Comparative Histomorphologic Studies of the Heart in Three Mammalian Species: Rabbits (*Oryctolagus guniculus*), Wistar Rats (*Rattus norvegicus*) and African Giant Rats (*Cricetomys gambianus*)

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Abstract

Comparative morphologic, morphometric and histological studies was carried out on adult male hearts of the three mamalian species namely the Rabbit (*Oryctolagus cuniculus*), Wistar rats (*Rattus norvegicus*) and African giant rats (*Cricetomys gambianus*). Five animals from each species were weighed, lightly anasthetized with chloroform and humanely sarcrificed. The thoracic cage was carefully opened and the hearts were removed, morphologically observed andfixed in Bouins fluid. The tissues were processed for histological studies and stained using Haematoxylin and eosin (H & E) and Rafferty's stain. The hearts were physically observed to be reddish in colour and pear shaped. The heart of the three mamalian species showed the aorta, superior and inferior vena cava and the pulmunary vessels similar to that of the humans. The mean total body weight of the African gaint rats, Rabbits and Wistar rats are 1.167±0.153 kg, 1.233±0.252 kg and 20.333±5.774 g respectively, which showed a significant difference (P≤ 0.01) between the body weight of the African gaint rats, Rabbits and the Wistar rats. The mean weight of the hearts of African giant rats, Rabbits and Wistar rats are 4.367±1.620, 3.306±0.115, 0.766±0.164 gms respectively which showed a significant difference (P≤0.01) between the hearts of African gaint rats, Rabbits and Wistar rat. Histologically, the atrial and ventricular parts of the heart of the three species showed great similarities in

terms of the arrangement of the cardiac muscle cells and the layersof the walls of the heart in the three mamalian species with little differences observed in the morpho-metrics of the muscle fibres and intra-muscular cellular distribu-tions in the different hearts studied which could be due to the sizes of the mammals in general.

Key Words: Comparative, Morphology, Heart, Wistar rat, African giant rat, Rabbit

Introduction

Comparative anatomy is the study of similarities and differences in the anatomy of different organisms and it is closely related to evolutionary biology and phylogeny. Histo-morphometry on the other hand is the quantitative study of the micro-scopic organization and structures of a tissue, an organ or a system by a microscope (Campbell and Reece, 2002; Caldwell, 2006). Com-parative histomorphometrics study the simila-rities and differences in microscopic organiza-tions and structures of the heart of the three species from the whole organ down to the tissue level by analysis of their measurements (Eroschenko, 2003; Perry, 2006).

Rabbits are small mammals found in several parts of the world, the male is the buck and the female is the doe. Rabbits have long ears which can be more than 10cm long which are probably an adaptation for detecting predators(Wilson and Burnie, 2001; Wilson and Reeder, 2005). They have large powerful hindlegs, the two front pairs have five toes, the extra called dew claw (Romer et al., 1977; Sharon and Crowell, 2010). They are pentigrade animals while at rest, however they move around on their toes while running, assuming a more digiti-grade form(Wilson and Burnie, 2001). The fur is most commonly long and soft, with colors such as shades of brown, gray, and buff, the tail is a little plume of brownish fur with white on top for cotton tails. Rabbits have very complex needs, being aware of how wild rabbits live can help you understand what rabbits need (Dyce et al., 1996, Sharon and Crowell, 2010). Rabbits are herbivores, that feed by grazing on grass, forbs, and leafy weeds.

The Gambian pouched rats (*Cricetomys gambianus*), known as the African giant rat, are nocturnal rats and are among the largest mucoids in the world, growing up about 0.9 meters (3ft) long including their tail which makes up half their length (Perry, 2006; Sharon and Crowell, 2010). It is wide-spread insub-saharan Africa and has a very poor eye-sight and so depends on its senses of smell and hearing (Happold,

1987; Kingdon,1997). It is not a true rat but is part of a uniquely African branch of mucoid rodents. It typically weighs between 1 and 1.4 kg in its native Africa. The African giant rats have cheek pouches which allow it to gather up several kilograms of nuts per night for storage underground (Ajayi, 1975; Kingdon,1997; Perry, 2006). The burrow consists of a long passage with side alleys and several chambers.They are completely omnivorous, feeding on vegetables, insects, crabs, snails, and other items but apparently prefering palm fruits and palm kernels (Ajayi, 1975; Kingdon, 1997; Perry, 2006). These rats store a considerable amount of foods as well as non-edible items such as coins, metals, and bits of cloth.In a study of captives, Ajayi observed one female to give birth 5 times in 9 months, and he thought that probably females could produce 10 litters annually (Ajayi, 1975; van der Straeten, et. al., 2008). The gestation period was 27-36 days. Litters numbered 1-5 and Sexual maturity is attained at about 20 weeks of age (Perry, 2006).

