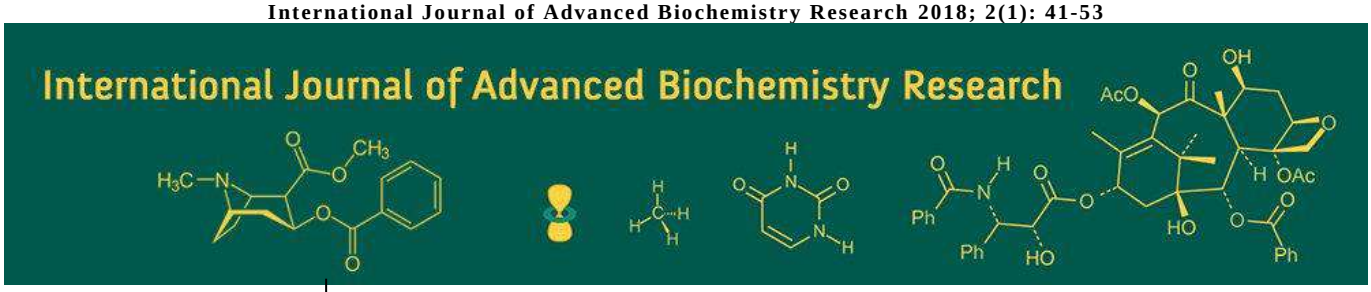

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Time-dependent alterations of nucleic acids, intracellular proteins and electrolytes in tissues of post-mortem rats

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Abstract
The study is to determine post-mortem changes in nucleic acids in brain and liver, electrolytes and protein levels in brain and kidney, and to evaluate its relation with post-mortem interval (PMI). Twenty-five male Wistar rats (122.2 ± 8.29g) were sacrificed by cervical dislocation, five rats used for immediate dissection (PMI=0); the remaining 20 rats were kept in their cages under the same environmental conditions in other to allow natural decomposition. Four more groups of five rats each were taken and dissected PMI of 15, 18, 24 and 48 hours. The intracellular levels of DNA and RNA in brain and liver were determined by UV spectrophotometry. The sodium (Na), potassium (K), calcium (Ca), phosphate (PO_4^{2-}), and iron (Fe) concentration, were determined. Brain nucleic acids and proteins showed significant changes at $p<0.05$ with increasing PMI, while K showed significant changes throughout the 48 hours with very high correlation values (r = -0.9). There was decrease in kidney K, and protein while calcium levels increased significantly. The relationship for rat PMI =-109.409 x K^+ concentration in mg/g of kidney + 161.05) was obtained. These results suggest that brain intracellular electrolytes, nucleic acids and Kidney K, and proteins may be useful for determining PMI.

Keywords: Post-mortem interval, DNA, RNA, electrolytes, ion selective electrolytes

1. Introduction
Animal tissues are some of the most prominent sources of high quality proteins in diets. Unfortunately however, from the moment an animal is slaughtered for dietary purpose, the race against deterioration begins. Evidences from biochemical investigations have shown that numerous changes commence in all tissues at the onset of death. Fundamentally, the cessation of the flow of blood to tissues leads to the accumulation of toxins, ischemia or anoxia, alteration of pH, membrane damage with diffusion, disruption of homeostasis, and eventually cessation of cellular biosynthetic activity. More specifically, anoxia leads to accumulation of lactic acid, which in turn decreases the tissue pH with increasing post-mortem interval. The cellular pH is important in determining the level of activity of specific subsets of proteases and consequently the level of protein degradation. The integrity of the plasma membrane has also been shown to be compromised after death, thus allowing the diffusion of ions in and out of the cell (Blair *et al.*, 2016, Despina *et al.*, 2003) [4, 17]. All these alter the quality and preservation of meat.
With this press for "high quality" tissue comes the need to determine the critical markers of quality for human post-mortem tissue (Deep-Soboslay *et al*., 2005; Hargrove and Schmidt, 1989) [66, 67]. Several laboratories have approached the evaluation of human postmortem brain cases with suggestions for markers of tissue quality (Lee *et al*., 2005; Preece and Cairns, 2003; Tomita *et al*., 2004) [68, 69, 60]. Several of these newer measures of tissue quality have been taken and tested in human postmortem tissue in a variety of situations to detect the relationship of the markers to the real quality of the postmortem tissue. Traditionally, a low postmortem interval (Barton *et al*., 1993; Ferrer-Alcon *et al*., 2003; Harrison *et al*., 1995; Lewis, 2002; Trotter *et al*., 2002) [6] has been the hallmark of high tissue quality. More recently tissue pH (Hynd *et al*., 2003; Kingsbury *et al*., 1995; Li *et al*., 2004) [72, 73] has been used, and even more recently, specific markers of RNA quality have been introduced (Imbeaud *et al*., 2005; Johnston *et al*., 1997; Miller *et al*., 2004) [74].

In a cohort of human post-mortem cases, we have determined these standard tissue quality measures have been determined, checked their collection correlates, and tested them for an association with RNA quality and with quantification of representative proteins. Attempts have been made to identify which ones are the most sensitive and reliable markers of tissue quality in human post-mortem cases.

Increasingly, human post-mortem brain tissue is being used to quantify cellular and molecular markers of neural processes in the pursuit of understanding normal brain function and correlates of dysfunction in CNS illnesses (Casanova and Kleinman, 1990; Castensson *et al*., 2000; Harrison *et al*., 1994; Mirnics *et al*., 2001; Ravid *et al*., 1992; Torrey *et al*., 2000) [10]. Because of the inaccessibility of the human brain and its unavailability for biopsy, fresh tissue is rarely obtainable. Consequently, the translation of new findings from basic laboratory studies to human brain diseases relies heavily on the use of post-mortem tissue. Although studies using human tissue have been done for decades (Johnson *et al.*, 1986; Wester *et al.*, 1985), the sensitivity and specificity required for today's molecular measures are greater, therefore calling for better tissue characteristics. This requires not only collecting better tissue than before, but also identifying measures of tissue quality and setting quality thresholds.

