Comparative Micromorphological Studies of the Cerebrum in Three Mammalian Species: Rabbits (*Oryctolagus cuniculus*), Wistar Rats (*Rattus norvegicus*) and African Giant Rats (*Cricetomys gambianus*).

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Abstract

Comparative morphologic, morphometric and histological studies were carried out on the cerebrum of three adult male mammalian species namely the African giant rat (*Cricetomys gambianus*), Rabbit (*Oryctolagus cuniculus*) and Wistar rat (*Rattus norvegicus*). The animals were acclimatized for two weeks, weighed and lightly anaesthetized with chloroform and humanly sacrificed. The brains were removed through midline incision through the skull and then fixed in Bouin's fluid, processed routinely and stained using Haematoxylin and eosin method and Special stains; Cristal violet and Silver stain. The brains were observed to be milky in colour and globular in shape in the three mammalian species though the size differed in the animals. The mean body weight of the African giant rat, Rabbit and Wistar rat was 1.167±0.153kg, 1.233±0.252kg and 20.333±5.774g respectively. The result showed a statistical significant difference between the mean body weight of the African giant rats and Wistar rats ($p<0.05$) and between the Rabbit and Wistar rat ($p<0.05$). The mean volume of the cerebrum of African giant rat, rabbit and Wistar rat was 22.967±1.620, 25.067±0.115, 11.167± 0.289 which showed a significant difference between the African giant rat and the Wistar rat and between the Rabbit and Wistar rat respectively ($p<0.05$). The cerebrum of the three mammalian species shows sulci and gyri but vary in their extent and sizes. The result of histological observation of the cerebral cortices shows similarities among the three species. The result showed pyramidal cell layer, plexiform layer and granular layer in the cerebrum which vary in size of the layers and cellular population. The results from the present study can be used as a baseline research data in comparative neuroanatomy for related rodent species.

Key Words: Comparative, Micromorphology, Cerebrum, Brain, Wistar rat, African giant rat, Rabbit

Introduction

Comparative morphology has long served as evidence of evolution and indicates that various animals shared a common ancestor. This can assist scientists in classifying animals based on similar characteristics or diversity of their structures (Campbell and Reece, 2002). The Gambian pouched rat (*Cricetomys gambianus*), known as the African giant rat, is a nocturnal rat and among the largest muroids in the world and is widespread in Sub-Saharan Africa (Perry, 2006). In rain forest zone, they are restricted to farmlands, grasslands and human habitations (Happold, 1987; Kingdon, 1997). African giants rat have poor eyesight and so depends on its senses of smell and hearing to explore its environment (Ajayi, 1975; Kingdon, 1997; Perry, 2006). They are useful in some areas for detecting land mines, as their sense of smell is very effective in detecting explosives (Wilson and Burnie, 2001). Rabbits are small mammals found in several parts of the world. More than half of the world's rabbit population resides in North America and are also native to Africa and South America (Wilson and Burnie, 2001). They have large, powerful hind legs and the two front paws have five toes, the extra toe is called the dew claw. The hind feet have four toes and they are plantigrade animals while at rest and they move around on their toes while running, assuming a more digitigrade form (Dyce et al., 1996, Sharon and Crowell, 2010).

Albino Wistar rats are the most common type of Laboratory rats, belonging to the species *Rattus norvegicus*. This strain was developed at the Wistar Institute in 1906 for use in biological and medical research, and was the first rat strain developed to serve as a model organism in the laboratories. Their qualities include: tolerant to crowd, calmness, breeds early, less likely to bite and produce more offsprings within a short period (Kemp, 1982; Jan et al., 2006). Wistar rats are currently one of the most popular rat strains used for laboratory research

(Young, 1981; Wilson and Reeder, 2005).

The cerebrum is the largest part of the brain and is the part of the brain involve in reasoning, learning, sensory perception and emotional responses. It consists of two connected cerebral hemispheres (Singh, 2006).Some areas of the cerebral cortex can be assigned specific functions. These areas can be defined in terms of sulci and gyri (Shoshani et al., 2006). The Broadmann's scheme represents different areas by numbers, which include the Motor area, in the pre-central gyrus on the superio-lateral surface of the hemisphere which corresponds to area four on Broadmann classification (Saladin, 2001). Premotor area is anterior to the motor area and occupies the posterior part of the superior, middle and inferior frontal gyri, which constitute the motor area (Russel, 1979; Singh, 2006). Sensory areas are located in the post-central gyrus. Auditory Area is situated in the temporal lobe and lies in that part of the superior temporal gyrus, which forms the inferior wall of the posterior ramus of the lateral sulcus (Saladin, 2001).

