Original Article

Sahel Journal of Veterinary Sciences

Sahel J. Vet. Sci. Vol. 20, No. 4, Pp 20-26 (2023)
http://doi.org/10.54058/saheljvs.v20i4.375

Article History
Received: 17-03-2023
Revised: 08-07-2023
Accepted: 18-12-2023
Published: 31-12-2023

The Effect of High Ambient Temperature on Acid-Base Balance and Electrolyte Parameters in Rats

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ABSTRACT

Exposure to high environmental temperature could lead to different heat-related illness as well as worsening some disease conditions. Thirty rats were used to determine the nature of the disturbances of the acid-base balance and electrolytes balance of blood serum after exposure to high ambient temperature. Twenty rats which formed the experimental group were exposed to sun for 30 minutes daily for two weeks, while the remaining rats served as the unexposed control group. The serum pH, serum HCO_3^- and electrolytes of both the groups in the first week and second week were obtained using pH meter, back titration, flame photometry, mercurite titrimetric and spectro-photometry. Histopathology of the lungs and kidneys of rats from each group was carried out using basic histological technique. Results show decreased level of serum HCO_3^- and increased level of serum pH in the ambient temperature exposed group. In the second week there was significant difference in HCO_3^- levels ($p<0.05$). The serum pH in 1st and 2nd week did not show any significant difference about change in the temperature. The findings also showed that high ambient temperature has significant effect on serum sodium (Na^+), serum chloride (Cl^-) and serum potassium levels (K) ($p<0.05$); but did not affect serum calcium (Ca^+) level ($p>0.05$). There was glomerular degeneration, interstitial haemorrhage, and tubular distension due to fluid retention in the kidney as revealed by the photomicrograph of the exposed rat kidneys. In the lungs of the exposed rat, there was mild interstitial haemorrhage and peribronchiolar epithelial shading. In conclusion, this study indicates that serum calcium is not significantly affected like in the case of serum sodium, chloride and potassium during heat stress and respiratory alkalosis is the predominant acid-base disorder in rats exposed to sun due the occurrence of hyperventilation in heat stress. This research provided some references of acid-base and electrolytes disorder in rats exposed to high ambient temperature.

Keywords: Acid-base balance; Electrolytes; Rats; Serum; Temperature

INTRODUCTION

During exposure to environmental extreme heat, body water and electrolyte imbalances are common (Sawka *et al*., 2007) and high sweat rate for many hours results in electrolytes imbalances (Montain *et a*l., 2006). Heat related illness and mortality are significant health problem in the world today (Grogan and Hopkins, 2002). As global warming has resulted in heat waves in climates that were previously temperate, there has been an increase in weather-related heat deaths (McGeehin and Mirabelli, 2001; Bernstein and Rice, 2013). Predisposing factors in deaths attributed to high-environmental temperature include lack of familiarity with environmental conditions, excessive clothing, prolonged sun exposure, use of certain drug and medication, acute alcohol intoxication, obesity, underlying dementia, alcoholic liver disease, living alone, advanced age, kidney or respiratory problem, pre-existing cardiac psychiatric disease and (possibly) epilepsy (Gauer and Meyers, 2019). While in Europe, Australia and USA excessive exposure to the sun occurs for cosmetic reasons among the Caucasians; in a nation like Nigeria, it is more for economic reasons e.g. street trading (Swerdlow and Weinstock, 1998). Hyperthermia could lead to heat stress, progressing to heat exhaustion, heat stroke, and culminating in all organ dysfunction and death in some instances (Bouchama and De Vol, 2001).

The term pH is defined as the negative base 10 logarithm of the hydrogen ion concentration expressed in equivalents per liter or the base 10 logarithm of the reciprocal of the hydrogen ion concentration: pH = log1o [1/[H^+]] (Madias *et al*., 1982). The first line of defence against disease is a proper pH balance. The blood pH is important because even a minor deviation from normal range can severely affect many organs. When pH deviates from its normal range, pH-dependent enzymes and membrane transport proteins may not work properly and

metabolic pathways can be negatively affected. Acidemia, which is defined as arterial pH lower than 7.35, can cause a variety of disturbances including arterial vasodilation, insulin resistance, compromised immune function, and reduced neuronal excitability. Alkalemia, which is defined as arterial pH greater than7.45, can also cause many disturbances including reduced myocardial blood flow and seizures (Quade *et al.*, 2021). Studies in animals suggest that the acid-base responses to heat stress is dynamic and progresses in a predictable pattern. Oxen, dogs and rats passively exposed to heat developed respiratory alkalosis (Hales and Webster, 1967; Magazanik and Shapiro, 1980).

