271 The biochemical analysis of serum total protein is relatively important in assessing the  
272 health state of mammals (Vasile et al., 2009). The decrease of total protein post irradiation might  
273 be due to the disturbances of the vital biological processes or because of progress in the  
274 penetrability of the liver, kidney and other tissues resulting in leakage of protein via the kidney  
275 (Muhammad et al., 2015). The decline in the level of albumin concentration could be due to  
276 enhanced degradation as well as enhanced loss of albumin through the gastrointestinal tract  
277 (Shabon, 2005). Moreover, the extensive damage to the lymphoid organs following  $\gamma$ - irradiation  
278 was probably related to the drop in serum globulin levels (Moulder et al., 2004). Earlier  
279 investigations by Wheeler and Bernard (1994) have reported that irradiation leads to proteinuria,  
280 which is associated with low serum total protein and albumin. An improvement in the levels of  
281 serum total proteins and albumin in the pretreated groups with propolis and combination  
282 maintain their levels near the normal level at the different time intervals. The pretreatment with

283 honey only shows slight improvement. The effect of pretreatment with propolis is in harmony  
284 with Saleh (2012) who reported that propolis significantly improved the total protein content of  
285 the liver and kidney and indicated more profound therapeutic effects.

286 The increased uric acid level in rats exposed to  $\gamma$ -radiation compared with the normal  
287 group is supported by other works (Ferreira-Leach and Hill, 2001; Saleh, 2012). Uric acid is the  
288 end product of the purine metabolism and is normally eliminated by the kidney in urine. Excess  
289 uric acid may be a side effect of some cancer treatments and may lead to a condition called  
290 tumor lysis syndrome (Crohns et al., 2009). The excess uric acid forms crystals which may  
291 deposit in the tiny tubes of the kidney and cause acute kidney damage, leading ultimately to  
292 kidney failure (Abd elhalim and Moussa, 2013). This result might be ascribed to the hindrance  
293 of glomerular selective properties caused by irradiation (Berry et al., 2001).

294 The mechanism by which the natural product honey and propolis anticipate renal  
295 oxidative stress may incorporate several mechanisms. One of them is the induction of  
296 glutathione GSH synthesis which is consumed when bind to free radicals. Others are by a  
297 scavenger effect of antioxidants phenols and flavonoids (naringenin, pinostrombin and galangin)  
298 found in honey and propolis to the electrons of ROS. This effect could have accumulated in the  
299 cells of the proximal convoluted tubules of the kidney where propolis was reported to be  
300 collected and secreted (Saleh et al., 2013). Instead of ROS, their free electrons will be captured  
301 with the antioxidant (Newairy, et al., 2009). So there must be a massive concern to clarify the  
302 clinical role of the propolis.

303 Osmotic fragility of RBCs membranes showed an increase in hemolysis percentage and  
304 osmotic fragility of RBCs membrane in irradiated groups which was detected through the shift

305 of the osmotic fragility curves towards the higher NaCl concentration. Also, the reduction in  
306  $W_{\text{hmax}}$  is an evidence of decreasing the flexibility of RBCs. This result is in coincidence with  
307 Selim and his coworkers ([Selim et al., 2009](#)). It is interesting to confirm that these changes in  
308 osmotic fragility can be omitted by pretreating with honey, propolis, and their combination.

309 The increase in the osmotic fragility may be attributed to some changes in the properties  
310 of the RBCs membrane. Ionizing radiation causes a disturbance in energy metabolism,  
311 disorganization of lipoprotein structure of biological membranes, peroxidation of membrane  
312 lipids and inactivation of various bound enzymes ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^+$ ) ATPase. It has also been  
313 suggested that radiation induces perturbation in  $\text{Na}^+$  and  $\text{K}^+$  transport system which is connected  
314 with oxidation of membrane protein sulfhydryl groups ([Jóźwiak and Helszer 1981](#)).  
315 Modification in the physical condition of the membrane proteins may lead to change in the  
316 permeability of the RBCs membrane. Some proteins of the cell membrane act as pores (ion  
317 channels) through which the aqueous solutions carried inside the cell ([Hemida et al., 2011](#)).