The cerebrum is larger relative to the rest of the brain in mammals; such as primates and as such show some levels of intelligence (Jenkins, 1978; Ridgway, 2001). In these animals indications of intelligence include the ability to learn, matched with behavioral flexibility (Peters and Jones 1984; Sharon and Crowell, 2010). Rats, for example, are considered to be highly intelligent, as they can learn and perform new tasks (Sharon and Crowell 2010). The aim of the present work was to study the comparative micro-morphology of the cerebrum of three mammalian species namely the Rabbits, African giant rats and Wistar rats.

Material and Methods

Experimental Animals

The animals used for the research were five adult male rabbits, five adult male Wistar rats and five adult male African giant rats, were obtained from Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, and were transferred using animal cages to the Department of Human Anatomy Laboratory, Ahmadu Bello University Zaria. The animals were acclimatized for a period of two week before the commencement of the study.

Animal Sacrifice

The animals were weighed using weighing balance and anaesthetized lightly using chloroform. The animals were then sacrificed humanely and the head detached immediately. A longitudinal incision was made along the mid-cranial line to allow the penetration of Bouins fluid as a fixative. The brains were removed by gentle traction with scalpel and were then weighed using a digital scale. The components of the brain were then detached, weighed and recorded.

Morphologic Studies

The structural characteristics of the animals were examined; the weights of the animals were measured before sacrifice and the area of the head and the brain were examined with the naked eyes after sacrifice. This includes the shape, sizes, volume, weight of the brain and the presence of sulci and gyri were evaluated in each of the brains.

The fixed brain tissues were processed routinely and stained using three different stains namely, Haematoxylin and eosin stain (H and E), a general stain and two special stains were used namely, Cresyl fast violet and silver stain, according to the methods of Itabashi et al. (2011).

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.

Results

Morphologic Observation

The brain of the three species, the rabbit, African giant rat and Wistar rats are milky in colour, it shows two major depressions separating the cerebrum from the cerebellum and the other separating the cerebrum into two hemispheres. The cerebrum was observed to be the largest part of the brain and it is the largest among each of the three animals species. The mean number of sulci on the cerebrum of the rabbits was 23.3±0.41, African giant rat was 16.0±0.36 and Wistar rat was 10.6±0.28 and the mean amount of gyri on the cerebrum of Rabbit was 24.4±0.37, African giant rat was 17.0±0.59 and Wistar was 11.6±0.85. The sulci and gyri of the rabbit were more depressed significantly when compared to that of the African giant rat and the Wistar rats, and between the African giant rat and Wistar rat ($P<0.05$) as shown in Table 1.

Morphometric Study

The morphometric study shows the mean and standard deviation of length, height, width and volume of the whole brain and cerebrum in the African giant rat, Wistar rat and Rabbit. There was significant difference in the length of the whole brain between the Rabbit, Wistar rat and

African giant rat (P<0.05), but there was no significant difference in the length of the cerebrum between the African giant rat and the Rabbit. However, there was an increase in the length of cerebrum of rabbit and African giant rat over that of Wistar rats.

There was a significant difference in the height of the whole brain and the cerebrum between the Rabbit, African giant rat and Wistar rat (P<0.05). There was a Statistical difference between African giant rat, Rabbit and Wistar rat in the volume of whole brain, while in the volume of the cerebrum, there was a statistical difference between that of rabbit and Wistar rat (P<0.05). There was statistical significant difference in the width of the whole brain between the Rabbit and Wistar rats, and African giant rat and Wistar rat only (P<0.05), and there was no significant difference between that of Rabbit and African giant rats.

The results in Table 2, showed the mean and standard deviation of the total body weight, whole brain weight, weights of the cerebral cortices. There was significant difference in the total body weight, between the Wistar rats and African giant rats, and between Wistar rats and Rabbits (P<0.05), but there was no significant difference in the body weights between the rabbits and the African giant rats. The weight of the whole brain showed significant difference between the rabbits and Wistar rats, African giant rats and Wistar rats but there was no significant difference between the whole brain weights of the rabbits and African giant rats (P<0.05). There was a significant difference in the right and left hemispheres of the cerebrum (P<0.05) between the African giant rats, Wistar rats and Rabbits.