Apart from post-mortem studies of diseases, tissue quality and shelf-life, determination of post-mortem interval (PMI) upon discovery of a possible homicide victim is still a subject of major interest in forensic – medicine. This is important in the vindication or indictment of a homicide suspect. Since majority of the victims are found within the first two days, it is therefore important to research and develop methods of determining time of death quickly and accurately. In this light, this work has been designed into a two (2) hour post-mortem interval study of the brain, kidney, tongue, and liver. Currently, various methods of determining PMI have been developed but none of these provide better than an 8hour window time estimate (Johnson and Ferris, 2002) [33]. The time of death of an individual can easily be determined if the post-mortem interval can be assessed. Although livor mortis, and to a lesser degree, algor mortis have been used to estimate the post-mortem interval. These methods are hinged on the fact that biomolecules continually degrade after death. Upon death, all tissues lose the ability carry out homeostasis. The results of this loss are the obvious changes usually observed in cadavers, minutes after death. At this point, degradation of molecules sets in and fast. Nucleic acids start fragmenting (Mona, *et al.*, 2009) [42], proteins denature and enzymes gradually lose their activity, apoptosis sets in. This study therefore reports the in situ alterations of DNA and RNA in post-mortem rat brains and livers and in situ alterations of proteins, potassium, sodium, phosphates, iron and calcium in post-mortem rat brains and kidneys.

Materials and Methods

Rats collection and acclimatization

Twenty-five (25) male albino rats of the Wistar rats were purchased and acclimatized for 30 days at the animal house of the National Institute for Medical Research (NIMR), Iyaba, Lagos, Nigeria. The long period of acclimatization was to bring all the rats to the same level of physiological state i.e. a disease-free, physically fit and active state. Average weight of rats at the end of acclimatization was 122.2g

Method of inducing death

The rats were sacrificed by cervical dislocation.

Collection of organs and preparation of samples

The rats were all sacrifice by the method stated above at a noted time. Five (5) of these rats were separated and their organs (brain, kidney and liver) were immediately removed. This was taken as zero hour. The remaining dead rats were kept in their cages to undergo natural degradation. At 15hours after death, another five (5) rats were taken and their organs harvested. This procedure continued for 18hrs, 24hrs and 48hrs. The organs were immediately kept in their appropriate buffer and under ice. This was done in other to halt any further post-mortem changes. The temperature range of the environment for the study was 26 ^{0}C to 32 ^{0}C

Sample Preparation for the Estimation of Electrolytes (Na^+, K^+, Ca^{2+}, PO_4^{3-}, Fe^{2+})

Cytoplasm Extraction

0.5g of brain and 1.0g of kidney were cut out weighed**.** The organs were rubbed on filter paper to remove red blood cells. They were allowed to drain in order to remove much of the extracellular fluid and washed in double distilled-deionized water twice. Then, they were kept in 2ml 0.25% sucrose solution in plain bottles and refrigerated at -20^{0}C for 5minutes. The organs were homogenized and centrifuged at 14,000rpm15 minutes (to pellet nuclei and other cytoplasmic structures) in a total volume of 5ml sucrose solution. The supernatant (intracellular fluid) was transferred to fresh plain tubes and stored at -20^{0}C.

Calcium Estimation

Using an autoanalyzer (HTACHI COBAS C 502), Calcium ion concentration was assyed in the tissues based on the reaction of calcium with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA.

$$Ca^{2+} + \text{NM-BAPTA} \xrightarrow{\text{Alkaline pH}} \text{calcium-NM-BAPTA Complex}$$

$$\text{Calcium-NM-BAPTA+ EDTA} \longrightarrow \text{NM-BAPTA + calcium-EDTA complex}$$

The change in absorbance is directly proportional to the calcium concentration and is measured photo metrically.

Na+, K^+, Estimation

An auto-analyzer (HTACHI COBAS C 502) was used to assay for the concentrations of Na and K. This was based on the principle of An Ion-Selective electrode (ISE) which makes use of the unique properties of certain membrane materials to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The electrode has a selective membrane in contact with both the test solution and an internal filling solution. The internal filling solution contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions will closely associate with the membrane on each side. The membrane EMF is determined by the difference in concentration of the test ion in the test solution and the internal filling solution. The EMF develops according to the Nernst equation for a specific ion in solution.

1. $E = E_0 + RT/nF. \ln (f - C_t) / (f - C_i)$

Where
E = electrode EMF
E_0 = standard EMF
T = temperature
R = constant
n = charge of the ion
F = Faraday's constant
In = natural logarithm (base e)
f = activity coefficient
Ct = ion concentration in test solution
Ci = ion concentration in internal filling solution

Sodium, potassium and chloride, which all carry a single charge, R, T, n, and F are combined into a single value representing the slope (S). For determination on a cobas c 501 analyzer where the sample is diluted 1:31, the ionic strength and therefore the activity coefficients are essentially constant. The concentration of the test ion in the internal filling solution is also constant. These constants may be combined into the E_0 term. The value of E_0 is also specific for the type of reference electrode used. Equation (1) can hence be rewritten to reflect these conditions:

$$E = E^1_0 + S. \ln (C_t) \quad (2)$$

The complete measurement system for a particular ion includes the ISE, a reference electrode and electronic circuits to measure and process the EMF to give the test ion concentration.
The sodium and potassium electrodes are based on neutral carries and the chloride[5] electrode is based on an ion exchange.

Inorganic Phosphate Estimation
Molybdate UV
Inorganic phosphate concentrations assay involved the formation of an ammonium phosphomolybdate complex having the formular $(NH_4)_3\{PO_4\ (MoO_3)_{12}\}$ from the reaction of inorganic phosphate with ammonium molybdate in the presence of sulfuric acid.

$$PO_4^{3-} + \text{ammonium} \xrightarrow{H_2SO_4} \text{Ammonium phosphomolybdate complex molybdate}$$

The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photo metrically.

Estimation of Total Protein
Total Protein was assayed using an auto-analyzer HITACHI/COBAS C501 based on the reaction of divalent copper ions in alkaline solution with protein peptide bonds to form the characteristic purple-coloured biuret complex. Sodium potassium tartrate was added to prevent the precipitation of copper hydroxide and potassium iodide prevents auto-reduction of cooper.

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{Alkaline}} \text{Cu-protein complex Solution}$$

The colour intensity is directly proportional to the concentration of proteins which can be determined photometric ally.