318 In the presence of honey, propolis and their combination protective effect against RBCs  
319 membrane damage were observed. This observation could be attributed to the presence of some  
320 coenzyme minerals such as Zn for superoxide dismutase and Fe for catalase. Both enzymes  
321 protect the cell membrane from oxidative damage. Since propolis contains both elements, it  
322 promotes the formation of the dismutase and catalase and as a consequence decreases the  
323 hemolysis. Additionally, Potassium is the principle cation in intracellular fluid and functions in  
324 the regulation of the osmotic pressure and co-enzyme of  $\text{Na}^+/\text{K}^+$  ATPase ([Achikanu et al., 2013](#);  
325 [Shah, et al., 2014](#)). It was found that protective effect of propolis on RBCs membrane was  
326 related to the higher phenolic content of propolis ([Moreira et al., 2011](#); [Valente et al., 2011](#)).

327 These results mean that propolis act as a powerful antioxidant compound which prevents the  
328 oxidative damage of lipoproteins of the cell membrane ([Yousif et al., 2012](#)).

329 RBCs are well known that they are large cells having a biconcave structure which is  
330 naturally flexible and bendable ([El-marakby et al., 2013](#)). They do not stick together as a result  
331 of the Coulomb repulsive forces between the positive electrostatic charges on their outer surface  
332 membranes. The electrostatic charges are formed on the surface of the normal healthy RBCs  
333 subsequently of the K ion pump which forms the resting potential across cellular membranes  
334 ([Hemida et al., 2012](#)). RBCs cell membranes are particularly sensitive to the effects of  
335 irradiation. The sticking of several cells together after irradiation is due to the oxidative damage  
336 of the free radicals. They destruct the processes within both layers of RBCs membrane and that  
337 alters the membrane structure and function ([El-marakby et al., 2013](#)). Also, the sticking can be  
338 attributed to the changes in the packing properties of the phospholipid bilayer and  
339 macromolecules forming the cellular membrane. This will cause changes in the membrane  
340 permeability to ion transport and the liquid crystalline phase of the membrane will change.  
341 Therefore, one may state that changes in the membrane permeability will result in the change of  
342 the membrane bioelectric potential and surface electrostatic charges ([Hemida et al., 2012](#)). The  
343 loss or decrease of the surface electrostatic charges on the cellular membrane will deteriorate the  
344 repulsive forces between adjacent RBCs membranes and cause the sticking. Moreover, the  
345 oxidation of membrane proteins leads to red cell vesiculation ([Moreira et al., 2011](#)). It is noticed  
346 that pretreating of the animals with honey decreases the sticking of cells and reaching mostly to  
347 normal shape by propolis and their combination. This is because the hydroxyl groups of the  
348 phenolic and flavonoids compounds have anti-lipid peroxidant and rheologically protecting  
349 against oxidative stress ([de Kok et al., 2010](#)).



350 **Conclusion**

351 The administration of natural antioxidants such as honey and propolis mixture mitigates  $\gamma$ -  
352 induced oxidative stress in rat blood.

353 UV absorption spectra and dielectric measurement were found to be good tools to support the  
354 data given by biochemical analysis, such as total proteins. The obtained data indicated that honey  
355 and propolis mixture display an in vivo antioxidant activity, which is evidenced experimentally  
356 by ameliorating the osmotic fragility, sticking and aggregation of red blood cell as well as serum  
357 total protein and albumin.

358 Further investigations are required to elucidate the mechanisms of propolis and honey actions.

359

360 **Declaration of interest**

361 The authors declare that there are no conflicts of interest.

362 **Captions of figures**

363 Figure (1): Absorption spectra of flavonoid (A) UV-Vis of different honey and propolis samples  
364 at concentration 0.1 mg/ml and (B) UV-Vis of different honey samples at concentration 10 mg/  
365 ml (C) FTIR spectrum pattern of honey, (D) FTIR spectrum pattern of propolis.