The results in Table 3 show the mean and standard deviation of cranial width, ear-to-ear measurement, cranial length, side-to-side and eye-to-eye measurement. There was a significant difference in the cranial length between the Rabbit and Wistar rats, African giant rats and Wistar rats while there was no significant difference between the rabbits and African giant rats. There was a significant difference in the cranial width, ear-to-ear measurement, and the mandible-to-mandible length between the African giant rats, Rabbits and Wistar rats. In the side-to-side measurements, there was a significant difference between the Rabbits and Wistar rats, African giant rats and Wistar rats (P<0.05), but no significant difference between the African giant rats and Rabbits. There was a significant difference in the eye-to-eye measurements (P< 0.05) between the African giant rats and Wistar rats, and there was no significant difference between the African giant rats and rabbits, and between the African giant rats and Wistar rats.

Histological Observations

The cerebral corticesin the three mammalian species were similar in terms of their structural composition as they are consisting of six distinguished layers. The plexiform or molecular layer consists of few cells, the second layer, the outer granular layer consist of small, if any polymorphous cells, the third layer consist of medium pyramidal cells or layer of outer pyramidal cells, the fourth layer or the granular layer consist of many granular cells.The fifth layer of large pyramidal layer, consist of large pyramidal cells, while the sixth layer consists of polymorphic cells as shown in Figures 1 to 9.

Discussion

Morphologically, the shape, colour and arrangement of sulci and gyri of the cerebrum of African giant rats, rabbits and Wistar rats are similar though there were differences in the numbers and sizes of sulci and gyri in the African giant rats, Rabbits and Wistar rats which may result in the differences in their behavioral system (Russel, 1979; Sharon and Crowell, 2010). The study showed that the larger the size of the cerebrum, the more the number of sulci and gyri on it, which shows that the Rabbits have higher and many numbers of sulci and gyri than the African giant rats and Wistar rats which may be the reason why Rabbits are more intelligent than the other rodents (Peters and Jones, 1984; Sharon and Crowell, 2010).

Histologically, the cerebrum of the African giant rats, Rabbits and Wistar rats are similar, showing pyramidal cell layer, plexiform layer and granular layer which are similar to that of humans (Windle, 1958; Eroschonko, 2003). The results revealed that the pyramidal cell layer contain giant pyramidal cells in the Rabbits followed by those of the African giant rats and then those of Wistar rats. This may be the reason why Rabbits have more sense of understanding and intelligence followed by the African giant rats when compared to those of Wistar rats (Russel, 1979; Kermack and Kermack, 1984; Kelly and Stick, 2003).

Morphometrically, the total weight of the body and that of the brain is higher in the Rabbits than that of the African giant rats, and that of the African giant rats are higher than that of the Wistar rats. The length of the cerebrum of the Rabbits are greater than that of the Wistar rats and African giant rats which agrees with the findings of Kelly and Stick (2003), Jan et al. (2006).

The study shows that the total body weight of the African giant rat has correlation with the brain weight, cerebrum weight and the brain volume (Caldwell, 2006). The total body weight of the rabbit has a positive correlation with the brain weight and a negative correlation with the brain volume. The total body weight of the Wistar rats has a negative correlation with the brain weight and a positive correlation with the brain volume (Affi and Bergman, 1998; Donalson, 1999; Jan, et al., 2006).

Conclusion

This study has shown the similarities and differences between the cerebrum of three mammalian species; African giant rats, Wistar rats and Rabbits. Correlation between the total body weight, cerebral weight and other parameters were observed in the three rodents. The result obtained from the present study can be used as research baseline data for neuro-pharmacology and animal neuro-psychiatry. It can also be used for comparative neuro-anatomy for other animals.

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Table (1): The colour, shape, brain weight and mean number of sulci and gyri

ANIMALS	COLOUR	SHAPE	TBWT	BWT	M/SULCI	M/GYRI
RABBIT	Milky	Globular	1.23±0.25kg*	6.14±0.12gm*	23.3±0.41*	24.4±0.37*
AGR	Milky	Globular	1.17±0.15kg*	3.94±0.15gm*	16.0±0.36*	17.0±0.59*
WSTRAT	Milky	Globular	203.3±5.8gm	6.14±0.21	10.6±0.28	11.6±0.85

*P≤0.05; AGR=African Giant Rat, WST RAT=Wistar rat, TBWT=total body weight, BWT=brain weight, M=mean

Table (2): The Mean and Standard Deviation of height, length, Volume and width of the Cerebrum

