EFFECT OF X-RAYS ON THE ELECTROLYTE CONCENTRATION OF BOVINE AQUEOUS AND VITREOUS HUMOUR

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Abstract

The eye interacts constantly with a wide range of physically occurring materials like x-rays, air pollutants and toxins. The purpose of this study was to investigate the effects of X-rays on the electrolyte concentration of bovine aqueous and vitreous humour. Aqueous and vitreous humours were obtained from the eyes of certified healthy cows freshly slaughtered in the government abattoir at Ikpoba Slope, Benin City, Edo State, Nigeria. These cows' eyes were enucleated within 2 hours after the death of the animals, and the right eyes were separated from the left eyes. The aqueous humour (AH) and vitreous humour (VH) samples were collected using a 12ml syringe and a needle through scleral puncture made on the lateral canthus and the total extractable aqueous or vitreous humour aspirated from each eye separately. The eyes were transferred to the University of Benin Teaching Hospital (UBTH) Radiology Department where they were irradiated with X-rays machine (VARIAN Medical System model 93061 -7t). Before irradiation, the eyes were removed from ice and allowed to thaw for about 3 minutes. Determination of electrolyte concentration were determined using E110111 flame Photometer (Antex Diagnostics, Beijing, China). Results show that radiation had no statistically significant effect on the mean concentrations of cations and anions in both AH and VH in this study ($p > \{0.05\}$). It is therefore concluded that radiation had no statistically significant effect on the mean concentrations of potassium ion and chloride ion in both AH and VH.

Keywords: X-rays, electrolyte concentration, Bovine humours, radiation

Introduction

The eye is constantly exposed to a wide range of physical occurring materials like X-rays, chemical toxins, air pollutants and biological materials such as microbes on daily basis. X-radiation (composed of X-rays) is an electromagnetic radiation which has a wavelength range of 0.01-10nM and it emanates from the electronic part of the atom. Its energies exist in the region of 120 eV to 120 keV[1]. The eye does not differentiate between any material i.e. microbes, X-rays or toxins, it interacts with; thus it adopts certain mechanisms to deal with all of these agents. Although radiations could have detrimental effects on the eyes, artificial radiations such as; ultrasound, X-rays, and lasers are constantly used for medical diagnosis and therapy [2]. The eye uses a combination of barriers and mechanisms to deal with radiations and microbes, but not all radiations (for example X-rays) can be tolerated, especially at high doses, thus affecting the eye.

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The aqueous humour (AH) is a clear, gelatinous fluid similar to plasma but with a lower protein concentration. The AH is secreted from the ciliary epithelium, and is continuously being produced and reabsorbed[3]. Exposure of the eye to X-rays leads to the formation of cataracts and breakage of Deoxy ribonucleic acid (DNA) in the lens and AH; production of hydroxyl radicals and peroxides that subsequently lead to DNA damage[4].

The Vitreous humour (VH) is responsible for the oval shape of the eye; thus preventing friction, collapse and vibration of the eye organs and tissues. It allows light to be guided to the retina and reduces bleeding in the eye. Ueno et al. (1987)[5] studied the effect of visible light irradiation on the vitreous structure in the presence of a photo-sensitizer. They claimed that the active, charged species of oxygen i.e. the singlet oxygen, superoxide anion, hydrogen peroxide and the hydroxyl radicals generated by the photo-dynamic action of the radiation in the presence of the sensitizer, caused significant liquefaction of the calf vitreous.

When biological tissues are exposed to ionizing radiation, their structural properties such as: shape, size, charge distribution and dipole moment are modified[5]. The resultant change in physicochemical and biological properties can be used to study and characterize the alteration. The physical properties of biological tissues include; electrical conductivity, dielectric constant or relative permittivity, electric potential or self-potential. Analysis of cations and anions present in the fluid, as well as, levels of triglycerides, total protein and antioxidant activities are indicators of the chemical properties.

The effects of different types of radiations on biological tissues have been discussed by several authors[6,7]. Radiations increase the activity of free radicals which initiate degenerative changes that can lead to debilitating conditions[8]. Radiations have been associated with systemic diseases e.g. central nervous system diseases and ocular diseases e.g. cataract. Radiations carry energies, some of which are deposited in materials along its. Thus, it is important to determine the damaging effects of radiations on the eyes.