The four most important electrolytes of physiological significance are sodium ion (Na^+), chloride ion (Cl^-), potassium ion (K^+) and calcium ion (Ca^{2+}) (Sawka *et al*., 2007). Sodium and chloride ions are the principal extracellular cation and anion respectively. Among other functions, they are both critical for the maintenance of acid-base balance and osmolality (Carlson, 1997). Potassium plays a key role in regulation of acid-base balance, osmolality, activation of several enzymes and in nerve transmission (Pohl *et al.*, 2013). Calcium ion plays a key role in many physiologic processes including contraction of skeletal, cardiac and smooth muscles, blood clotting and transmission of nerve impulse (Hall and Hall, 2020). These electrolytes in combine functions help in regulation of body heat. For instance, potassium and calcium involve in transmission of nerve impulses to the temperature-regulating centre located in the hypothalamus and stimulation of sympathetic pathway resulting in sweating, brought about by vasodilatation of peripheral blood vessels. On the other hand, sodium and chloride ion involved in the maintenance of body fluid and blood pressure, blood volume and pH regulation (Wiig *et al*., 2018; Seifter, 2019). Many conditions including congestive heart failure, liver failure, kidney failure and pneumonia are commonly associated with a low sodium concentration in the blood (Rosner and Kirven, 2006). Animal deficient in electrolytes licks almost any object, including the sweat of other animals (Dow *et al*., 1987).

Very few works or perhaps none was recorded in this part of the world on the effect of high environmental temperature on acid-base balance and electrolytes. The aim of this study is to determine the effect of high environmental temperature on acid-base balance and electrolytes in rats.

MATERIALS AND METHODS

Study Area

This research was conducted in Maiduguri the capital city of Borno State. The state is in the North-Eastern part of Nigeria with a land mass of 69,435 sq.km. It lies between latitudes 10° -13°N and longitudes 12°-l5°E (weether.com). The seasons includes the cold-dry (harmattan) season (October-February), the hot-dry season (March-June) and rainy season (July-September) (Ishaku and Majid, 2010). Temperatures are high all year round especially during the daytime, with hot season mean temperature, ranging between 39°C and 40°C under the shade in the Northern part of the state. Two vegetation zones are identified as the Sudan and Sahel Savannah to the South and North respectively (www.bornostate.gov.ng). The research was carried out in the hot dry season between April and May with temperature ranging between 39 °C and 42 °C.

Experimental Animals

Thirty (30) albino rats of both sexes with average weight of 150g were used for the study. They were kept in plastic cages and provided feed and water ad libitum, at the Department of Veterinary Physiology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri, Borno state, Nigeria. The rats were divided at random into two groups: 20 rats in group A (ambient temperature exposed) and 10 rats in group B (unexposed control).

Effect of Ambient Temperature Exposure on the Animals

The rats that were in group A (experimental group) were exposed to temperature of 41°C for 30 minutes daily for two weeks and the rats in the group B (control group) were kept at normal room temperature of 37°C.

Sample Collection

Blood samples were collected on weekly basis for a period of 2 weeks from ten (10) rats per week into sterile, plain vacutainer sample bottles and transported in plastic flask to University of Maiduguri Teaching Hospital (UMTH) Laboratory for analysis of serum pH, bicarbonate, and electrolytes. The lungs and kidneys of the sacrificed rats at the second week were collected and stored in 10% formalin, until processed. Handling of animals and sample collection were done in compliance with the International Guiding Principles of Biomedical Research Involving Animals by the Council for International Organisations of Medical Sciences and International Council for Laboratory Animal Science (2012).

Laboratory Procedure

The blood samples were centrifuged at 4000 revolution per minute for five minutes. The serum obtained was stored at -20°C until required for analysis.

Measurement of Blood pH

Blood pH was measured potentiometrically using the pH meter.

Estimation of Serum Bicarbonate

Control tube was prepared by mixing 0.1 ml of serum to 5.0 ml of 1% normal saline, 2 drops of phenol red indicator was added until it turns to bright pink colour. This colour corresponds with the end point of the titration.