	AGR	WRT	RBT
Length (cm)			
LWB	3.23±0.06*	2.23±0.12	4.17±0.21*
LCBR	2.20 ±0.10*	1.30±0.61	2.43 ±0.91*
Height (cm)			
HWB	1.33±0.08*	0.78±0.06	1.78±0.06*
HCBR	1.32±0.06*	0.75±0.06	1.73±0.06*
Width (cm)			
WWB	2.07±0.06*[a]	1.40 ±0.0	2.37±0.23*[b]
WCBR	2.07±0.06*	1.33±0.12	2.03±0.81*
Volume (cm^3)			
VWB	24.77±0.32*	16.27±0.25	26.27±0.30*
VCBR	22.97±1.62*	15.07±0.12	25.17±0.29*

*p<0.05=significant, a&b=no significant, L=length, H=height, W=width, V=volume, CBR=cerebrum, LWB=Length of Whole Brain.

Table (3): The Mean and Standard Deviation in the Weight of the Brain

WEIGHT	AGR	WRT	RBT
TBW	1170±0.15*[a]	203.33±5.77	1230±0.25*[b]
WWB(G)	39.4±0.15*	6.14±0.21	53.7±0.09*
CRBRM	32.10±0.13*	5.07±0.13	47.61±0.03*
LFT CRBM	16.30±0.08*	2.55±0.07	24.67±0.02*
RT CRBM	15.80±0.06*	2.28±0.06	24.60±0.07*

*p<0.05=significant, a&b=no significant, TBW=total body weight, LFT=left, RT=right, CBRM=cerebrum.

Table (4): Mean and Standard Deviation of External Morphology

mm	AGR	WRT	RBT
CL	8.70±0.46*[a]	4.87±0.15	9.93±1.97*[b]
CW	3.30 ±0.10*	1.96±0.06	4.36±0.40*
EE	5.32±0.15*	1.83±0.05	2.50±0.20*
SS	7.27±1.70*	4.66±0.15	13.03±1.05*
MM	4.60±0.27*	1.93±0.05	3.13±0.23*

*p<0.05=significant, a&b= no significant, CL=cranial length, CW=cranial width, EE=ear to ear,
SS=side to side, MM= mandible-to-mandible, mm= millimeter.

Table (5): Correlation between total body weight , brain volume and brain weight.

RABBIT	PARAMETERS	BWT	CBLWT	CBWT	BV
5	**TBWT**	**0.327**	**0.986***	**0.629***	**0.866***
5	BWT	-------	0.470*	0.529*	0.756*
5	CBLWT	-------	-------	0.500*	0.933*
5	CBWT	-------	-------	-------	0.156
5	BV	-------	-------	-------	-------
AGR					
5	TBWT	0.378	0.419	0.0339	0.0339
5	BWT	-------	0.999*	0.938*	0.938*
5	CBLWT	-------	-------	0.922*	0.922*
5	CBWT	-------	-------	-------	1.000*
5	BV	-------	-------	-------	-------
WISTAR					
5	TBWT	0.826*	0.322	0.886*	0.500*
5	BWT	-------	0.800*	0.470*	0.0751*
5	CBLWT	-------	-------	0.154	0.659*
5	CBWT	-------	-------	-------	0.845*
5	BV	---------	--------	-------	-------