The eye is one of the body's organs particularly sensitive to radiations from various sources [9,10]. It is also important to note that the effects of radiations on the eye not only depend on the sensitivity of the tissue itself but also on the mode of radiation delivery [11].

This study was aimed at determining the effect of X-rays on electrolyte concentration and biochemical parameters of bovine aqueous and vitreous humour.

MATERIALS AND METHODS

Sample Collection

Aqueous and vitreous humours were obtained from the eyes of certified healthy cows freshly slaughtered in the government abattoir at Ikpoba Slope, Benin City, Edo State, Nigeria. The cows used were between the ages of 10-14yrs (mature cows) and they were all reared in the same environment. These cows' eyes were collected within 2h after the death of the animals, and the right eyes were separated from the left eyes. These eyes were placed on ice to maintain the normal biological state after death[12,13].

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The aqueous humour (AH) and vitreous humour (VH) samples were collected by the method proposed by Coe (1989)[14]. This involved the use of a 12ml syringe and a needle to make scleral puncture on the lateral canthus to allow the total extractable aqueous or vitreous humour to be aspirated from each eye separately.

The eyes were transferred to the University of Benin Teaching Hospital (UBTH) Radiology Department where they were irradiated with X-rays machine (VARIAN Medical System model 93061 -7t). Before irradiation, the eyes were removed from ice and allowed to thaw for about 3 minutes.

The eyes were divided into four (4) groups of 40 eyeballs consisting of 20 eyeballs of left and right and labeled A to D for processing. Sample A were non-irradiated eyes; sample B were bovine eyes irradiated with a low dose (skin dose) of 0.04mSv, which is within the limit of radiation normally used for skull diagnosis; sample C were irradiated with a medium dose of 0.08mSv; and sample D irradiated with a high dose of 0.1mSv.

Determination of AH and VH Electrolytes (Na^+, K^+, HCO_3- and Cl-)

Both the cations and anions in AH and VH were determined using E110111 flame Photometer (Antex Diagnostics, Beijing, China). A combination of the ions (Na^+, K^+ HCO_3^- and Cl-) present in AH/VH can be measured together since all of their electrodes are combined in this auto-analyzer.

Standard comparison was the measurement methodology used by the auto-analyzer; the electrode potentials measured by this instrument was converted to the ion concentration by its microprocessor. The ion concentration was derived by equations. The ions (Na^+, K^+, HCO_3^- and Cl-) present in AH/VH were measured by calibrating the flame photometer and determining the concentrations of the desired ions respectively.

Methods of Data Analysis

All data obtained in this study were analyzed using SPSS version 15. Analysis of Variance (ANOVA), Post hoc Fisher's LSD and student's t-test was used to determine significant differences in measured parameters. Results are presented as Mean ± standard errors of means (Mean± S.E.M). Significance was declared when probability values were $P < 0.05$ or $P<0.01$ [15].

RESULTS

For the aqueous part (Figure 1), normal samples had mean concentration of Na+ for the right eye as 132.40 ± 2.38 mg/dl, for the left eye, it was 133.30 ± 0.86 mg/dl. On radiating it at low dose, there was a decrease in the mean Na+ concentration 130.40±2.50 mg/dl of the right eye, which on increasing the dose to the medium level caused an increase in the mean concentration of Na+ (134.40 ± 0.60 mg/dl), while at high radiation dose, the mean Na+ concentration decreased to 132.40 ± 1.29 mg/dl. For the left eye, on radiating at low dose, the mean Na+ concentration decreased to 132.20 ± 1.02 mg/dl, which at medium radiation dose increased to 135.80 ± 0.58 mg/dl and finally decreased to 131.80 ± 1.11 mg/dl at high radiation.