Wistar rats or laboratory ratsare of the species *Rattus norvegicus* which is bred and kept for scientific research (Young, 1981; Kriegs et al., 2006). Wistar rats differ from wild rats in many ways, they are calmer and less likely to bite and can tolerate greater crowding (Kemp, 1982; Witmer, et al., 2010).They breed earlier and produce more off-springs. Over the past years, two breeding lines derived originally from outbred Wistar rats have been established that differ markedly and consistently in their anxiety related behaviour in the elevated plus maze (Witmer, et al., 2010; Davoust et al., 2013). Wistar rats are commonly fed and watered *ad-libitum*. They are fed with a laboratory quality rodent chow (Young, 1981; Wilson and Reeder, 2005). The average diet contains about 18-24% protein, 4-7% fat, and 60-75% carbon-hydrate respectively (van Zuphten, et al., 2001; Wilson and Reeder, 2005).

Rodents consume and damage human foods in the fields and in stores and they spoil the foods in stores by urine and droppings reducing their sale values (Baumans, et al., 2001; Perry, 2006). Their gnowing and burrowing habit, destroy many articles such as packaging, clothes, furnitures and structures like floors, buildings, and bridges, by gnowing through electrical cables they can cause fires (Betelak, et al., 2001; Cecie at al., 2009). Rodents can be responsible for transmitting diseases dangerous to man and are important animals used for research in psychology, medicine, and other

fields (Cooper, 2008; Davoust et al., 2013). The aim of the present work was to study the comparative morphology and histomorphometrics of the hearts of three mammalian speices namely the Rabbits, (*Oryc-tolagus cuniculus*), Wistar rats (*Rattus norvegicus*) and African giant rats (*Cricetomys gambianus*).

Material and Method

A total number of 5 animals of each specieswere obtained from the Faculty of Veterinary Medicine Animal house, Ahmadu Bello University, Zaria and was housed in the Depart-ment of Human Anatomy Laboratory in animal cages. The animals were physically observed and vital signs were checked to see if the animals were apparently healthy. The animals were then weighed and allowed to acclimatize for two weeks and the weights were again taken before the comencement of the study. The animals were fed with animal feeds and Water was given *ad libitum.*

The animals were sacrificed humanely by anaesthesizing them with chloro-form inhalation and were perfused using formal salaine, the thoracic cage was opened and the hearts were removed. Physical observations were made to see the position and orientation of the hearts and the vessels.

Morphologic Studies of the Rodents's Hearts

The structural and morphological characteristics of the hearts of each of the animals in each group were examined with the naked eyes. These include the shape, size, and the external landmarks of the atria and ventricles were evaluated in each of the hearts of the species.

Morphometric Studies of the Rodents's Hearts

The animals were anaesthesized using chloroform, and then weighed using a sensitive digital balance. They were sacrificed and their hearts were removed and immediately placed into the bouin's fluid fixative. The hearts were then weighed using a digital micro scale, followed by the measure-ment of heart volumes and the hearts were dissected and the chambers as well as the thickness of the walls and of the heart valves. The fixed hearts were processed routinely and stained using H&E staining method, as well as Rafferty's stain (Weiss et al., 2010).

Statistical Analysis

Data obtained were expressed as means ± standard deviation (SD). Differences between group means were estimated using Students' T-test and one-way analysis of variance (ANOVA) followed by Post-hock Turkey's test using SPSS 12.0 for windows. A P value less than or

equal to 0.05 was considered to be significant (Sellke et al., 2001).