*Significant correlation

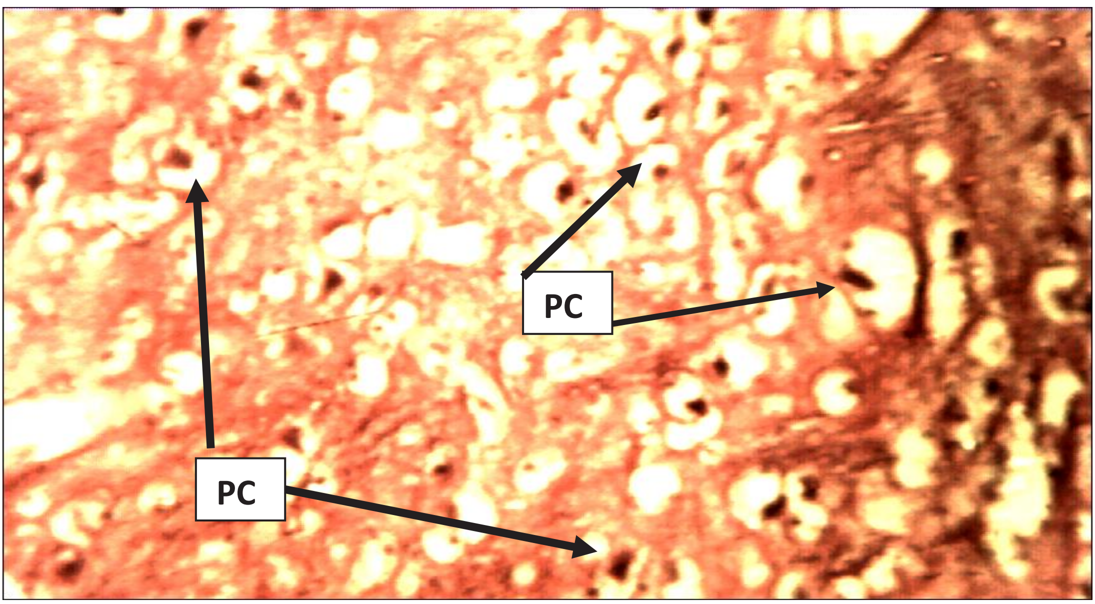

Fig (1): A section of cerebral cortex of African Giant rat, showing many pyramidal cells (PC). H&E (X 250).

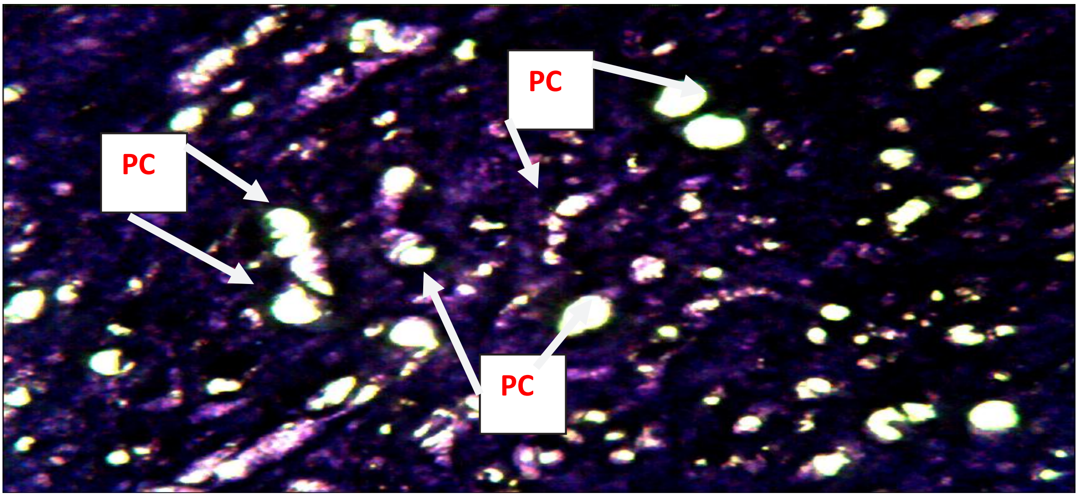

Fig (2): A section of cerebral cortex of African Giant rat, showing numerous pyramidal cells (PC), Cresyl fast Violet, (X 250).

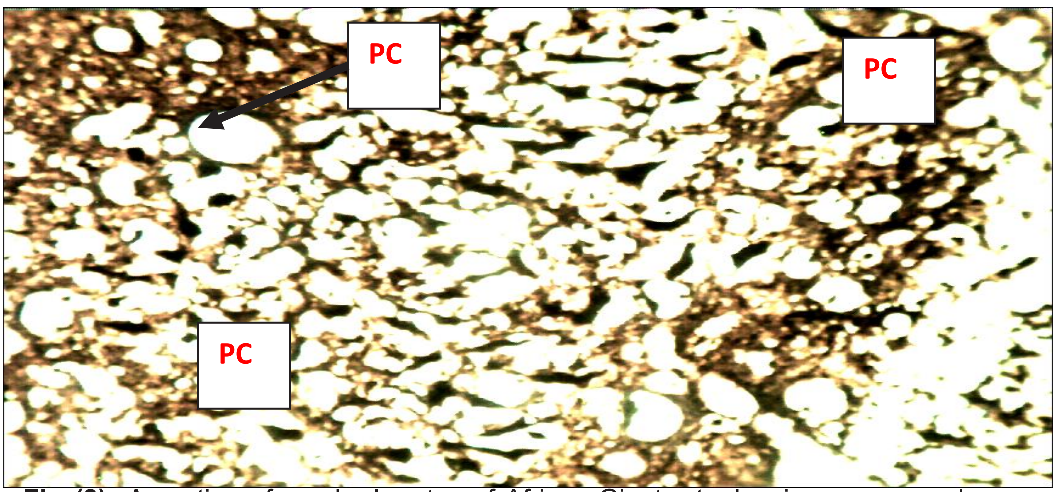

Fig (3): A section of cerebral cortex of African Giant rat, showing numerous large pyramidal cell (PC), Silver stain, (X 250).