However, the mean changes in the mean Na+ concentration in the aqueous part of both eyes were statistically not significant (P>0.05)

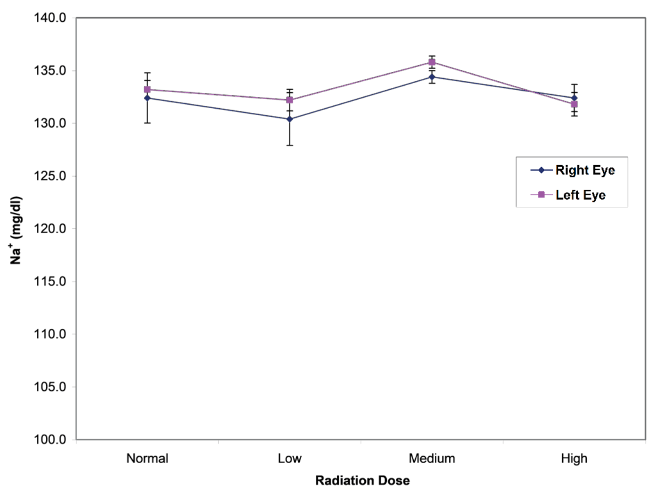

Figure 1: Effect of Radiation on Na^+ of Aqueous part of Right and Left Eye

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For the vitreous part (Figure 2), normal samples had mean concentration of Na^+ for the right eye as 114.75±2.59 mg/dl, for the left eye was 117.80±4.81 mg/dl. On radiating it at low dose, there was a decrease in the mean Na^+ concentration (110.00±6.72 mg/dl) of the right eye, which on increasing the dose to the medium level caused a decrease in the mean concentration of Na^+ (97.40±3.23 mg/dl), while at high radiation dose, the mean Na^+ concentration increased to 104.40±6.62 mg/dl. For the left eye, on radiating at low dose, the mean Na^+ concentration decreased to 117.80±4.81 mg/dl, which at medium radiation dose increased to 110.20±2.94 mg/dl and finally decreased to 109.80±3.68 mg/dl at high radiation.

For the aqueous part (Figure 3), normal samples had mean concentration of K^+ for the right eye as 6.16±0.47 mg/dl, for the left eye was 5.98±0.23 mg/dl. On radiating it at low dose, there was a decrease in the mean K^+ concentration (4.96±0.28 mg/dl) of the right eye, which on increasing the dose to the medium level caused an increase in the mean concentration of K^+ (5.36±0.37 mg/dl), while at high radiation dose, the mean K^+ concentration increased to 6.30±0.80 mg/dl. However, the mean changes in the mean K^+ concentration in the vitreous part of both eyes were statistically not significant (P>0.05).