Result

Physical Observations

Physical and Gross observations showed that the heart of the three species have red coloration due to presence of blood vessels and reddish coloration of the heart muscles. The orientation of the heart were seen to be lying in the midline plane and was tilted to the left side partially thereby making the right lungs having a larger surface area than the left. The apex of the hearts of the three mammals were seen facing down-wards as seen in that of humans.The aorta was clearly seen as well as the cranial and caudal vena cava, the pulmonary vessels were also seen from mere viewing of the rodent's heart with the naked eyes.

Mophological Observations

The morphological observation seen in the three hearts revealed a significant differences in the measurements of the heart parameters namely; heart weight, heart volume, lenght and width of the four heart chambers as well as thickness of heart walls and valves. Based on the morphology, the African giant rats have the biggest heart and have the largest sizes of the chambers of the heart excluding the left ventricle where the Rabbits have the largest size as shown in Table 1. The result showed that the African giant rats have the largest heart chambers apart from the right atrium in which the rabbit predominates, this is due to the origin of the longitudinal sulcus dividing the heart of the African giant rats that deviates to the right at the arterial part of the heart making the right atrium remarkably smaller than the left one.

Mophometric Observations

The result of the morphometric study showed the African giant rat posse-ssed the highest values in most of the parameters taken, apart from the right atrium in which the rabbit had the highest value in terms of width and thickness of the walls of the right atrium and right ventricle as well as the intra-ventricular septum as shown in Table 2. for the width of the various heart chambers in which the African giant rats have the highest values for the width of the right and left atria and have the highest value of the right and left ventricles which showed a significant difference ($P \leq 0.01$), between the African gaint rats and Wistar ratsand between the Rabbits and Wistar rats. In the right ventricles, left atria and left ventricles of the three mammals, there was a

significant difference (P≤0.01) between the African gaint rats and Wistar rats and between the Rabbits and Wistar rats.

The result of the thickness of the heart walls of the three species showed that the Rabbits had thickest wall of the right atrium and ventricles whereas a smaller value in terms of thickness on the left side while the African giant rats showed the highest values in the left compartment. The Rabbitsalso had the thickest intra-ventricular septa compared to the two. In both the right and left atrio-ventricular septa, the African giant rats predominates as shown in Table (3).

The results presented inTable 4 show the correlation between the total body weight in relation to the heart weight and heart volumes in the three species. The rabbit showed a significant correlation between the total body weight and the heart weight and heart volume and also between the heart weight and heart volume. In the African giant rats the correlation observed was in the total body weight and heart volume as well as between the heart weight and heart volume. In the wistar rat the only correlation observed was in the total body weight and heart weight.

Histo-Morhpometrics

This is the measurement of the lenght and width of a single ventricular cardiac muscle fibre in the heart of the three rodents as well as the intercellular space between two adjacent single muscle fibres, both of which were measured in micrometers. These measurements were taken under a magnification of X 400.The lenght of the ventricular cardiac muscle fibres showed great variations and as a result of these, five ven-tricular cardiac muscle fibres were randomly selected, measured and then analysed for cardiac muscle lenght in the three mammalian species. Wistar rat heart muscle fibres hada length ranging from 37.5μm to 72.5μm and width ranging from 30μm to 42.5μm, with intercellular space of 15.5μm to 20μm.Rabbit heart muscle fibres have a length ranging from 57.5μm to 100μm and width ranging from 32.5μm to 50μm, with inter-cellular space which ranges from 17.5μm to 25μm.The Afrcan giant rat heart muscle have a length ranging from of 72.5μm to 115μm and width ranging from 47.5μm to 62.5μm, with intercellular space which ranges from 25μm to 32.5μm. The results of the histomorphometric measurements of cardiac muscle fibres are shown in Table5.

Histological Observation of the Rodent's Heart

The result of the histological observation of the hearts of the three Rodent species stained with the H&E and Rafferty's special staining proce-dures show both cross-striational arrangement of the heart muscles with the intercalated disks and cardiac muscle cells. Figure (1) shows the heart muscle of the Wistar rats showing the cardiac muscles with striated muscle fibres and nuclei while Figure (2) shows the heart muscle of the Wistar rats with cardiac striated muscle fibres and nuclei of muscle cells stained with Rafferty Special stain. Figure (3) shows the heart muscle of the African giant rats showing cardiac muscle with striated muscle fibres and nuclei stained using heamatoxylin and eosine stain while Figure (4) shows the heart muscle of the African giant rats showing the cardiac muscle with striated muscle fibres and nuclei using rafferty special staining procedure. Figure (5) shows the heart muscle of the rabbit showing the cardiac muscle with striated muscle fibres and muscle nuclei using heamatoxylin and eosine stain while Figure (6) shows the heart muscle of the rabbit showing the cardiac muscle with striated muscle fibres and muscle nuclei using rafferty staining procedure.