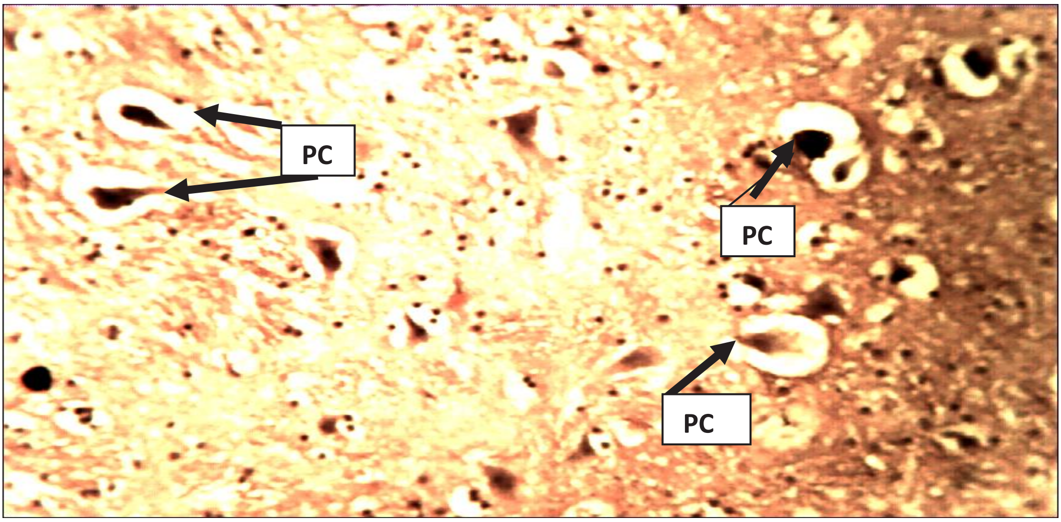

Fig (4): A section of cerebral cortex of Rabbit, showing Few giant large pyramidal cells (PC), H&E, Mg X 250.

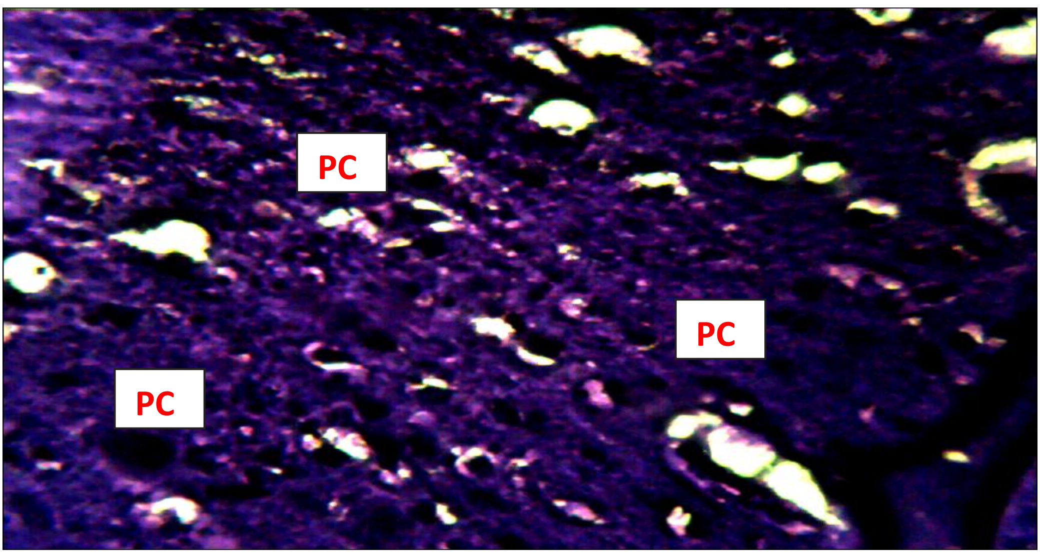

Fig (5): A section of cerebral cortex of Rabbit, showing few giant pyramidal cell, (PC), Cresyl fast Violet, (X 250).

