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SPECTROPHOTOMETRIC ANALYSIS OF STORED X-IRRADIATED RED BLOOD CELLS AND THE ROLE OF HONEY AS A RADIOPROTECTIVE AGENT

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ABSTRACT

Radioprotective ability of honey was considered on X-radiation red blood cells using UV-Spectrophotometry. Radiotherapy and exposure to ionizing radiation like X-ray damages red blood haemoglobin and human tissue hence there is need for radioprotection. An adult human blood was collected and stored for three weeks. The blood was divided into eight groups, centrifuged to collect the RBCs. Three were treated with natural honey, three were without honey and all irradiated at 50, 70, 100 kVp respectively using X-ray machine (MDX-100- RMS). The two remaining groups, one was neither treated nor irradiated and the other was treated but not irradiated to serve as normal control and positive control respectively. The RBCs spectra were recorded using a UV-Vis spectrophotometer over a wavelength range of 700 – 250 nm. The RBCs absorbance spectrum in the UV domain was obtained as follows: control sample (409.00 nm, 5.447), positive control sample (409.00 nm, 3.792), sample irradiated without honey at 50, 70 and 100 kVp (409.00 nm, 3.103), (376.00 nm, 5.069), (407.00 nm, 2.092), sample irradiated with honey at 50 70 and 100 kVp (404.00 nm, 2.133), (411.00 nm, 1.093), (402.00 nm, 1.347) respectively. The result suggests that X-radiation has affected the hemoglobin content of the different RBCs groups and honey partially protected the hemoglobin content from the damage caused by the free radicals created as a result of exposure to X-radiation when compared with the control RBCs.

KEYWORDS
Spectrophotometry, x-irradiated red blood cells, honey, radioprotective agent

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INTRODUCTION

Radioprotectors are agents that, when given before or during irradiation, reduce the likelihood of early and/or late effects from developing (Poljsak et al., 2013). Radioprotectors are compounds that are designed to reduce the damage in normal tissues caused by radiation. These compounds are often antioxidants and must be present before or at the time of radiation for effectiveness (Deborah et al., 2010). Honey is a sweet, viscous food substance produced by bees and some related insects (Crane, 1990). Honey is readily available, affordable and well accepted and useful for improving lives (Orsolic et al., 2010).

It is noticed that pretreating of the patients with honey decreases the sticking of cells and reaching mostly to normal shape by propolis and their combination. This is because the hydroxyl groups of the phenolic and flavanoids compounds have protective ability against oxidative stress (de Kok et al., 2010). Blood is a body fluid found in humans and other animals that delivers vital substances such as nutrients and oxygen to the cells and also removes metabolic waste products away from them. The storage of red blood cells intended for transfusion alters their survival and function. Red blood cells (RBCs) are dark red, flexible biconcave cells containing the protein hemoglobin (Beutler et al., 2001).

Red blood cells can be considered as sacks of hemoglobin, the oxygen carrying molecules which gives the cell its red colour. Approximately 97% of the total red cell protein is hemoglobin with a cell mass fraction of approximately 0.3 (Beutler et al., 2001). Its primary function is to transport molecular oxygen (O_2) from the lungs to the tissues and to carry carbon dioxide (CO_2) from the tissues to the lungs. Its flat shape keeps the hemoglobin molecules closer to the membrane which allows for a more efficient transfer of oxygen (Nonoyama, 2004). Red blood cells generally exhibit a diameter of approximately $7.5 - 8.7$ μm, and are found at a concentration of 5×10^6 cells per microliter of blood (Alberts et al., 1989).

Furthermore, mature human red blood cells lack a nucleus and mitochondria and exhibit minimal metabolism yet maintain their existence for an average of 100-120 days (Hillman and Finch, 1996). Various cellular changes occur as a result of RBC storage which is believed to contribute to the reduced viability of post-transfused red blood cells. These changes include the loss of adenosine triphosphate, potassium, oxidative injury to proteins, lipids and carbohydrates. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of

a material as a function of wavelength. It deals with visible light, near-ultraviolet, and near-infrared. However, they can also be designed to measure the diffusivity on any of the listed light ranges that usually cover around 200 ─ 2500 nm using different controls and calibrations (Allen et al., 2009). Within these ranges of light, calibrations are needed on the machine using standards that vary in type depending on the wavelength of the photometric determination (Schwedt, 1997).