Discussion

Morphologically the shape and orien-tation of the heart of the three mammalian species look alike but the most significant difference lies in the size of the hearts which was justi-fiable owing to the body sizes and the body weights of the three animals differed as a whole (Romer et al., 1977; Cecie et al., 2009). Compa-ratively, it was observed that there were significant differences regarding the heartbeats of the three animals pecies. It was observed that the smaller the mammal, the faster the heartbeat per minute which showed that the heart of the Wistar rats beat was faster than the African giant rats and then followed by that of the Rabbits which is in agreement with the observation made by Kriegs et al. (2006). From the result, it can be observed that the African giant rats have the largest and the thickest heart amongst the three mammals while the Wistar rats heart was the smallest. Morphometrically, it was be observed from the study that the African giant rat has the largest heart amongst the three mammalian species which was evident from the mean value obtained which showed a significant difference between the animls. As for the thickness of the walls of the heart chambers and valves showed significant differences between the species of the

bigger the animal, the bigger the heart and the thicker the heart walls of the animals (Donalson, 1999; Utsumi et al., 2004; Müller et al., 2010; Davoust et al., 2013).

Histologically, the heart of the three rodent species are similar in the nature and arrangement of the cardic muscle fibers of which various sections through the hearts showed cardiac muscles fibres as well as intercalated disks which are similar to that of humans (Kermack and Kermack, 1984; Eroschenko, 2005; Witmer et al., 2010). The shape and structure of the heart muscle cells are similar but the size differed due to the differences in the sizes of their body and their body weight. This is true since the size of the body determines the size of the other organs and tissues of the body (Venzke, 1975; Ibegbu et al., 2014). The length of the muscle fibers can be used to determine the length of the heart and as such the longer the cardiac muscle fibers the longer the heart of the specie (Utsumi et al., 2004).

Conslusion

Significant differences were observed in the morphometrics of the various hearts of the three rodent species. It could also be derived from the study that the differential rates of heartbeats of the mammalian species could be associated to their body weight and their body sizes. The heart muscles of rodents showed great simi-larities to that of humans and other related animal species and as such could be very useful in the comparative anatomy of other mammalian species.

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Table (1): Descriptive statistical analysis of the lenght of the heart chambers in the three species.

Animals	RV(Mean±SEM) (n=5)	LV(Mean±SEM) (n=5)	LA(Mean±SEM) (n=5)	RA(Mean±SEM) (n=5)
AGR	1.93±0.03***	1.63±0.03*	1.27±0.15**	0.93±0.067***
Rabbit	1.37±0.29***	1.10±0.07*	0.90±0.06**	1.13±0.03***
Wistar R	0.93±0.03***	0.70±0.06*	0.43±0.03**	0.27±0.03***

* =P < 0.05 ** = P < 0.01 *** = P < 0.001; RV = right ventricle; LV = left ventricle; RA = right atrium; LA = left atrium.

Table (2): Descriptive statistical analysis of the width of the heart chambers of the three species.

Animals	RV(Mean±SEM) (n=5)	LV(Mean±SEM) (n=5)	LA(Mean±SEM) (n=5)	RA(Mean±SEM) (n=5)
AGR	0.33±0.09*	0.33±0.33***	0.38±0.02***	0.27±0.03**
Rabbit	0.12±0.06*	0.30±0.00***	0.22±0.06***	0.20±0.00**
Wistar rat	0.10±0.33*	0.08±0.03***	0.07±0.03***	0.1033±0.03**

* =P < 0.05 ** = P < 0.01 *** = P < 0.001; RV = right ventricle; LV = left ventricle; RA = right atrium; LA = left atrium.

Table (3): Descriptive statistical analysis of the thickness of the heart chambers and valves in the three species.