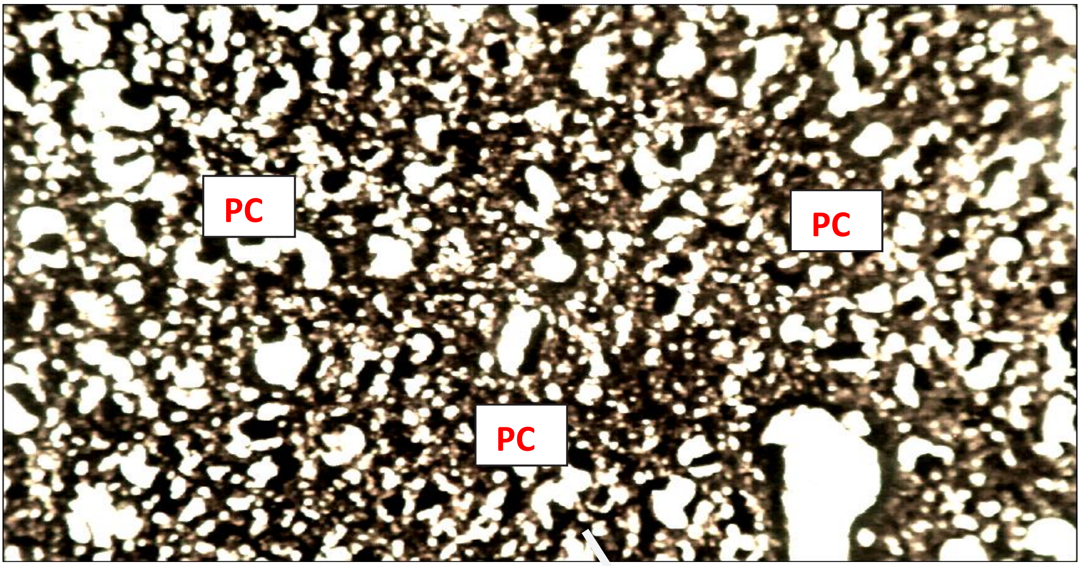

Fig (6): A section of cerebral cortex of rabbit, showing large pyramidal cells (PC), Silver stain, (X 250).

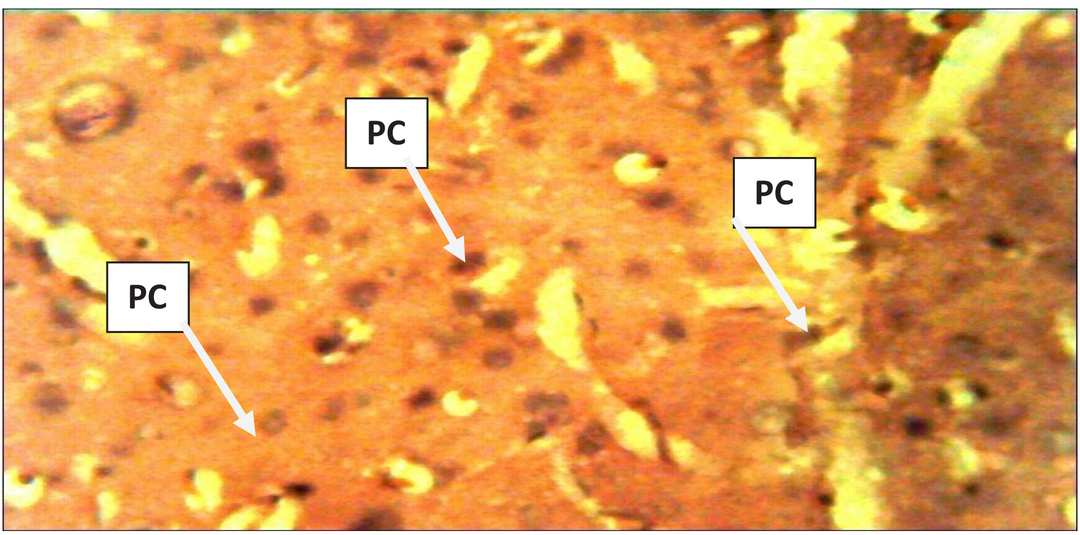

Fig (7): A section of cerebral cortex of Wistar rat, showing many small sized pyramidal cells, (PC) H&E (X 250).