Absorption of UV-Vis light excites molecules that are in ground-states to their excited-states (Ninfa and Ballou, 2004). The ultraviolet light is a type of electromagnetic radiation that comes from the sun and transmitted in waves or particles at different wavelengths and frequencies. Spectrophotometry is widely used for various types of chemical and biological analysis, and by its nature spans a broad range of wavelengths. This project focuses on applying the significant potential of ultraviolet Spectrophotometry to analyse stored X-irradiated red blood cells.

MATERIALS AND METHODS

A pint of blood was obtained from the blood bank of the Ahmadu Bello University Medical Center (ABUMC) Main Campus, Zaria. The blood was screened for viruses, and then stored for duration of 20 days. Natural honey was also purchased from a local bee farmer. Experimental procedures were as follows:

Preparation of Red Blood Cells Sample

The blood was transferred into eight EDTA bottles and then centrifuged to separate the red blood cells from whole blood. The centrifuge was done at the Department of Toxology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The blood was centrifuged at 3000 x g for 10 minutes after which the plasma was aspirated. The red blood cells were divided into four groups: control, positive control (red cell mixed with honey), irradiated cells without honey, irradiated cells mixed with natural honey.

X-Irradiation

The red blood cells were exposed to medical diagnostic X-rays generated by a medical diagnostic X-ray machine (MDX-100- RMS) available at the Diagnostic Imaging Center, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The diagnostic X-ray machine operated at 50, 70, and 100 kVp with the current tube at 100mAs. The red blood cells were placed 100 cm

from the X-ray tube. The field of view was 10 cm x 10 cm. The non-irradiated sample served as the control.

Spectrophotometric analysis

The RBCs spectra were recorded using the UV-Vis spectrophotometer (Cary-300 UV-Vis spectrophotometer series, Alingent technology) available at the Multi-users laboratory, Ahmadu Bello University, Zaria. The spectra were recorded over a wavelength range of 700-250 nm.

RESULTS AND DISCUSSION

The hemoglobin absorbance band in the UV domain (376.00 - 411.00 nm) determines the characteristics spectrum of the RBC. Figures 1, 2, 3, 4, below present the characteristic spectrum of the different RBCs sample in UV domain.

Results of Hemoglobin Absorbance of Non- Irradiated Red Blood Cell (RBC)

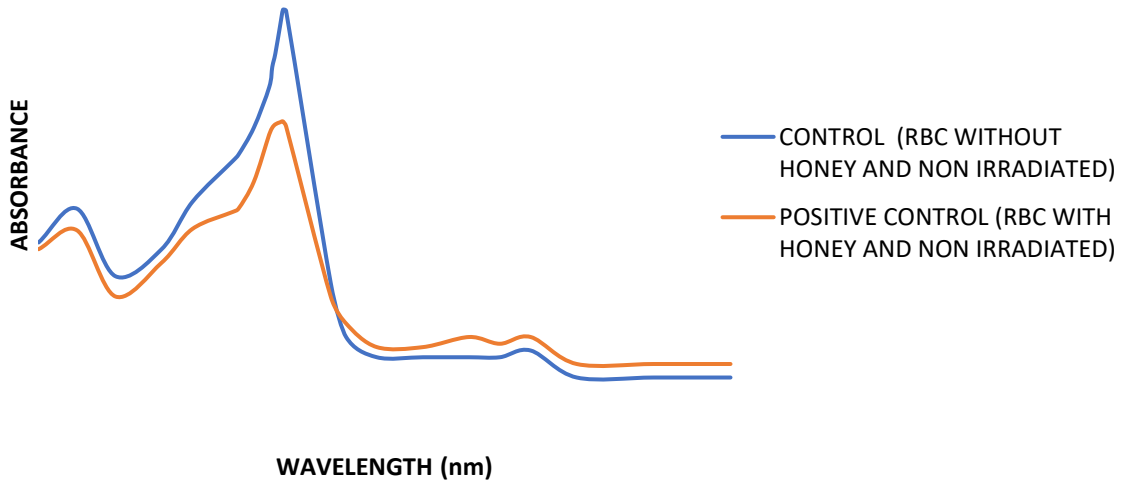

Figure 1: RBC spectrum in UV domain (control sample and positive control sample)