Then 4ml of 1% saline was put into another tube. 1m1 of 0.0 1N HCI was added and then followed by 0.lml of serum and 2 drops of phenol red indicator. The above mixture (in the 2nd tube) was titrated against 0.0 1N NaOH till the colour changed to red from yellow. The colour change of the end point was compared with the initial control tube (1st tube) and the reading (Xml) was noted (i.e., the required ml of 0.0 1N NaOH to change the colour). The serum bicarbonate was obtained by the following calculation: Serum bicarbonate mmol/L = (1-Xml of 0.01N NaOH required) x 100 (Ochei and Kolhatkar, 2000).

Determination of Serum Sodium and Potassium by Flame Photometry Method

Sodium or potassium solution (under carefully controlled conditions) was finely sprayed (aspirated) into a burner. The flame de-solvate the solution leaving solid (salts) which later

dissociate to give natural ground state atoms and some of these atoms are excited in the flame thus moving into a higher energy state which emits light of characteristic wavelength (590nm for sodium and 770nm for potassium). This light, which is directly proportional to the concentration of sodium or potassium in the solution, was measured after being directed through a suitable filter onto a photosensitive element.

Determination of Serum Chloride by Mercuric Titrimetric Method (Schales and Schales, 1971)

Mercuric nitrate solution was added to a solution containing chloride, unionized but soluble mercuric Chroride was formed. At the end point-point, the first excess mercuric ions combined with the indicator-diphenyl carbazone to give violet-blue coloured complex.

$$\text{Calculation, Mg\% chloride} = \frac{ml\ mercuric\ nitrate\ required\ for\ the\ unknown}{ml\ mercuric\ nitrate\ required\ for\ the\ standard}$$

Normal values 95-110 Mmol/L

Determining of Serum Calcium by Spectro-photometry Method

The content of the test tubes with the solution were mixed and incubated at room temperature for 10 minutes. Calcium reacted with methylthymol to produce deep blue coloured complex whose absorbance was read at 578nm. Normal value 2.3-2.7 Mmol/L

Tissue Preparation for Histology

Tissues collected from the kidneys and lungs were immerse-fixed in 10% formalin for 24–36 hours, dehydrated in a graded series of alcohol, embedded in paraffin blocks and sections of 4µm thick were made and collected on glass slides. The slides were stained with Haematoxylin and Eosin (H & E), for histological assessment. Tissue sections were viewed at 10x and 40x magnification, using a microscope fitted with a digital camera, coupled to a computer with Analysis Imaging Processing software (Winsor, 1994).

Statistical Analyses

The data collected were presented as mean ± SD and the differences between the means of the two groups were analysed by student 't' test using computer statistical software GraphPad InStat® Inc., San Diego California USA (Motulsky, 1998). P values greater than or equal to 0.05 was considered not significant.

RESULTS

The serum bicarbonate concentration of rats exposed to high ambient temperature (41 °C) shows a significant difference ($p < 0.05$) in the second week, compared with the control group of rats (Table 1). Table 2 shows the serum pH of the rats exposed to high ambient temperature and control group of rats; the pH did not show any differences ($p > 0.05$) due to the change in temperature.

Table 3 shows the serum sodium concentration of rats exposed to high ambient temperature and control group of rats. Table 4 shows significant difference in serum chloride concentration level ($p<0.05$) and Table 5 shows significant difference in serum potassium while Table 6 shows no significant difference in serum calcium level ($p>0.05$) when compared with respective control groups.

Figure 1A and 2A are photomicrographs of rat's kidneys obtained after exposure to high ambient temperature. There was glomerular degeneration, interstitial haemorrhage and tubular distention due to fluid retention. Figure 3A and 4A are photomicrographs of ambient temperature, showing mild interstitial haemorrhage and peribronchiolar epithelia shading.

Table 1: Serum Bicarbonate Concentration of Rats Exposed to High Ambient Temperature and Control

Period of study (weeks)	Experimental group (exposed at 41 ºC) Serum HCO_3(mmol/L) (n) Mean ± SD	Control group (normal room temp. 37 ºC)
1	(5)19.60±1.14	(5)20.20±1.30
2	(6)20.00±0.63	(4)20.75±0.50*

*p<0.05 (n) number of rats

Table 2: Serum pH of Rats Exposed to High Ambient Temperature and Control

Period of study (weeks)	Experimental group (exposed at 41 ºC) Serum pH (n) Mean ± SD	Control group (normal room temp. 37 ºC)
1	(5)7.12±0.83	(5)6.32±1.30
2	(6)8.73±0.63	(4)8.70±0.20

(n) number of rats

Table 3: Serum Sodium Concentration of Rats Exposed to High Ambient Temperature and Control.