Quantitation of nucleic acids
DNA extraction: Nucleic acids extraction was done according to (salting out extraction method) of Aljanabi and Martinez, (1997) [2] and modification introduced by Hassab El-Naby, (2004) [26]. Where, a piece of 10mg of liver and brain, tissues was squeezed by blue tips and lysed with 600 microlitre lysing buffer (50 mM NaCl,1 mM Na2 EDTA,0.5% SDS, pH 8.3) and gently shaken. The mixture was incubated overnight at 37ºC then, 200 microlitre of saturated NaCl was added to the samples, shaked gently and centrifuged at 12,000 rpm for 10 min. The supernatant fluid was transferred to new eppendorf tubes and then DNA was precipitated by 600 microlitre cold isopropanol. The mix was inverted several times till fine fibers appear, and then centrifuged for 5min. at 12000 rpm. The supernatant fluid was removed and the pellets were washed with 500 microlitre 70%ethyl alcohol, centrifuged at 12000 rpm for 5min. After centrifugation, the alcohol was decanted or tipped out and the tubes blotted on Whatman filter paper, till the pellets appeared to be dry. The pellets were resuspended in 50 microliter or appropriate volume of TE buffer (10 mM tris, 1mM EDTA, and pH8)

DNA Estimation
DNA was quantified by ultraviolet absorption method.
Nucleic acid, nucleotide and their derivatives have the system of conjugated double bond, so the maximal ultraviolet absorption of RNA and DNA is at 260nm. Popularly at 260nm, the absorbance of 1μg/ml of RNA solution is 0.022～0.024, and that of DNA solution is 0.020. So we can nucleic acid concentration was determined by measuring the absorbance of DNA and RNA at 260nm.

Materials
Reagents
(1) Ammonium molybdate-perchloric acid reagent (precipitator): Add 7ml of perchloric acid and 0.5g ammonium molybdate into 193ml distilled water to get 200ml solution. (2) 5 % -6 % of ammonia water

DNA Estimation
Two centrifuge tubes (t1 and t2) were taken, and 2ml sample solution and 2ml distilled water were added into the first tube, and 2ml sample solution and 2ml precipitator (to remove macromolecular nucleic acid if there was any left as contaminant) were added into the second tube to be comparison. Each was mixed up, kept in ice bath for 30minutes, and then centrifuged at the speed of 3000rpm. Each was Sucked up; 0.5mls of supernatant from each of the two tubes were obtained; distilled water was added accurately to 10ml. Absorbances (A1, A2) at 260nm (corresponding to tubes t1 and t2) with 1cm of quartz cuvette were taken. The concentrations of the DNA were accounted for thus:

$$\text{Concentration of DNA (μg/ml)} = \frac{\text{A1-A2 x N}}{0.020 \text{ x L}}$$

Estimation of the Content of RNA by Orcinol Development Process

Principle

When RNA is heated with concentrated hydrochloric acid, it will degrade to ribose, which will transfer to furfuraldehyde later. Catalyzed by $FeC1_3$ or $CuCl_2$, furfuraldehyde will react with orcinol to give green complex whose maximal absorbance is 670nm. The relationship between absorbance and concentration of RNA is a straight line in range of 20-250µg/ml of RNA concentration. Using serial concentrations of RNA, a standard curve was obtained. The absorbance values for each sample obtained from the tissues samples were extrapolated on the RNA standard curve.

Statistical Analysis

These data were run on an IBM compatible personal computer by using Statistical Package for Social Scientists (SPPS) for windows 11 (SPSS Inc., Chicago, IL, USA). Data were compared by using two types of statistics; Descriptive statistics: e.g., mean (x) and standard deviation (SD) and analytical statistics: e.g., student's t-test (to compare two groups) was used to test association between variables. P value of <0.05 was considered statistically significant.

Results

DNA and RNA concentrations at different PMI's are presented in figures 1-4. DNA and RNA concentrations showed significant decrease (p<0.05) in brain but liver tissues showed significant decrease in DNA concentrations only while changes in RNA concentrations were not significant at (p<0.05). Figures1-4). However, Figure 3 showed that there was a significant decrease in the liver RNA concentrations in the first 15hours but this became insignificant from 15 to 48 hours. DNA and RNA showed high decrease in concentrations in the first 15hours in both brain and liver

Sodium concentrations in Kidney and Brain and did not show significant change at (p<0.05). Although there was an abrupt fall in the concentration of Na ions in the first 15 hours, this change did not continue after that time. (Figures7 and 8)

The values for kidney and brain potassium ion concentrations against PMI are presented in figures 8 and 9 respectively. Kidney and brain Potassium concentrations revealed that there were significant changes. Both organs showed significant decrease ((p<0.05). Meanwhile potassium ions concentrations showed the highest correlation values (r= -0.996) with PMI as revealed in Table1 (i.e. correlations tables)

Figures11 and 12 show the results for iron concentrations in kidney and brain respectively. Kidney iron concentrations showed no consistent change of any significance (at p<0.05). Brain Fe concentrations showed significant decrease from PMI of 0-18hours. While iron levels in the kidney increased slightly in the first 15 hours but thereafter became inconsistent in its change.

Figure 13 shows the values for calcium ion concentrations in the kidney. Results for calcium in kidney showed no significant change in concentration for the 48hours of study. But the first 15hours (PMI) showed a slight increase in the concentration of calcium.

Figure 14 however, showed a significant increase in the intracellular concentrations of calcium for up to 24 hours PMI

Figure15 presents the concentrations of proteins at different PMI's. The results obtained from kidney assays of protein concentrations revealed a significant decrease as PMI increased (p<0.05).

Figure 16 presents the concentrations of proteins in the brain for the 48hours of study. There was significant decrease in the protein concentration (p<0.05). The most marked change in protein concentration occurred during PMI 0- 18hours. The rate of decrease continued but gradually slowed down as PMI approached 48 hours.

Figure 17 shows the various concentrations of phosphate ions in the brain throughout the 48 hours of post-mortem study. The result shows that there is a significant decrease (p<0.05). in the phosphate concentration from 0-15 hours. There- after, the concentration started to increase up till the 48th hour. But there was no significant change in kidney phosphate concentration (at p<0.05) (Table1). However in brain tissues, phosphate concentration showed significant decrease in the first 15hours (at p<0.05).