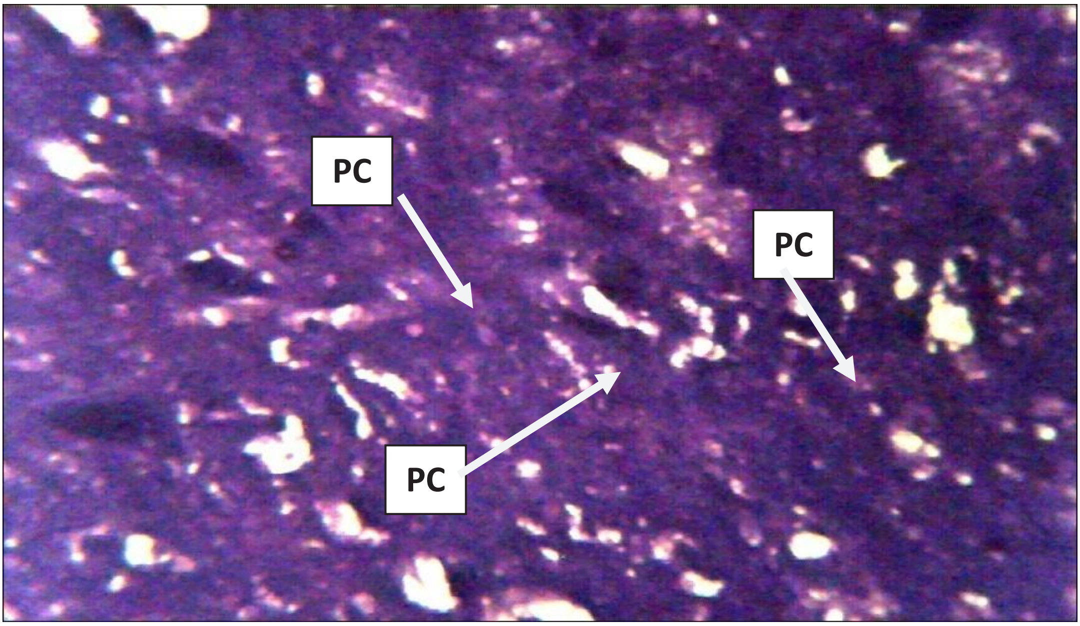

Fig (8): A section of cerebral cortex of Wistar rat, showing few pyramidal (PC), Cresyl fast Violet, (X 250).

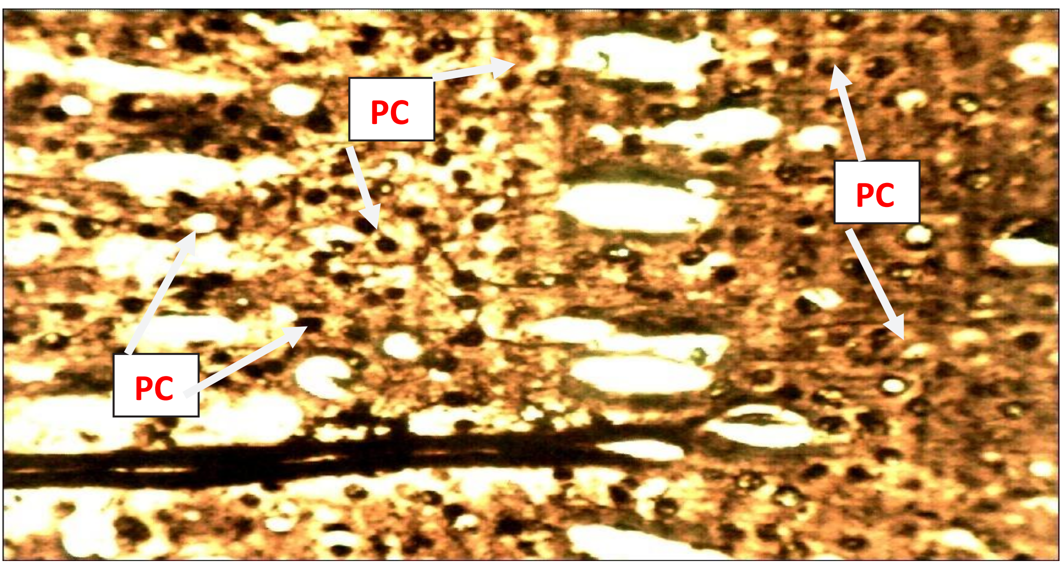

Fig (9): A section of cerebral cortex of Wistar rat, showing small sized pyramidal cells (PC), Silver stains (X 250).