Period of study (weeks)	Experimental group (exposed at 41 ºC) Serum sodium (Mmol/L) (n) Mean ± SD	Control group (normal room temp. 37 ºC)
1	(5)146.4±2.61	(5)140.0±2.61*
2	(6)142.67±2.42	(4)139.75±1.71*

*p<0.05; (n) number of rats

Table 4: Serum Chloride Concentration of Rats Exposed to High Ambient Temperature and Control.

Period of study (weeks)	Experimental group (exposed at 41 ºC) Serum chloride (Mmol/L) (n) Mean ± SD	Control group (room temp 37ºC)
1	(5)115.60±4.34	(5)102.00±2.00*
2	(6)113.67±4.27	(4)108.50±1.92*

*p<0.05; (n) number of rats

Table 5: Serum Potassium Concentration of Rats Exposed to High Ambient Temperature and Control

Period of study (weeks)	Experimental group (exposed at 41 ºC) Serum potassium (Mmol/L) (n) Mean ± SD	Control group (room temp 37ºC)
1	(5)6.42±1.26	(5)7.52±0.48*
2	(6)5.07±0.23	(4)5.58±0.29*

*p<0.05; (n) number of rats

Table 6: Serum Calcium Concentration of Rats Exposed to High Ambient Temperature and Control

Period of study (weeks)	Experimental group (exposed at 41 ºC) Serum calcium (Mmol/L) (n) Mean ± SD	Control group (room temp 37ºC)
1	(5)2.78±0.08	(5)2.86±0.05
2	(6)2.75±0.12	(4)2.95±0.26

(n) number of rats

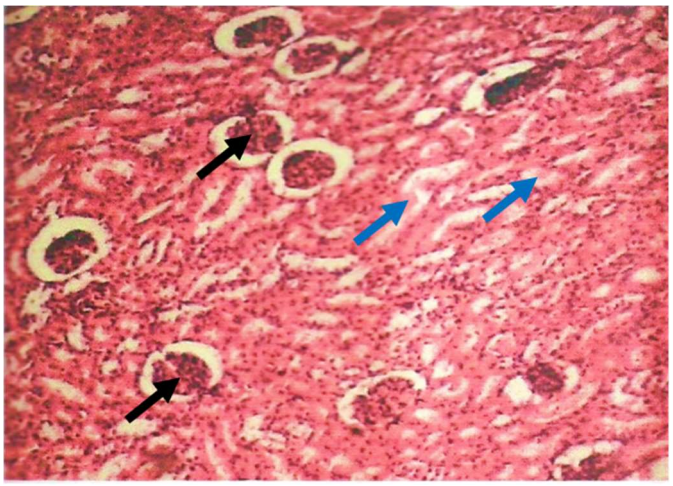

Figure 1A: Photomicrograph of rat kidney exposed to high ambient temperature showing mild glomerular degeneration (black arrows) and interstitial haemorrhage (blue arrows) in the cortex H&E, x 100

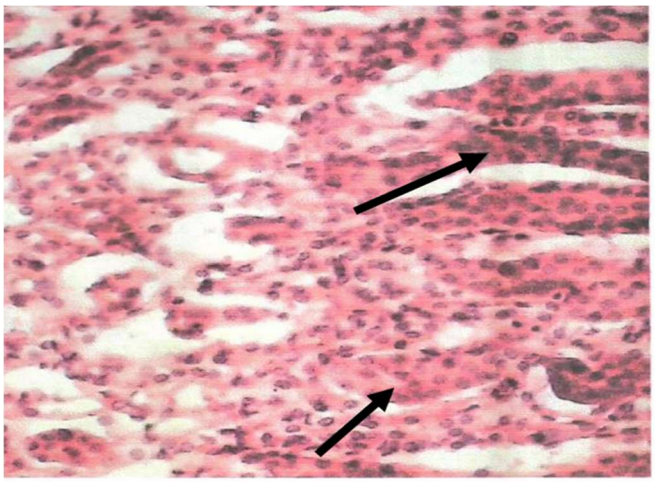

Figure 2A: Photomicrograph of rat kidney exposed to high ambient temperature showing tubular ballooning (black arrows) in the medulla H&E, x 400