Animals	RV(Mean±SEM) (n=5)	LV(Mean±SEM) (n=5)	LA(Mean±SEM) (n=5)	RA(Mean±SEM) (n=5)	IVS(Mean±SEM) (n=5)	LAV(Mean±SEM) (n=5)	RAV(Mean ±SEM) (n=5)
AGR	0.20±0.07	0.46±0.03	0.50±0.03***	0.18±0.02***	0.50±0.2	0.27±0.03***	0.37±0.02***
Rabbit	0.53±0.03**	0.33±0.03*	0.20±0.0***	0.63±0.03***	0.52±0.2	0.09±0.03	0.32±0.02
Wistar rat	0.17±0.03**	0.47±0.03*	0.46±0.03***	0.14±0.01***	0.43±0.04	0.01±0.***	0.01±0.3***

* = P < 0.05 ** = P < 0.01 *** = P < 0.001; RV = right ventricle; LV = left ventricle; RA = right atrium; LA = left atrium; I-V sep = intraventricular septum; L A-V sep = left atrio-ventricular septum,R A-V sep = right atrio-ventricular septum.

Table (4): Correlation between the total body weight and the heart weight and heart volume.

RABBIT	PARAMETERS	HWT	HV
5	TBWT	0.737*	0.918*
5	HWT	----------	0.945*
5	HV	----------	---------
AGR			
5	TBWT	0.371	0.866*
5	HWT	---------	0.786*
5	HV	---------	---------
WISTAR RAT			
5	TBWT	1.000*	---------
5	HWT	---------	---------
5	HV	---------	---------

TBWT- Total body weight; HWT- heart weight; HV- heart volume.*P<0.001

Table (5): lenght and width of cardiac muscle and intercellular space between two adjacent muscle fibres.

Anims	LOCM (µm)		WOCM (µm)		ICBN (µm)	
	Mean±SD N=5	Range	Mean±SD N=5	Range	Mean±SD N=5	Range
Rabb	74.51±12.11*	57.5-100	38.22±7.2*	32.5-50	23.12±5.1*	17.5-25
AGR	87.21±10.21*	72.5-115	51.15±8.1*	47.5-62.5	31.53±8.1*	25-32.5
Wistar	52.12±11.25	37.5-72.5	32.42±5.2	30-42.5	21.45±4.3	20-25

LOCM = length of cardiac muscle; WOCM = width of cardiac muscle; ICBN = intercellular space between the cardiac muscle fibers. *P≤0.05

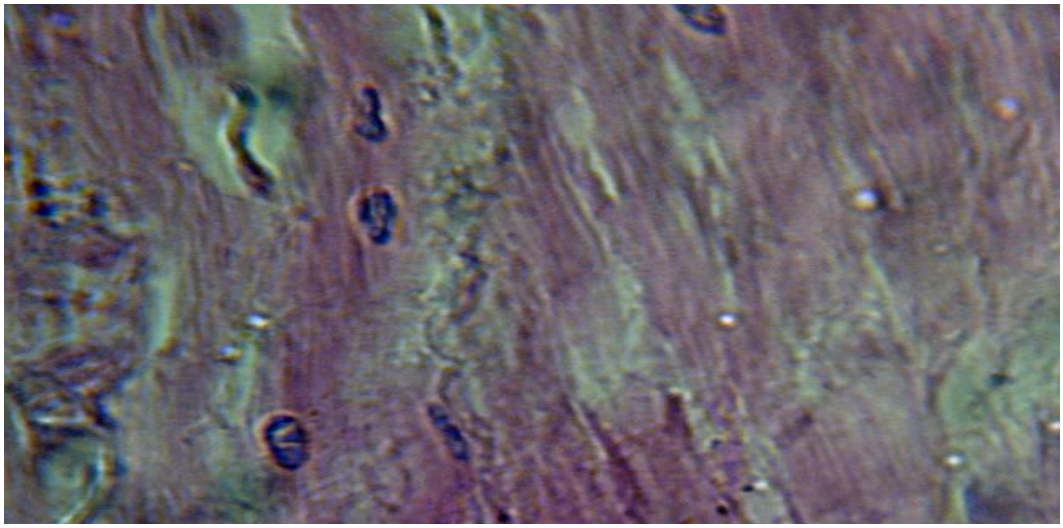


Fig (1): Showing the arrangements of the cross-striations of the cardiac muscle of the heart of adult Wistar rats, NC= nuclei of muscle cells, IC= intercalated disks H & E stain (mag. X400)