Results

Results for Nucleic acids concentrations in postmortem rat tissues illustrated in bar charts

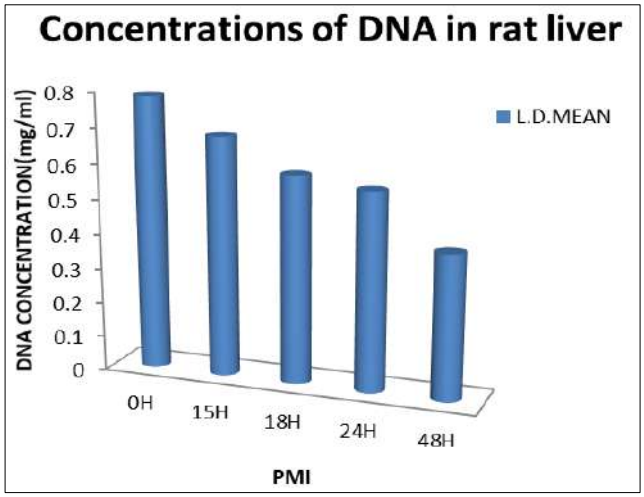

Fig 1: DNA (Mean ± SEM) values against PMI in postmortem liver tissues of rats. DNA significantly decreased in concentration (p<0.05)

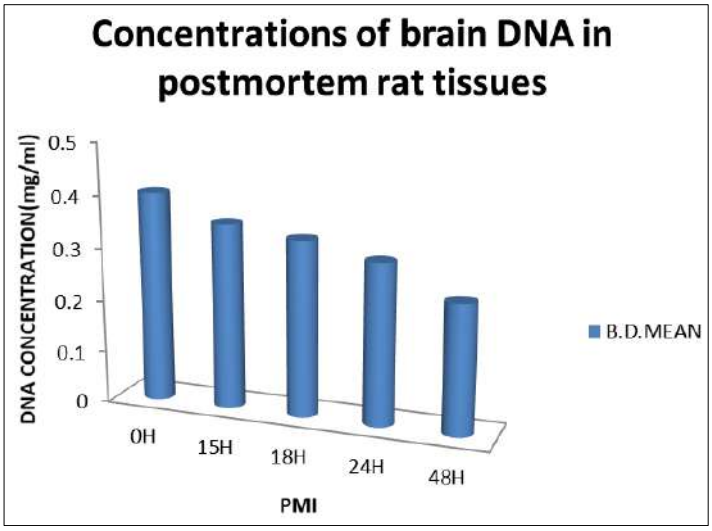

Fig 2: This shows the alteration of DNA in rat brain tissues. DNA concentrations decreased significantly (p<0.05)

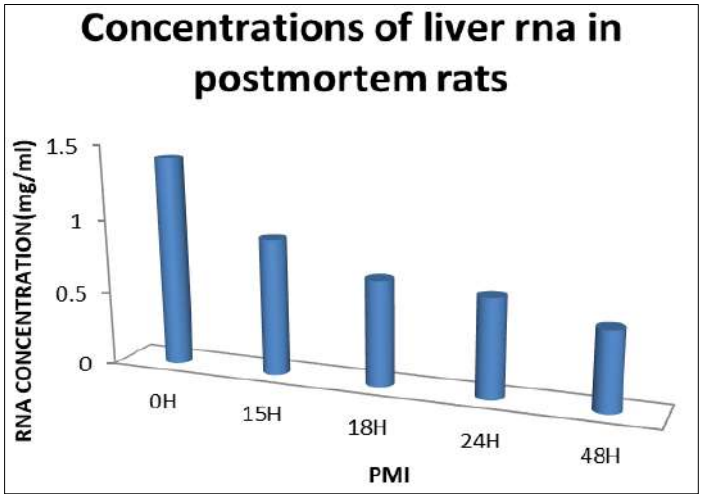

Fig 3: RNA alteration in liver tissues of postmortem rats. RNA concentrations showed no significant change at p<0.05 but there is significant change in the first 15hours

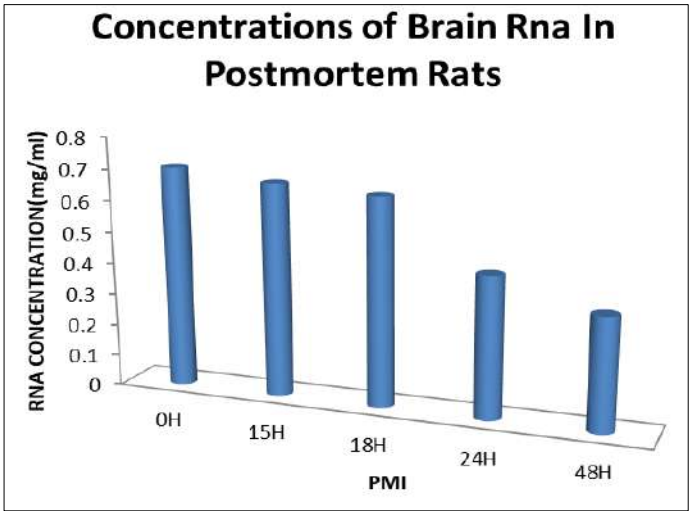

Fig 4: RNA alteration in Brain tissues of postmortem rats. RNA in brain showed significant decrease (p<0.05) in concentration as PMI increased.

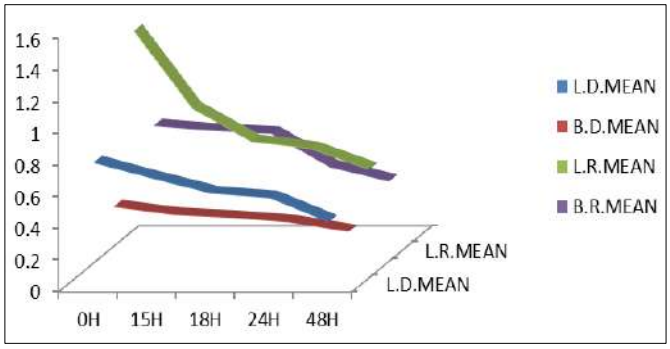

Fig 5: Comparing The Degradation of RNA and DNA In brain and liver. Degradation of nucleic acids in brain showed a more linear correlation when compared with nucleic acids in liver. Also, degradation of DNA generally was more consistent than RNA degradation.