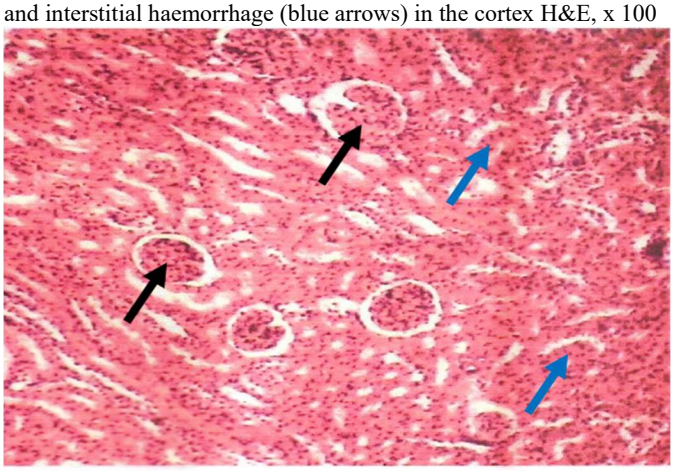

Figure 1B: Photomicrograph of rat kidney showing cortex with normal glomeruli (black arrow) and renal tubules (blue arrow) H&E, x 100 (control group)

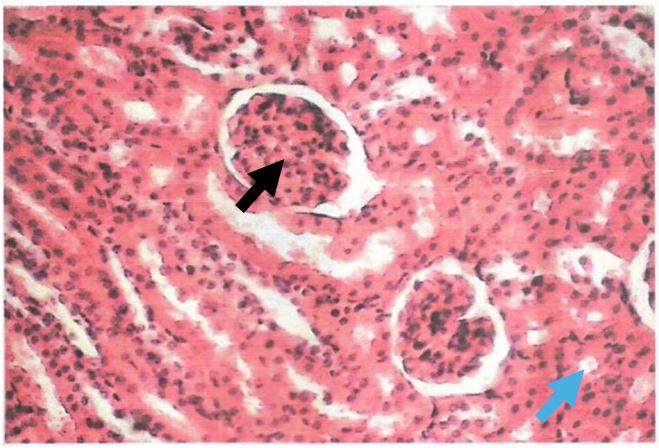

Figure 2B: Photomicrograph of rat kidney showing normal glomeruli (black arrow) and renal tubules (blue arrow) H&E, x 400 (control group)

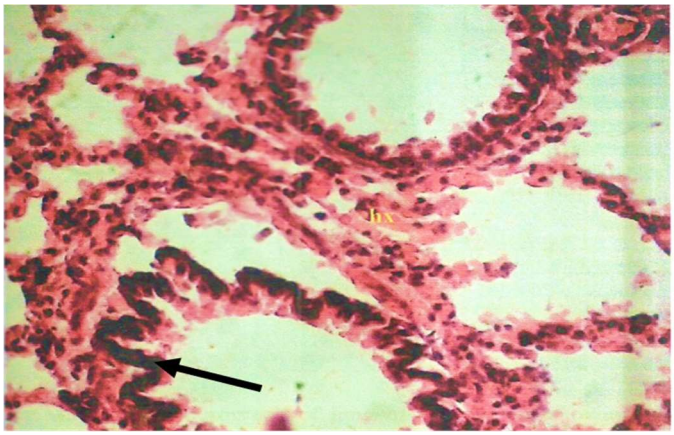

Figure 3A: Photomicrograph of rat lungs exposed to high ambient temperature showing interstitial haemorrhage (hx) and peribronchiolar epithelial (black arrow) H&E, x 400