366 Figure (2): (2 A and B) RBCs of control (A) Osmotic fragility curves and (B) Differentiation  
367 curves. (2 C and D) RBCs of 1<sup>st</sup> day post- irradiation (C) Osmotic fragility curves and (D)  
368 Differentiation curves (E and F) RBCs of 7<sup>th</sup> day post- irradiation (E) Osmotic fragility curves  
369 and (F) Differentiation curves (2 G and H) RBCs of 14<sup>th</sup> day post- irradiation (G) Osmotic  
370 fragility curves and (H) Differentiation curves.

371 **Figure (3 A-P):** Microscopic blood film observations of different animal groups RBCs (400X)  
372 and (96DPI). (3 A-D) Microscopic blood film observations of controls (A) Negative control rats,  
373 (B) Control rats administered honey, (C) Control rats administered propolis (D) Control rats  
374 administered combination. (3 E-H) Microscopic blood film observations of 1<sup>st</sup> -day post  
375 irradiation animal group; (E) irradiated (f) irradiated + honey (G) irradiated + propolis (H)  
376 irradiated + combination. (3 I-L) Microscopic blood film observations of 7<sup>th</sup> day post irradiation  
377 animal group; (I) irradiated (J) irradiated + honey (K) irradiated + propolis (L) irradiated +  
378 combination. (3 M-P) Microscopic blood film observations of 14<sup>th</sup> day post irradiation animal  
379 group; (M) irradiated (N) irradiated + honey (O) irradiated + propolis (P) irradiated +  
380 combination.

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**Table (1): Distinct bands of honey and propolis in FTIR spectra**

<b>Bands (cm<sup>-1</sup>)</b>	<b>Functional group</b>	<b>expected compounds</b>
3330-3373	C-H aromatics ,O-H	Phenolic, its esters, flavonoids
2933	C-H	Flavonoids, aromatic ring
2853	C-O ,C-H aromatics	Phenolic, flavonoids, aromatic ring
1630	C=O, C=C, N-H	Flavonoids, amino acids
1450	C-H, aromatic CH <sub>3</sub> , CH <sub>2</sub>	Flavonoids, aromatic ring
1260	C-C stretch	Carbohydrate structure
1160	C-O, C-OH	fatty acids, steroids, carboxylic acids
1043	C-O stretch in C-OH group	Carbohydrate structure
920	C-H bending	Carbohydrate structure

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**Table (2): The variation of bands intensity in FTIR spectra of honey and propolis**

Sample \ Band (cm <sup>-1</sup> )	H2	H3	P1	P2
	Intensity			
3373	0.67	1.56	44.45	36.60
2933	27.55	26.80	41.65	29.45
2853	—	—	48.38	34.49
1630	39.69	38.47	43.58	30.85
1450	30.28	25.29	49.37	34.78
1260	34.15	32.72	49.74	35.01
1160	—	—	47.55	34.76
1043	4.16	1.70	55.20	46.66

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567 **Table (3): Correlation coefficient of FTIR bands intensity**

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Bands (cm <sup>-1</sup> )	Correlation coefficient (R <sup>2</sup> )
3330-3373	0.9955 well correlation
2933	0.9831 well correlation
1630	-1.377 negative correlation
1450	0.498 poor correlation
1260	0.7065 poor correlation
1160	0.9594 well correlation
1043	0.9916 well correlation

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571 **Table (4): Concentration of the trace elements studied in honeys and propolis in**  
 572 **mg/100g dry weight.**