Figure 1 shows the result of hemoglobin absorbance of non-irradiated red blood cell. The hemoglobin absorbance of positive control sample (RBC with honey) was compared with control (that is RBC without honey). The hemoglobin absorbance of positive control sample is 3.792 and the hemoglobin absorbance of control sample is 5.447. The results show that RBC with honey was

able to reduce the hemoglobin absorbance of RBC without honey from 5.447 to 3.792 which amount to 30.4% reduction. This shows efficacy of honey as a radioprotective agent

Results of Hemoglobin Absorbance of Red Blood Cell (RBC) with Honey and without Honey when Irradiated at 50KVp

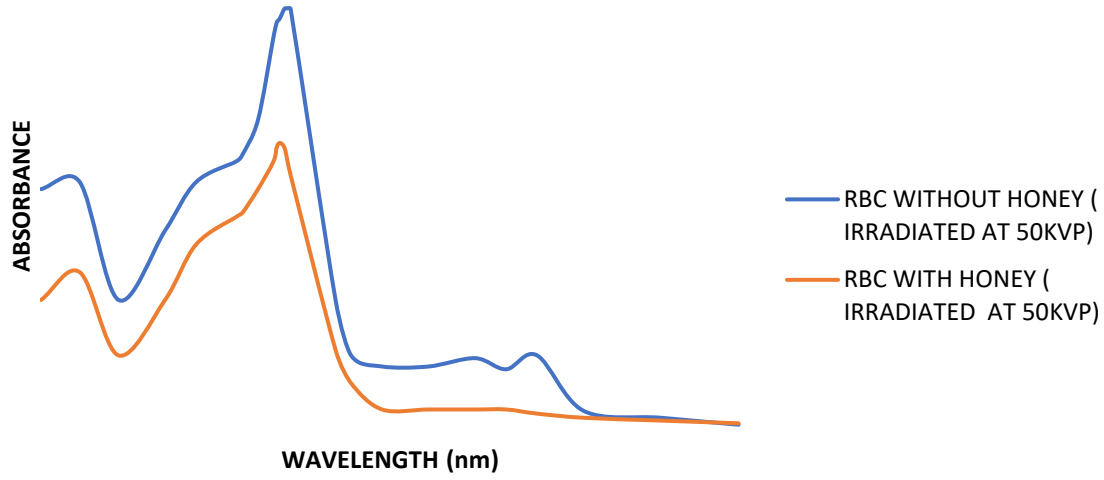

Figure 2: RBC spectrum in UV domain (Sample mixed with and without honey and irradiated at 50 kVp)

Figure 2 shows the results of hemoglobin absorbance of red blood cell with honey and without honey when both are irradiated at 50KVp. The hemoglobin absorbance of RBC with honey was compare with RBC without honey when the voltage across the X-ray was increase to 50KVp. The hemoglobin absorbance of RBC with honey is 2.133 and the hemoglobin absorbance of RBC without honey is 3.103. The results show that RBC with honey was able to reduce the hemoglobin absorbance of RBC without honey from 3.103 to 2.133 which amount to 31.3% reduction. This shows efficacy of honey as a radioprotective agent.

Results of Hemoglobin Absorbance of Red Blood Cell (RBC) with Honey and without Honey when Irradiated at 70KVp