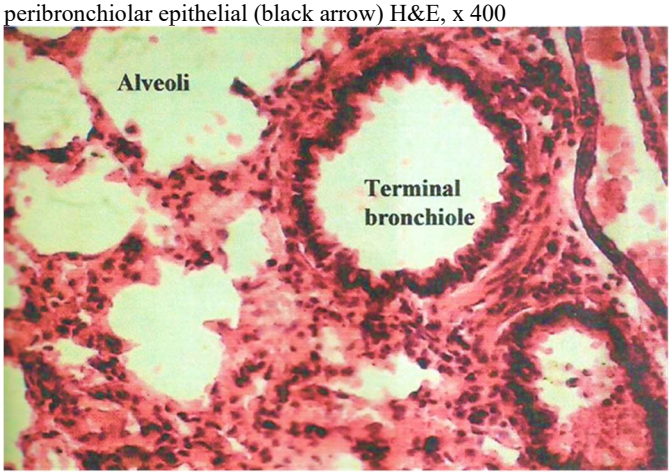

Figure 3B: Photomicrograph of rat lungs showing normal terminal bronchiole and alveoli H&E, x 400 (control group)

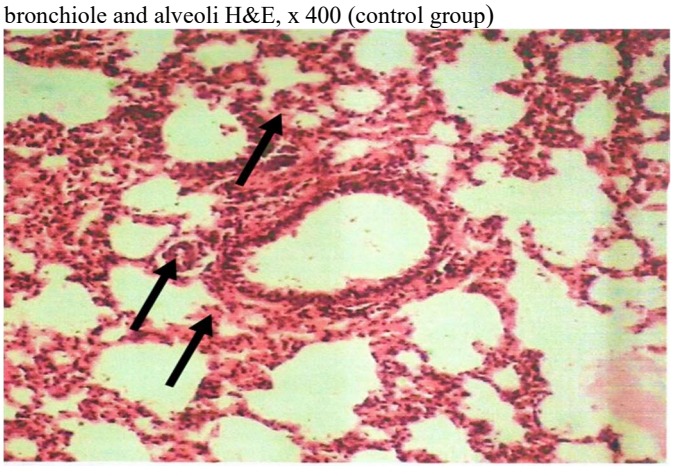

Figure 4A. Photomicrograph of rat lungs exposed to high ambient temperature showing interstitial haemorrhage (black arrows) H&E, x 100

DISCUSSION

In this study the high ambient temperature did not affect the bicarbonate level at first week but affected it during the second week. Gaudio and Abramson (1968), demonstrated that in volunteers exposed to heat, respiratory alkalosis occurred; they attributed it to an increase in alveolar ventilation. Their work agrees with this present work, because the rats were kept in open space with proper ventilation. It is apparent from the changes presented between the control and the test group in both table 1 and 2, that the results of this study support the suggestion that the major factor responsible for the disturbance of the acid-base balance of the blood in heat stress at the initial stage was hyperventilation. This is borne out by the lowered pCO_2, the elevated pH and the lowered ($BHCO_3$) in the experiments as reported by Wayne *et al.* (1938). Observations made upon blood obtained in the hourly experiments also support this view (Wayne *et. al*., 1938). This hypothesis is supported by other researchers, who have demonstrated a relationship between the rise in minute ventilation and body temperature (Barltrop, 1954; Gaudio and Abramson, 1968). Hales (1967), Magazanik (1980) and Zurovski (1991), passively exposed oxen, dogs and rats to heat respectively, developed respiratory alkalosis; though they suggested that metabolic acidosis predominates when threshold body temperature is reached, and if the exposure is prolonged. Bouchama and De Vol (2001), who disagree with this present work, suggested that the prevalence of respiratory alkalosis was independent of the degree of core temperature. To them, lactic acid production due to a heat-induced, increase metabolic rate and/or development of local hypoxia, resulted to metabolic acidosis in (prolong) extreme temperature rather than respiratory alkalosis in (short) moderate temperature elevation. The exposed rats were dehydrated which may have led to lactic acidosis, however this was not evident in the pH result. This may mean that even at high temperature, the rats were still able to maintain homeostasis by sweating. Regardless of the pathogenesis of heat-associated acid-base changes, the present study shows that heat-stress affects the kidneys and lungs. As reported by Knaus *et al*. (1985), more than one-third of heat-stroke patients had failure in more than two organ systems.