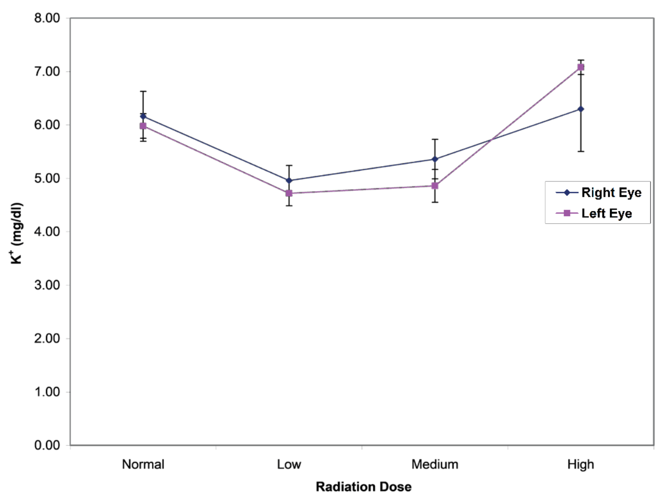

Figure 2: Effect of Radiation on K+ of Aqueous part of Right and Left Eye

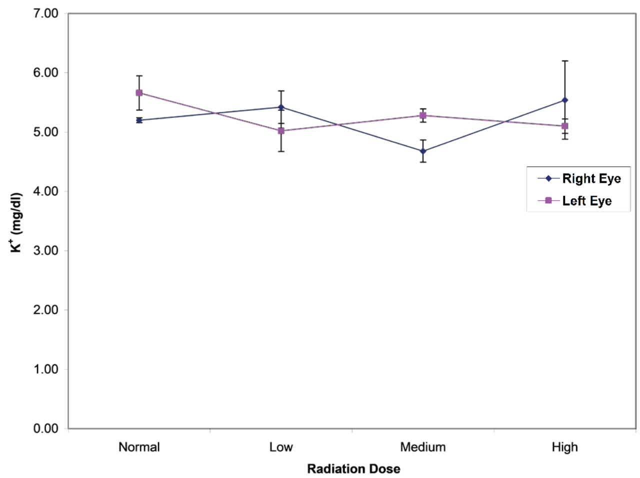

Figure 3: Effect of Radiation on K+ of Vitreous part of Right and Left Eye

For the aqueous part (Figure 4), normal samples had mean concentration of HCO_3- for the right eye as 16.40±0.68 mg/dl, while the left eye was 16.60±0.68 mg/dl. On radiating it at low dose, there was a decrease in the mean HCO_3- concentration (12.00±0.32 mg/dl) of the right eye, which on increasing the dose to the medium level also decreased the mean concentration of HCO_3- (11.60±1.03 mg/dl), while at high radiation dose, the mean HCO_3- concentration increased to 12.20±0.97 mg/dl. For the left eye, on radiating at low dose, the mean HCO_3- concentration decreased to (15.40±0.68 mg/dl), which at medium radiation dose was constant 15.40±1.29 mg/dl and finally decreased to 14.20±1.36 mg/dl at high radiation dose.

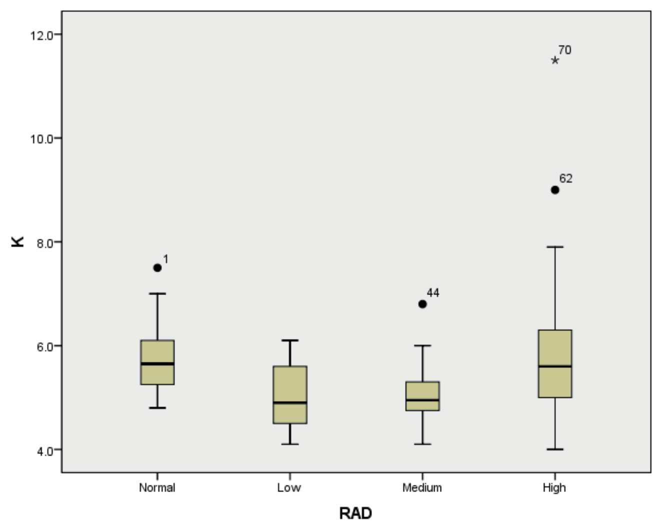

Figure 4: Mean and standard deviations of potassium concentration in both vitreous and aqueous humours. (P>0.05)

Chloride

For the aqueous part (Fig 5), normal samples had mean concentration of Cl- for the right eye as 101.20±2.42 mg/dl, for the left eye, it was 103.60±0.75 mg/dl. On radiating it at low dose, there was an increase in the mean Cl- concentration (107.60±2.14 mg/dl) of the right eye, which on increasing the dose to the medium level also increased the mean concentration of Cl- (111.60±1.47 mg/dl), while at high radiation dose, the mean Cl- concentration decreased to 108.80±1.02 mg/dl. For the left eye, on radiating at low dose, the mean Cl- concentration increased to (104.00±1.41 mg/dl), which at medium radiation dose was increased to 106.80±1.62 mg/dl and finally decreased to 106.00±1.10 mg/dl at high radiation dose. However, the mean changes in the mean Cl- concentration in the vitreous part of the left eye were statistically not significant (P>0.05), while at medium irradiation of the right eye showed statistical significance (P<0.05) with the normal.