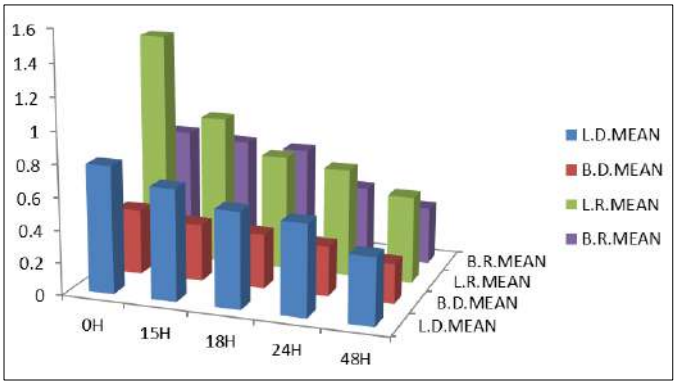

Fig 6: A comparison among nucleic acid concentrations in brain and liver

Results for Electrolytes and Proteins in the tissues of postmortem rats

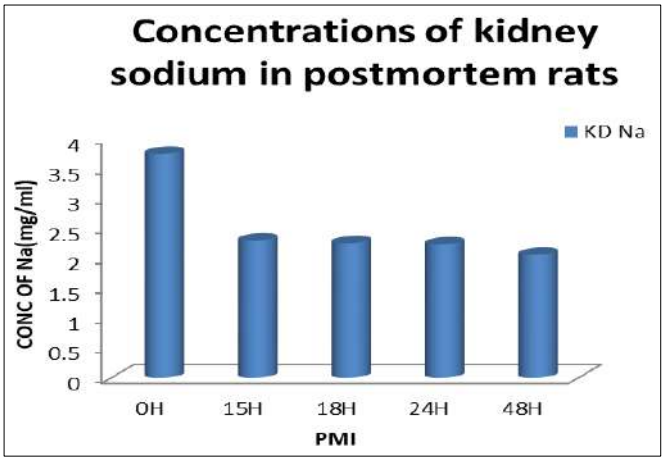

Fig 7: Alteration of Na in kidney tissues of postmortem rats (see table 1 for details) There was no statistical difference in the concentration of Na for the 48 hours of study.

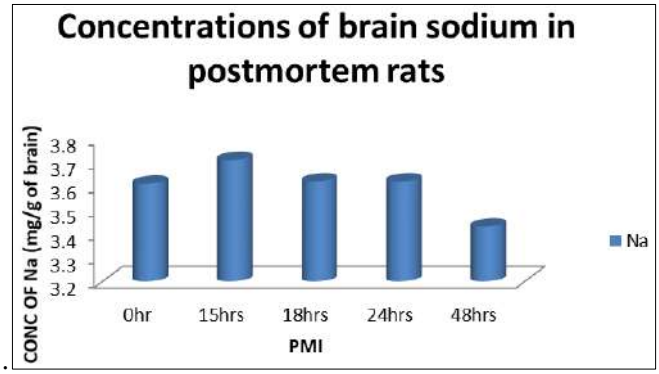

Fig 8: Alteration of sodium ion concentration in the brain. (see table 2 for more details). There was no significant change observed in the concentration of Na ions in the brain of the rats.

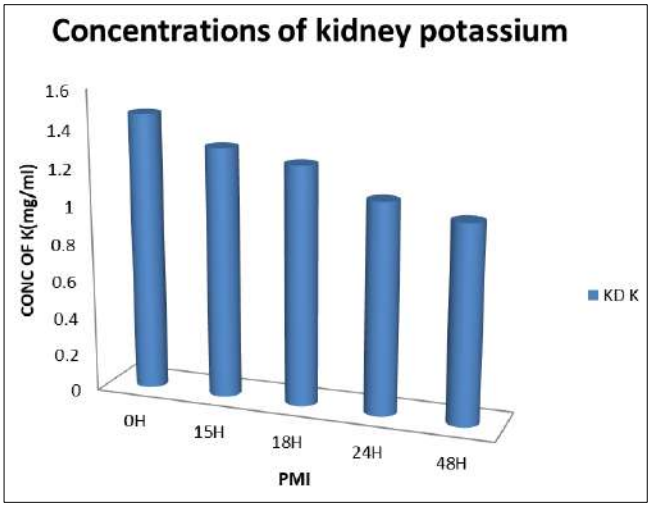

Fig 9: Alteration of potassium in kidney tissues of postmortem rats (see table 1). Potassium ion decreased significantly with PMI. Potassium used as a parameter for determining PMI because of its high correlation value with PMI

Derivation of Mathematical relationship for calculating postmortem interval (PMI) in rats

PMI =-109.409 [K^+] mg/g of kidney + 161.05 Regression Coefficient "b" was run on the table below Values

PMI	0H	15H	18H	24H	48H
KD K	1.472	1.322	1.257	1.114	1.04

Using the formula:

$$b = \frac{\sum(x_i - \ddot{x})(y_i - \hat{y})}{\sum(x_i - \ddot{x})^2}$$

X	$(x_i - \ddot{x})$	y	$(y_i - \hat{y})$	$(x_i - \ddot{x})(y_i - \hat{y})$	$(xi - \ddot{x})2$
0	-21	1.472	0.231	-4.851	441
15	-6	1.322	0.081	-0.486	36
18	-3	1.257	0.016	-0.048	9
24	3	1.114	-0.127	-0.381	9
48	27	1.04	-0.201	-5.427	729
			-11.193	1224	

Mean for PMI i.e. $\ddot{x}$= 21
Mean for [K^+] i.e. $\hat{y}$= 1.241mg/g of kidney:. B =-0.00914(hrs/mgg^{-1} of kidney) When b is inserted into the equation of straight line i.e. y= mx + c, the relationship for finding postmortem interval PMI is obtained
PMI =-109.409[K^+]in mg/g of kidney + 161.05