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Mineral	Sample				
	H1	H2	H3	P1	P2
Fe	1.97±0.3 <sup>d</sup>	1.51±0.8 <sup>e</sup>	3.58±0.6 <sup>c</sup>	37.98±2.4 <sup>a</sup>	28.78±1.2 <sup>b</sup>
Mg	7.78±1.6 <sup>c</sup>	4.04±2.9 <sup>c</sup>	7.57±2.4 <sup>c</sup>	153.24±9.1 <sup>a</sup>	41.60±1.7 <sup>b</sup>
Na	3.60±0.4 <sup>b</sup>	1.89±0.4 <sup>b</sup>	3.77±0.7 <sup>b</sup>	18.80±1.9 <sup>a</sup>	17.96±1.1 <sup>a</sup>
K	17.63±0.4 <sup>c</sup>	14.5±0.8 <sup>cd</sup>	9.15±1.6 <sup>d</sup>	105.82±7.4 <sup>a</sup>	75.37±2.4 <sup>b</sup>

<b>Ca</b>	35.56±1.9 <sup>cd</sup>	39.10±1.7 <sup>c</sup>	27.65±1.4 <sup>d</sup>	155.30±6.6 <sup>a</sup>	76.54±1.2 <sup>b</sup>
<b>Zn</b>	0.62±0.2 <sup>c</sup>	0.11±0.1 <sup>c</sup>	0.13±0.1 <sup>c</sup>	24.63±1.2 <sup>a</sup>	13.43±0.5 <sup>b</sup>

574 **Data expressed as mean ± SD, mg/100g of dried sample, n = 3**

575 \* **Significant different at p < 0.05.** Duncan test results are expressed as letters (a, b, c, d and e).

576 Values with different superscript letters are significantly differ. Values with same letters indicate  
577 no significant difference between samples. For each element separately (each row).

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584 **Table (5): Total serum protein levels in irradiated rats and those treated with honey,**  
585 **propolis separately and combined pre  $\gamma$ - irradiation.**

Treatment	Days after $\gamma$ - irradiation		
	1 <sup>st</sup>	7 <sup>th</sup>	14 <sup>th</sup>
<b>Normal</b>	5.44±0.26 <sup>a</sup>	5.50±0.87 <sup>a</sup>	5.34±0.85 <sup>a</sup>
<b>Honey</b>	6.00±0.30 <sup>a</sup>	5.44±0.14 <sup>a</sup>	4.65±0.71 <sup>a</sup>
<b>Propolis</b>	4.81±0.74 <sup>ab</sup>	5.30±0.23 <sup>ab</sup>	4.60±0.68 <sup>ab</sup>
<b>Combined</b>	5.00±0.16 <sup>ab</sup>	5.23±0.17 <sup>ab</sup>	5.04±0.34 <sup>ab</sup>
<b>Irradiated</b>	3.78±0.34 <sup>c</sup>	3.06±0.49 <sup>c</sup>	3.34±0.31 <sup>c</sup>
<b>Irradiated + honey</b>	4.97±0.78 <sup>b</sup>	4.30±0.55 <sup>b</sup>	4.21±0.81 <sup>b</sup>
<b>Irradiated + prpolis</b>	5.23±0.68 <sup>ab</sup>	5.49±0.63 <sup>ab</sup>	5.00±0.45 <sup>ab</sup>
<b>Irradiated + combined</b>	5.44±0.53 <sup>a</sup>	5.37±0.35 <sup>a</sup>	5.24±0.82 <sup>a</sup>

586 **Data expressed as mean ± SE, n = 10**

587 Duncan test results are expressed as letters (a and b). Values with different superscript letters are  
588 significantly different. Values with same letters indicate no significant difference between  
589 samples for each time separately (each coulomb).