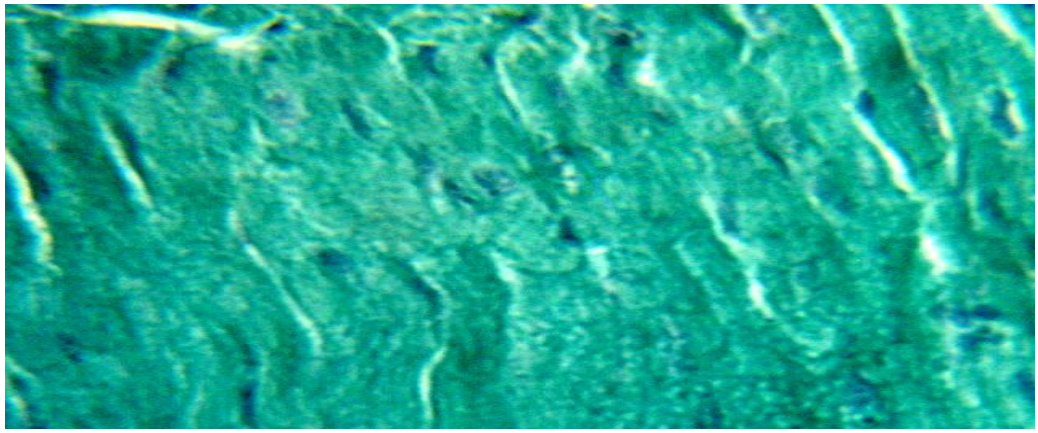


Fig (2): Arrangements in the cross-striations of cardiac muscle of the heart of Wistar rats, NC= nuclei of muscle cells, IC= intercalated disks. Rafferty special stain (mag. X400)

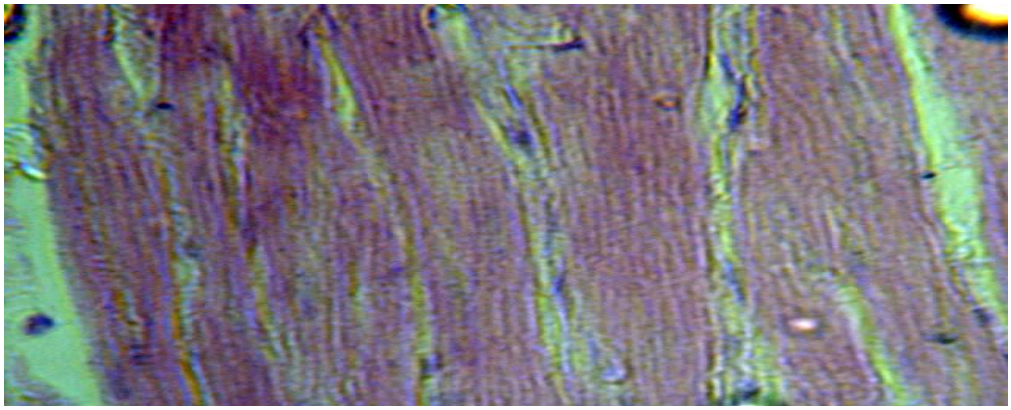
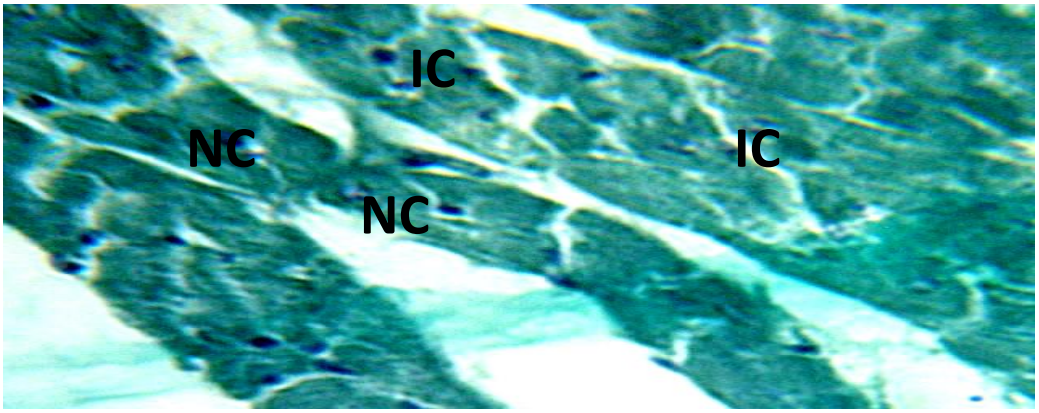