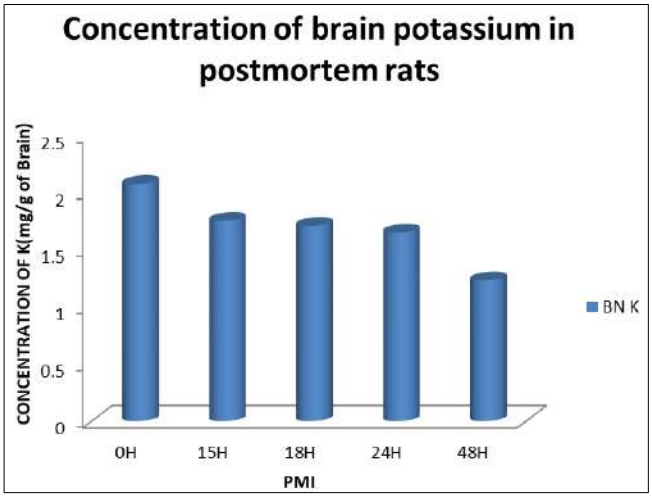

Fig 10: Alteration of potassium ion in the brains of postmortem rats. (see table 2 for details). Potassium concentration showed significant decrease in the brain of rat tissues (P<0.05)

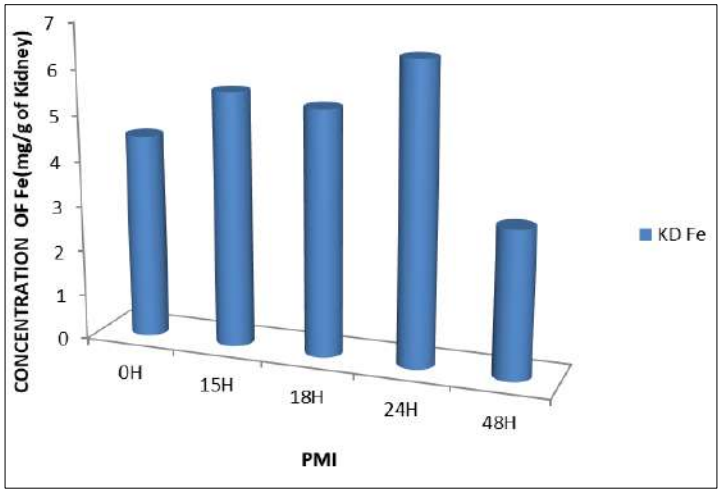

Fig 11: Alteration of Fe in kidney tissues of postmortem rats. There was no consistent change in the concentrations of iron in the kidney. Statistical analysis also proved that there was no significant increase in the concentration of Fe in kidney at (p<0.05).

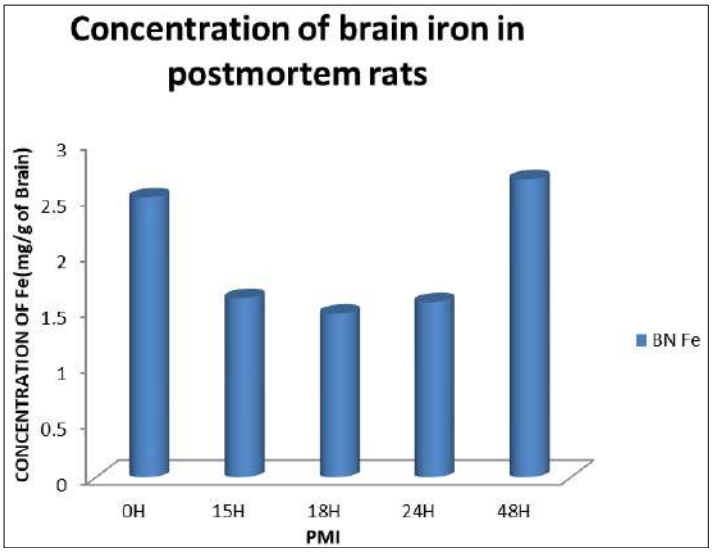

Fig 12: Alteration of iron ion in the brains of postmortem rats.

This figure shows the changes that occurred in Fe concentration of the 48hours study. Fe showed a significant decrease in the concentration 24hours (p<0.05). However in the last 24hours, the change in concentration was inconsistent.

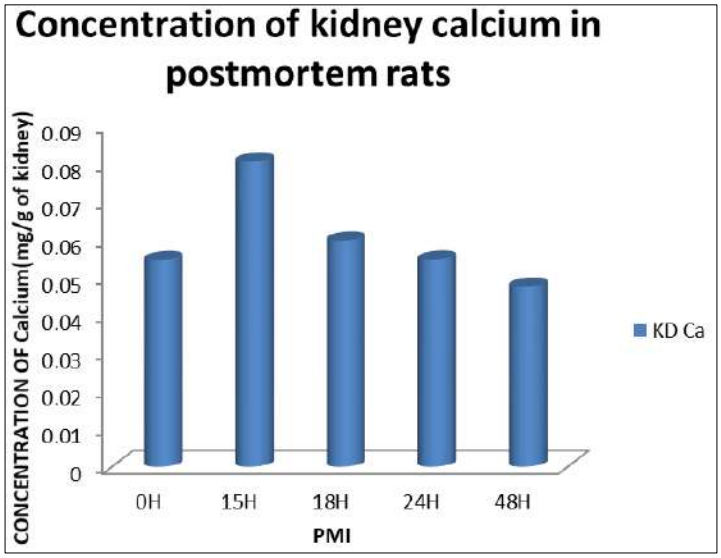

Fig 13: Alteration of calcium in kidneys of postmortem rats.