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**Table (6): Serum albumin levels in irradiated rats and treated with honey, propolis separately and combined pre  $\gamma$ - irradiation.**

Treatment	Days after $\gamma$ - irradiation		
	1 <sup>st</sup>	7 <sup>th</sup>	14 <sup>th</sup>
Normal	3.1±0.102 <sup>a</sup>	2.88±0.44 <sup>a</sup>	2.94±0.44 <sup>a</sup>
Honey	3.42±0.21 <sup>a</sup>	3.17±0.08 <sup>a</sup>	2.66±0.42 <sup>a</sup>
Propolis	3.23±0.39 <sup>a</sup>	3.00±0.08 <sup>a</sup>	3.21±.42 <sup>a</sup>
Combined	3.2±0.44 <sup>a</sup>	3.1±0.39 <sup>a</sup>	3.2±0.197 <sup>a</sup>
Irradiated	2.54±0.13 <sup>b</sup>	1.96±0.04 <sup>b</sup>	1.68±0.12 <sup>b</sup>
Irradiated + honey	3±0.10 <sup>a</sup>	2.93±0.44 <sup>a</sup>	2.95±0.46 <sup>a</sup>
Irradiated + prpolis	3.19±0.11 <sup>a</sup>	2.90±0.40 <sup>a</sup>	3.50±0.11 <sup>a</sup>
Irradiated + combined	2.82±0.43 <sup>a</sup>	2.99±0.11 <sup>a</sup>	2.95±0.43 <sup>a</sup>

621 **Table (7): Serum uric acid levels in irradiated rats and those treated with honey, propolis**  
622 **separately and combined pre  $\gamma$ - irradiation**  
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Treatment	Days after $\gamma$ - irradiation		
	1 <sup>st</sup>	7 <sup>th</sup>	14 <sup>th</sup>

<b>Normal</b>	1.43±0.04 <sup>b</sup>	1.32±0.03 <sup>b</sup>	1.48±0.08 <sup>b</sup>
<b>Honey</b>	1.38±0.03 <sup>b</sup>	1.51±0.031 <sup>b</sup>	1.59±0.05 <sup>b</sup>
<b>Propolis</b>	1.46±0.09 <sup>b</sup>	1.56±0.03 <sup>b</sup>	1.38±0.11 <sup>b</sup>
<b>Combined</b>	1.51±0.11 <sup>b</sup>	1.43±0.05 <sup>b</sup>	1.45±0.08 <sup>b</sup>
<b>Irradiated</b>	2.28±0.03 <sup>a</sup>	3.00±0.05 <sup>a</sup>	3.23±0.19 <sup>a</sup>
<b>Irradiated + honey</b>	1.41±0.09 <sup>b</sup>	1.40±0.07 <sup>b</sup>	1.52±0.06 <sup>b</sup>
<b>Irradiated + prpolis</b>	1.48±0.06 <sup>b</sup>	1.38±0.04 <sup>b</sup>	1.38±0.05 <sup>b</sup>
<b>Irradiated + combined</b>	1.38±0.05 <sup>b</sup>	1.17±0.04 <sup>b</sup>	1.15±0.08 <sup>b</sup>

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625 **Table (8): The values of MCF and Whmax for from different animal subgroups RBCs**

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<b>Group</b>	<b>MCF</b>	<b>Whmax(arbitrary units)</b>
<b>Control group</b>		
<b>N</b>	0.498	0.140
<b>H</b>	0.514	0.140
<b>P</b>	0.498	0.139
<b>C</b>	0.500	0.163
<b>1<sup>st</sup> day post irradiation</b>		
<b>R1d</b>	0.570	0.135
<b>HR1d</b>	0.520	0.139
<b>PR1d</b>	0.520	0.149
<b>CR1d</b>	0.520	0.150
<b>7<sup>th</sup> days post irradiation</b>		
<b>R7d</b>	0.560	0.130
<b>HR7d</b>	0.525	0.130
<b>PR7d</b>	0.515	0.140
<b>CR7d</b>	0.498	0.150
<b>14<sup>th</sup> days post irradiation</b>		

<b>R14d</b>	0.530	0.132
<b>HR14d</b>	0.520	0.137
<b>PR14d</b>	0.515	0.147
<b>CR14d</b>	0.500	0.150

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