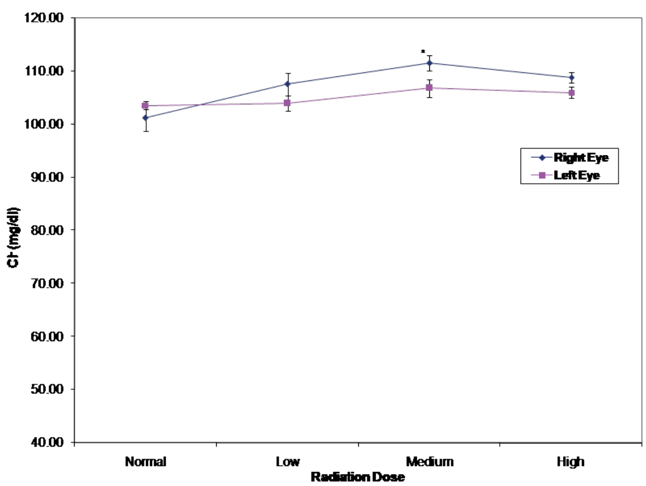

Figure 4: Effect of Radiation on Cl- of Aqueous part of Right and Left Eye

DISCUSSION

Biochemical analysis showed that sodium concentration observed in the aqueous part (Fig 1) for normal samples had mean concentration of 132.40±2.38 mg/dl for the right eye and 133.30±0.86 mg/dl for the left eye. Also, at high radiation dose, the mean Na^+ concentration was 132.40±1.29 mg/dl for the right and 131.80±1.11 mg/dl for the left eye. However, the mean changes in the mean Na^+ concentration in the aqueous part of both eyes were statistically not significant (P>0.05). For the vitreous part, normal samples had mean concentration of Na^+ for the right eye as 114.75±2.59 mg/dl, for the left eye was 117.80±4.81 mg/dl. High radiation dose showed 104.40±6.62 mg/dl for the right vitreous humour and 109.80±3.68 mg/dl for left vitreous humour. However, the mean changes in the mean Na^+ concentration in the vitreous part of both eyes were statistically not significant (P>0.05). Measured value of sodium concentration ranged between 80 mmol/L and 138 mmol/L. The mean changes in the mean Na^+ concentration in the aqueous and also in the vitreous part of both eyes when subjected to varying doses of irradiation were statistically not significant (P>0.05). In a research by Chandrakanth et al. (2013)[16], there was no significant difference in the differences in vitreous sodium, potassium, chloride levels and the sodium potassium ratio among males and females and between right and left eyes.

The potassium ion concentration in the aqueous humour for non- irradiated sample (Fig 4.36) was 6.16±0.47 mg/dl for the right eye and 5.98±0.23 mg/dl for the left eye. High irradiation gave 4.72±0.23 mg/dl for the right eye and 7.08±1.14 mg/dl for the left eye. For the vitreous humour (Figure 4.37), normal samples had mean concentration of K+ for the right eye as 5.20±0.04 mg/dl, while the left eye was 5.66±0.29 mg/dl. High irradiation gave 5.54±0.66 mg/dl for right eye and 5.10±0.12 mg/dl for the left eye. However, the mean changes in the mean K+ concentration in aqueous and vitreous humours of both eyes

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were statistically not significant (P>0.05). Measured value of potassium concentration ranged between 4 mmol/L and 11.5 mmol/L(Fig 4.38). Garg *et al*. (2004)[17] made similar observation that there was no difference in mean vitreous potassium level when the left eye was compared to the right eye. Also parameters like age, sex, temperature and humidity had no effect on the concentration of vitreous potassium. However, mean vitreous potassium showed a linear rise with increasing death interval[18].