Calcium concentrations did not show any significant change at $p<0.05$. but there was an increase in its concentration in at PMI 15hours

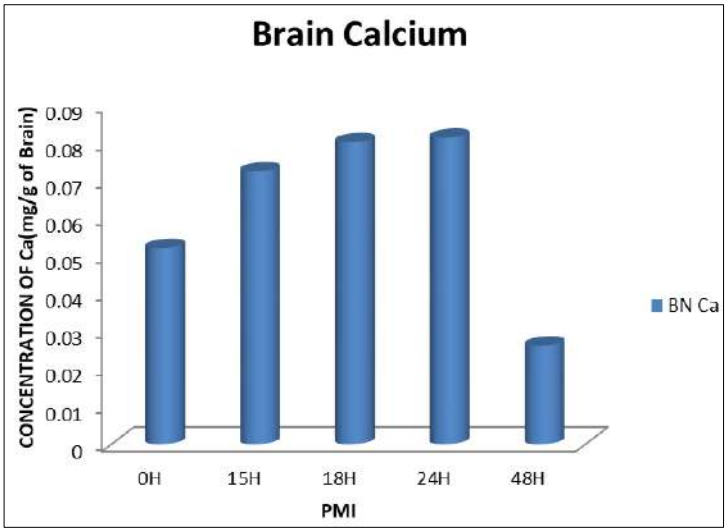

Fig 14: Alteration of calcium ion in the brains of postmortem rats. Calcium ion concentrations increased significantly in the brain of the rats, post-mortem ($p<0.050$)

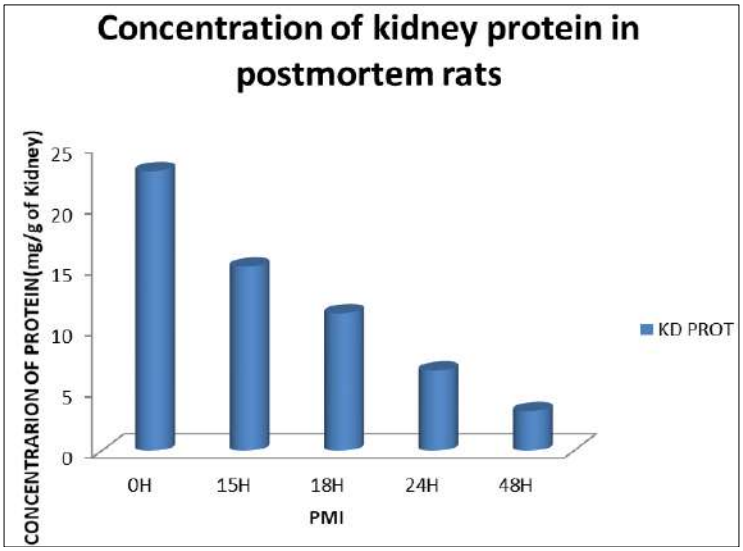

Fig 15: Alteration of protein in kidneys of post-mortem rats. Protein concentration decreased significantly ($p<0.05$).

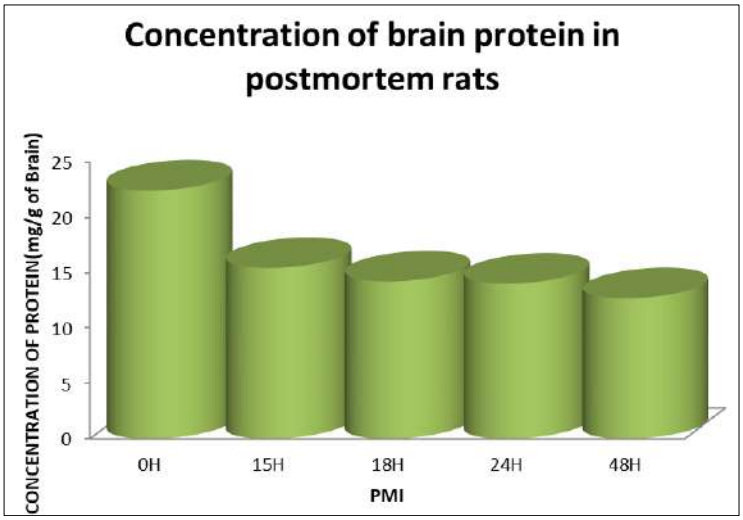

Fig 16: Alteration of protein concentration in the brains of postmortem rats. Protein results showed a gradual but significant decrease the brain of the rats, post-mortem ($p< 0.05$).

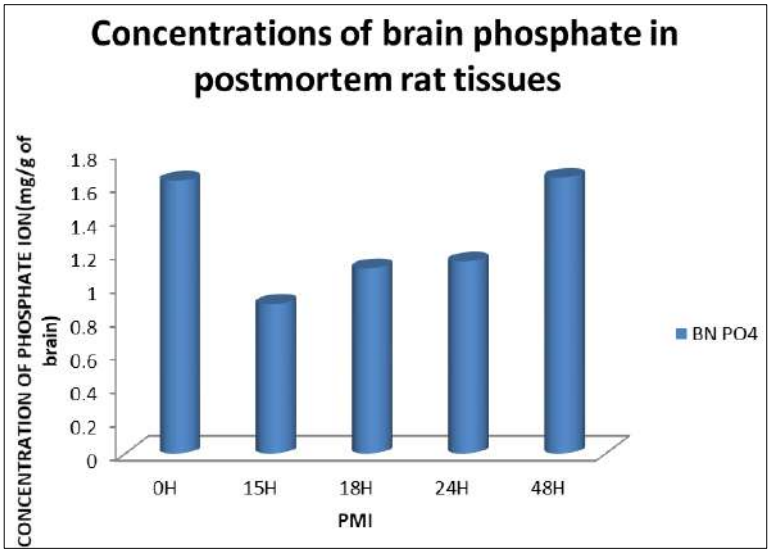

Fig 17: Alteration of phosphate ion in the brains of postmortem rats.

There was a significant decrease (p<0.05) in the concentrations of phosphate in the brains of the rats in the first 15hours and then the concentrations increase till the 48th hour.

Discussion

In this study, the changes and redistribution of eight (8) parameters were evaluated alongside post-mortem interval. The parameters are DNA, RNA, proteins, potassium, sodium, calcium, iron and phosphate ions.

Results obtained from this study using simple quantification of DNA and RNA at varying PMI showed that there is a general decrease in the concentrations of nucleic acids both in the liver and brain of the experimental rats. This is in consonance with findings from (Gilbert *et al.*, 2003) which showed that DNA decays after death, in biological samples, and the ensuring damage is manifested in many forms. There was also a good linear relationship between the nucleic acids and PMI for up to 18 hours. Some previous studies showed that DNA degradation can be linear with high correlation with PMI in brain tissues of drowned rats for up to 24hours. (Mona *et al.*, 2009) [42]. This relationship between DNA and PMI can be explored for the determination of post-mortem interval.