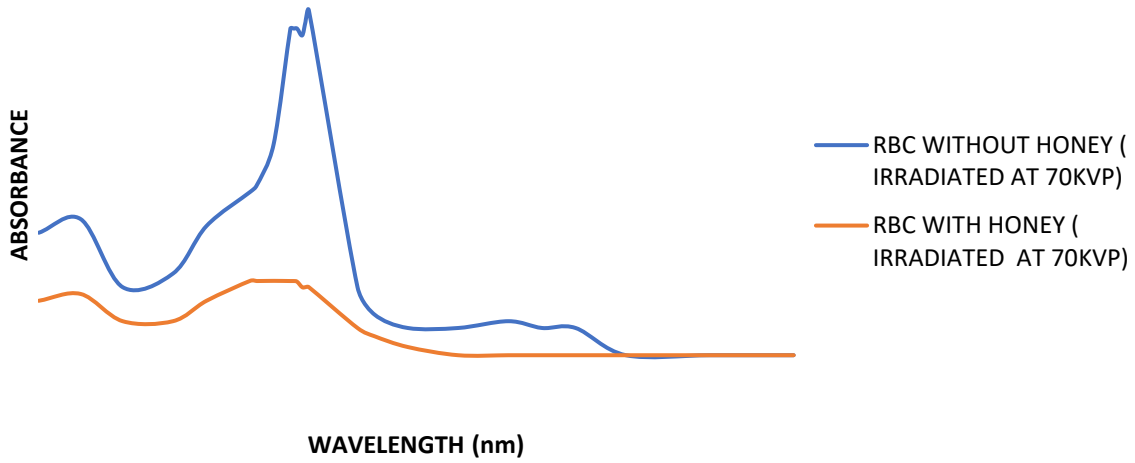

Figure 3: RBC spectrum in UV domain (Sample mixed with and without honey and irradiated at 70 kVp)

Figure 3 shows the results of hemoglobin absorbance of red blood cell with honey and without honey when both are irradiated at 70KVp. The hemoglobin absorbance of RBC with honey was compare with RBC without honey when the voltage across the X-ray was increase to 70KVp. The hemoglobin absorbance of RBC with honey is 1.1093 and the hemoglobin absorbance of RBC without honey is 5.069. The results show that RBC with honey was able to reduce the hemoglobin absorbance of RBC without honey from 5.069 to 1.1093 which amount to 78.1% reduction. This shows efficacy of honey as a radioprotective agent.

Results of Hemoglobin Absorbance of Red Blood Cell (RBC) with Honey and without Honey when Irradiated at 100KVp

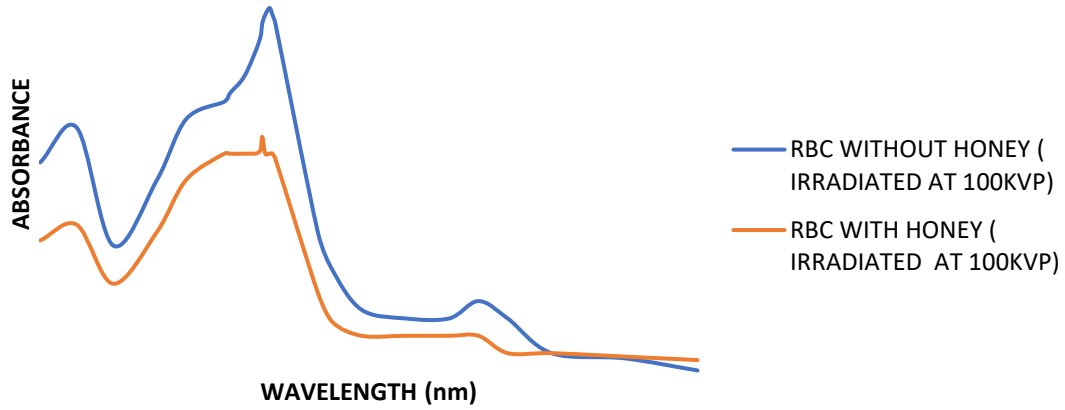

Figure 4: RBC spectrum in UV domain (Sample mixed with and without honey and irradiated at 100 kVp)

Figure 4 shows the results of hemoglobin absorbance of red blood cell with honey and without honey when both are irradiated at 100KVp. The hemoglobin absorbance of RBC with honey was compare with RBC without honey when the voltage across the X-ray was increase to 100KVp. The hemoglobin absorbance of RBC with honey is 1.347 and the hemoglobin absorbance of RBC without honey is 2.092. The results show that RBC with honey was able to reduce the hemoglobin absorbance of RBC without honey from 2.092 to 1.347 which amount to 35.6% reduction. This shows efficacy of honey as a radioprotective agent.

Summarily, all irradiated RBCs have decreased hemoglobin content as compared with the control samples. The absorbance peak for the irradiated sample with honey is lower than that of sample irradiated without honey. This indicates that the antioxidant has fought against the free radicals introduced by X-radiation.

CONCLUSION

The analysis of red blood cells spectrum for the different experimental samples showed that there are variations in the hemoglobin absorption spectra of the red blood cell samples when mixed with honey. The study shows that the spectrophotometric analysis of the red blood cells is a useful tool for the determination of the effect of X-radiation on the properties of RBCs. The hemoglobin absorbance band is within the range (376.00 - 411.00 nm) which determines the characteristics spectrum of the red blood cells. Therefore, the use of X-ray as a radiation sterilizer of blood and blood product and the use of honey as a radiation counter agent has yielded free radical scavenging ability.

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