The bicarbonate concentration for the aqueous humour in normal samples was 16.40±0.68 mg/dl for right eye while the left eye was 16.60±0.68 mg/dl. On irradiation at low dose, there was a decrease in the mean HCO_3^- concentration (12.00±0.32 mg/dl) of the right eye, which on increasing the dose to the medium level also decreased the mean concentration of HCO_3^- (11.60±1.03 mg/dl), while at high radiation dose, the mean HCO_3^- concentration increased to 12.20±0.97 mg/dl. For the left eye, on radiating at low dose, the mean HCO_3^- concentration decreased to (15.40±0.68 mg/dl), which at medium radiation dose was constant 15.40±1.29 mg/dl and finally decreased to 14.20±1.36 mg/dl at high radiation dose. However, the mean changes in the mean HCO_3^- concentration in the aqueous part of the left eye were statistically not significant (P>0.05), while at low, medium and high irradiation of the right eye showed statistical significance (P<0.05) with the normal. In the vitreous, normal samples had mean concentration of HCO_3^- for the right eye as 19.25±0.48 mg/dl, while the left eye was 19.40±0.40 mg/dl. On radiating it at low dose, there was a decrease in the mean HCO_3^- concentration (15.60±1.03 mg/dl) of the right eye, which on increasing the dose to the medium level also decreased the mean concentration of HCO_3^- (14.00±1.14 mg/dl), while at high radiation dose, the mean HCO_3^- concentration increased to 16.60±1.72 mg/dl. For the left eye, on radiating at low dose, the mean HCO_3^- concentration decreased to (14.60±0.93 mg/dl), which at medium radiation dose was increased to 15.40±1.33 mg/dl and finally decreased to 15.40±0.68 mg/dl at high radiation dose. However, the mean changes in the mean HCO_3^- concentration in the vitreous part of the right eye were statistically not significant (P>0.05), while at low, medium and high irradiation of the left eye showed statistical significance (P<0.05) with the normal.

Measured values of concentrations of carbonate ions ranged between 9.0 mmol/L and 20.0 mmol/L. The importance of bicarbonate ions has been highlighted in literature, they are important for proper retina function when they are at normal levels in the vitreous 15-25 mmol/L but when the levels in vitreous increases to about 35-45 mmol/L, it inhibits retina function [19]. From the results, the mean changes in the HCO_3^- concentration in the vitreous part of the right eye were statistically not significant (P>0.05), while at low, medium and high irradiation of the left eye showed statistical significance (P<0.05) with the normal. This means that there was no statistically significant effect of radiation on the bicarbonate concentration in the vitreous, which was within the normal range. Dufour (1982)[20] observed that there was no significant change in vitreous calcium postmortem nor significant correlation between vitreous calcium and antemortem serum calcium.

Chloride ion concentrations for normal samples had 101.20±2.42 mg/dl for the right and 103.60±0.75 mg/dl for the left. High radiation for the right was 108.80±1.02 mg/dl and 106.00±1.10 mg/dl for the left. The mean changes in the mean Cl^- concentration in the aqueous part of the left eye were statistically not significant (P>0.05), while at medium irradiation of the right eye showed statistical significance (P<0.05) with the normal. For the VH , normal samples had mean concentration of Cl^- for the right eye as

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91.00±0.58 mg/dl, the left eye was 92.00±3.41 mg/dl. On irradiation at low dose, there was a decrease in the mean Cl- concentration (84.40±4.79 mg/dl) of the right eye, which on increasing the dose to the medium level also decreased the mean concentration of Cl- (70.80±2.94 mg/dl), while at high radiation dose, the mean Cl- concentration increased to 77.80±6.04 mg/dl. For the left eye, irradiation at low dose, the mean Cl- concentration decreased to (80.40±3.87 mg/dl), which at medium radiation dose was increased to 85.20±2.06 mg/dl and finally decreased to 84.40±3.19 mg/dl at high radiation dose. However, the mean changes in the mean Cl- concentration in the vitreous part of the left eye were statistically not significant (P>0.05), while at medium irradiation of the right eye showed statistical significance (P<0.05) with the normal. Measured value of concentration of chloride ions ranged between 9.0 mmol/L and 20.0 mmol/l. This shows that radiation does not have significant effect on the chloride concentration of the aqueous and vitreous humours. The difference between the right eye and the left eye could be due to biochemical variations in the eyes of the animals.

Conclusion

Electrical currents in biological tissues are carried by components and structures that have a net electric charge and/or an electric dipole moment. Therefore in colloid-disperse system such as biological tissues, an electric field is determined by the concentration and behaviour of its chemical constituents. In the eyes, the structural macromolecular tissues such as, the tissues of the vitreous humour are still subjects of intensive investigation on how they interact to form the vitreous gel. Radiation had no statistically significant effect on the mean concentrations of potassium ion and chloride ion in both AH and VH in this study. One limitations to the study included the fact that irradiation would have been done immediately the eyes were enucleated to avoid freezing and subsequent thawing.