Figure 1 showed a rapid decrease in the liver DNA concentration in the first 18hours after death at the rate of 0.01079mg/ml/hour. This then slowed down to 0.006013mg/ml/hour in the next 30hours. These changes were statistically significant at p<0.001(see table11). According to earlier reports, during post-mortem autolysis, cellular organelles and nuclear DNA break down into their constituent parts (Boy *et al.*, 2003) [5]. This degradation occurs as a result of the activities of deoxyribonucleases. (Leonard *et al.*, 1993) [35]

Similarly, brain DNA degraded rapidly in the first 18hours and then the rate of degradation slowed down in the following 30 hours. This is shown in figure 2. The intracellular fluids of brain and liver cells contain numerous globular proteins some of which are enzymes of degradation. These are released after death in cells due to apoptotic processes. DNA in the liver showed faster degradation than DNA in the brain. This also is in accordance with the findings of earlier researchers who found that brain DNA degraded slower than the rate in other organs (Leonard *et al.*, 1993) [35]. This high rate of degradation in the liver may be attributed to the presence of many enzymes and up- regulation of proteolysis- related genes (Sanodou *et al.*, 2004) [58]. Gel electrophoretic studies revealed that there is continuous fragmentation DNA up 24 hours post-mortem in the liver (Lin *et al.*, 2000) [37]

Figure 7 is a bar chart that shows the relationship between sodium ions in the cytosol of rat kidney and post-mortem interval. There was no significant change for 48hours at p-value < 0.05. In figure 8, potassium concentration was shown to decrease in accordance with increase in PMI (significance at p < 0.019 see table3 for details). This decrease showed a near-perfect-linear relationship with correlation of -0.996 (See table 1) since the correlation was high for potassium-PMI, an attempt was thus made to derive a mathematical relationship for post-mortem interval using the potassium-PMI values from figure 8, using the regression formula:

$$p = \frac{\sum(x_i - \ddot{x})(y_i - \hat{y})}{\sum (x_i - \ddot{x})^2}$$

A regression coefficient b= -0.00914(hrs/mg/g of kidney). This was used to obtain relationship below:

PMI =-109.409 x [K^+] concentration in mg/g of kidney + 161.05

The changes observed in the concentration of kidney iron shown in Figure 9 were not statistically significant. Meanwhile there was a significant increase in cytosolic calcium ions in the first 15hours

Results obtained from this study (Figures 11 and 14) showed that protein concentrations in the kidney significantly decreased with increase in PMI. Proteins degraded very fast in the first 15hours after death. Also the changes observed in the concentration of protein brain were statistically significant (p<0.025).

There was a significant decrease in the concentrations of potassium ions in the brain in the full 48hours period of the study while results for calcium revealed a significant increase in concentration in the first 18hours. This is supported by a previous study by (Gemma *et al.*, 1980) [21]

which revealed that the cellular concentrations of Na, Ca and Cl increased marked, those of Mg and K decreased; no significant changes were found in the concentrations P and S and calcium increased both in the mitochondria and in the cytoplasm and endoplasmic reticulum. From results in this study Fe ions showed significant decrease in their concentrations for the first 18hours (p<0.045) while changes observed in sodium ions concentration were insignificant. (Figures 13, 14, 15 and 16)

Brain potassium concentrations were observed to decrease significantly (p<0.001) as PMI increased. This is shown on Table2 and in Figure 12. According to a study by researchers, the integrity of the plasma membrane has been shown to be compromised after death, thus allowing the diffusion of ions in and out of the cell leading to an alteration of the concentration of ions in the intracellular environment (Despina *et al.*, 2003) [17]. This is probably the mechanism involved the change in concentrations of ions in post-mortem tissues

Conclusion

The data of the study suggest that levels brain intracellular electrolytes, DNA and RNA, Kidney electrolytes and proteins and liver DNA may be useful for determining PMI. The most significant degradation of nucleic acids in the organs occurred in the first 15 hours post-mortem suggesting that there is a high proteolytic activity at about this window time. This leads to rapid loss of bio-molecules and loss of quality dietary animal tissues. It will be more convenient to develop a working relationship for post-mortem interval using tissues that are hardly ever missing in cadavers. For example, Sturner's PMI and Medea and Hensage's PMI are based on the concentration of potassium ion in the vitreous humour but these formulae may not be useful in cases of ritual killings were the victims' eyes have been excised. Therefore it is advised that more attention should be given to other organs.

Meanwhile, the results of this study, a relationship for post-mortem interval in rats is being proposed:

PMI =-109.409 x [K^+] concentration in mg/g of kidney + 161.05

Recommendations

Since the highest degradation of DNA, RNA and intracellular proteins occurred during the early post-mortem hours, it would be logical therefore, if more attention is given to animal tissues obtained at abattoirs for dietary purpose. Processing or preservation should be done immediately after the death of the animal in order to forestall the usual rapid degradation nutrients. Further research may be carried out on the levels of DNA, RNA, intracellular Fe, Ca, globular proteins, PO4 and K in different organs with increase in PMI using human samples.

In addition, degradation of nucleic acids in different climatic conditions may also be carried out. Various manner of deaths may also be studied in relation to alterations of biomolecules and ions.

Lastly, the submission made by this study for post-mortem interval i.e.

PMI =-109.409 x [K^+] concentration in mg/g of kidney + 161.05 may be a guide into the development of a better formula for finding longer PMI than the already existing ones. Changes in potassium ion in organs may just be the key.

It is also recommended that, a more detailed study on the levels of Fe, Ca, PO_4 and K in rat brain, liver and kidney tissues including a larger number of rats should be planned in further researches. Also, the relationship between calcium and phosphate may be explored in the future because the high correlation values (table1). Other methods of inducing death may be tried and degradation under environmental conditions (such as temperate conditions, tundra climate etc.) that are different from the one mentioned in this study may be studied using human samples. It can be recommended that animals slaughtered for food should be preserved immediately by freezing or prepared as meals instantly. This will help to retain the most of the nutrients

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