Fig (3): Showing the arrangements in the cross-striations of cardiac muscle of the heart of African giant rats, NC= nuclei of the larger muscle cells, IC= intercalated disks. H & E stain (mag. X400).



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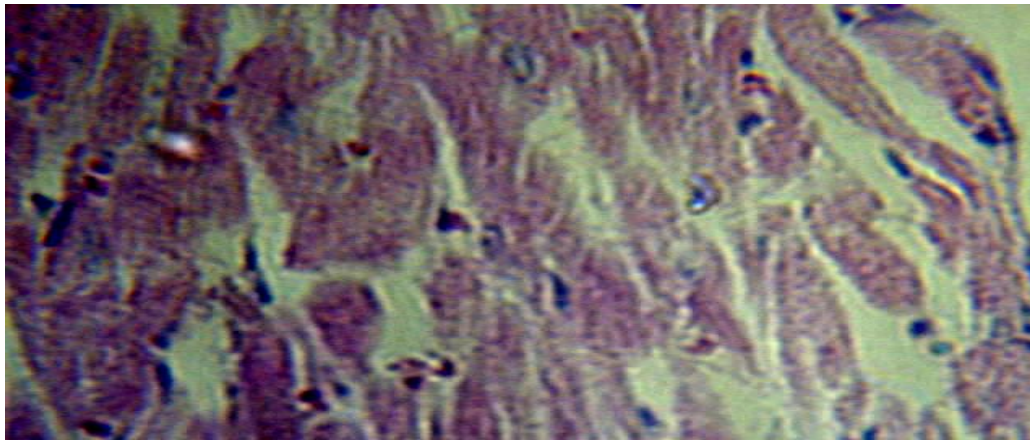

Fig (5): Showing the arrangement of the cross-striations of cardiac muscle of the heart of Rabbits. NC= nuclei of larger muscle cells, IC= intercalated disks. H & E stain (mag. X400)

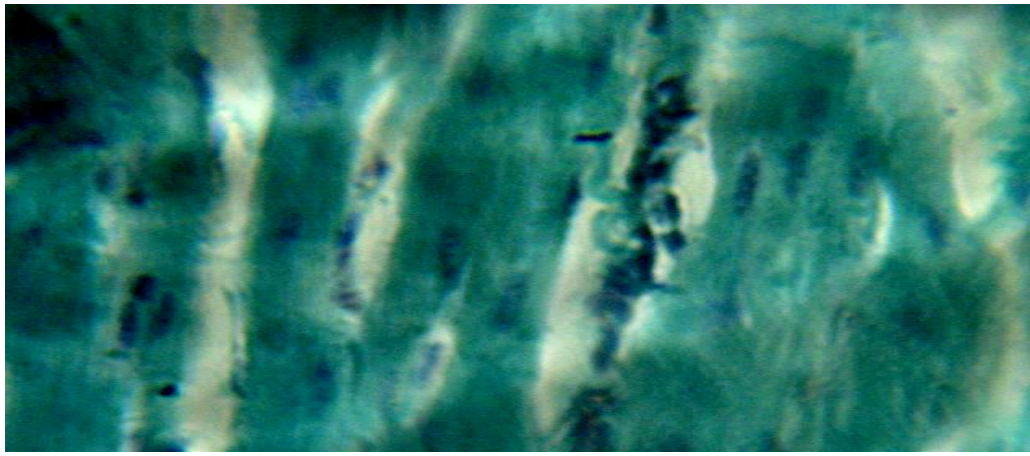

Fig (6): Showing the arrangement of the cross-striations of cardiac muscle of the heart of Rabbits. NC= nuclei of larger muscle cells, IC= intercalated disks .Rafferty special stain (mag. X400)