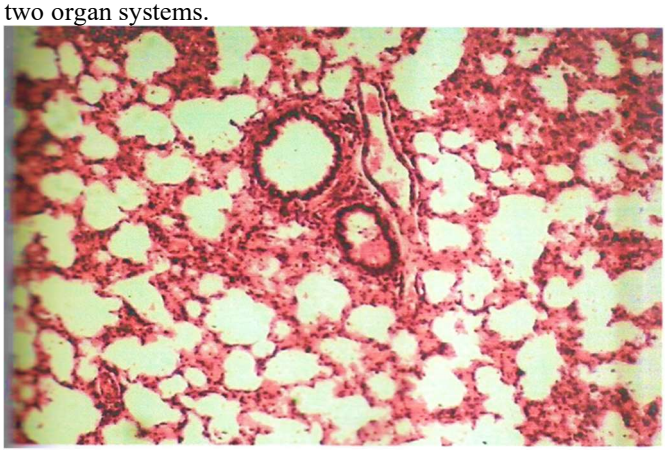

Figure 4B. Photomicrograph of rat lung of control group showing normal alveoli H&E, x 100 (control group)

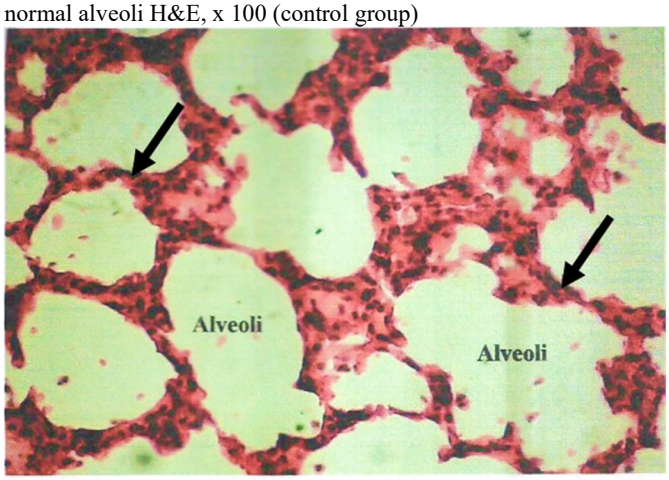

Figure 4C. Photomicrograph of rat lungs showing normal alveoli and septae (black arrow) H&E, x 400 (control group)

In this study the high ambient temperature has effect on the serum sodium and chloride concentration both at first and second weeks. This agrees with the work demonstrated by Senay (1968), and Kubica et. al. (1983) which stated that sweat-induced dehydration will decrease plasma volume and increase plasma osmotic pressure in proportion to the amount of fluid loss. Sodium and chloride primarily responsible for the elevated plasma volume in dehydrated individuals. The work is also in agreement with Sawka and Greenleaf (1992), research, which stated that heat acclimated persons have hyper osmolality to exert an osmotic pressure and redistribute and redistribute fluid from the intracellular space.

The study also shows that high ambient temperature has effect on serum potassium level at both first and second weeks. This agrees with the work demonstrated by Sevastos *et. al*. (2006) which stated that pseudohyperkalemia occurs due to excessive leakage of potassium from cells, during or after blood is drawn, typically cause by haemolysis. In the same vein, Sawka and Greenleaf (1992), deduced that acclimated persons showed hyperosmolality which causes shift of potassium into extracellular space, resulted into hyperkalemia.

The study showed further that high ambient temperature has no effect on serum calcium level at both first and second weeks. The finding agrees with the research conducted by (Lin *et al*., 2000) which stated that the concentration of calcium after exposure to high ambient temperature is relatively normal while Allahverdi et al. (2013), reported that there is significant decrease in Ca^{2+}concentration due to decrease feed intake during heat stress. Generally, the rate of absorption of divalent ions such as calcium ion (Ca^{2+}) is relatively slower (Rastogi, 2007).

Conclusion

The results of this study suggest that high ambient temperature can alter the serum levels of HCO^{3-} in rats. This shows that respiratory alkalosis is the predominant acid-base disorder in the initial (short) period of elevated temperature in rats. High ambient temperature also has significant effects on serum sodium, chloride and potassium levels but does not have effect on serum calcium concentration.

Competing Interests

The authors have no conflict of interest to declare.

Acknowledgements

The authors appreciate University of Maiduguri Teaching Hospital laboratory for assisting in the laboratory analysis.

Author's Contributions

MUS and SBA conducted the research, collected samples, and wrote the manuscript. AMW analysed histological data and reviewed the manuscript. WB and IMW provided laboratory assistance. UKS designed and supervised the research, analysed data and reviewed the manuscript. All authors have read and approved the final manuscript.

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