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Comparative Histomorphometric and Biochemical Analysis of Cerebral Cortex in Hedgehogs (*Atelerix albiventris*) and Pangolins (*Manis tricuspis*)

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ABSTRACT

The aim of this present study is to compare the gross anatomy, morphometry, histology and biochemical analysis of the cerebral cortex in African hedgehogs and pangolins. In this comparative study, six hedgehogs and five pangolins were used followed by histopathological and biochemical analysis. The average body weight and body length of hedgehog and pangolins were 181.50 g, 15.80 cm and 1558.40 g, 115.75 cm, respectively. The average brain weights and volume of hedgehog and pangolin were 1.42 g, 1.27 mL and 10.56 g, 66.38 mL, respectively while their Relative Tongue Weight (RTW) were determined to be 0.67 and 1.92%, respectively. The biochemical analysis of Lactate Dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PDH) in hedgehog and pangolin were found to be 12449.80, 11728.60 and 17090.75, 14937.00 U L⁻¹, respectively. The findings of this study provide baseline data that could be relevant in understanding the anatomical adaptation for their diet, cerebral and carbohydrate metabolism in these insectivorous mammals.

Key words: Morphometry, biochemical, comparative, morphology, neocortical

INTRODUCTION

These two mammals were chosen because of the inaccessibility and dearth of documented data on their relationship which correlate cerebral histomorphology and biochemical analysis. Hedgehog, *Atelerix albiventris* and Pangolin, *Manis tricuspis*, are insectivorous terrestrial mammals (Ofusori *et al.*, 2008; Adeniyi *et al.*, 2010; Stevens *et al.*, 2005; Hildebrand and Goslow, 2001). However, study has been done on the comparative anatomy of cerebral histology of Hedgehog, bat and rat (Adeniyi *et al.*, 2009). Three types of pangolins exist in Africa the giant pangolin, the tree pangolin and the most widespread, the ground pangolin. Pangolins have small heads and long, broad tails. They are toothless and have no external ears but they have a good hearing ability. Their sense of scent is well-developed but their sight is poor. The weight of the protective keratinous scales and skin make up about 20% of the pangolin's weight. The animal preens itself by scratching with the hind legs, lifting its scales so the claws can reach the skin. It also uses its tongue to remove insects from under the scales (African Wildlife Foundation, 2011). Enzyme metabolism is a fundamental biological process that is vital for the survival of all species. Their specific function is to catalyze chemical reactions. Enzymes have found wide and diverse applications at which enzymes increase the rate of reactions which approach to equilibrium (Mohamed and

Metwally, 2009). Enzymes play critical role in the metabolic activities of all living organisms whether humans, animals, plants or microorganisms and are widely applied in microbial technology and their diagnosis processes. Glucose-6-phosphate dehydrogenase is the rate-limiting enzyme of the Hexose Mono Phosphate (HMP) shunt pathway producing NADPH (Abo-Kassem, 2005; Mohamed and Metwally, 2009; Raja *et al.*, 2011).

To the best of our knowledge, this might be the first time, any work is been done to relate all these differences with the micro-and macro-anatomical adaptation which the cerebral cortex of these two insectivorous mammals have adopted during the evolutionary advancement. The soft internal parts of invertebrate along with their flesh do contain protein and fat which provide the nutrient for Hedgehog, Pangolin and other ant-eaters (Abayomi *et al.*, 2009; Scally *et al.*, 2001). The sand and other materials adsorbed together with the termite has been reported to add bulk to the digestive load of insectivorous and thus, reduced the caloric proportion of their digestive content (Adeniyi *et al.*, 2010).

This study comparatively elucidate the morphology, macro-and micro-structural organizational and biochemical analysis of the cerebral cortex of the two insectivorous mammals on the successfully manipulate their different diets to cope with the morphological differences.

MATERIALS AND METHODS

Care of the animals: Six hedgehogs weighing 178.00 g in average and five Pangolins weighing 1558.40 g in average of both sexes were used. The hedgehogs were procured for local sellers in Ilorin, Nigeria following ethical clearance and maintained in the animal holding of the same Department. The Pangolins were as well procured from Asejire Community of Oyo State, Nigeria. They were fed with insects and allowed to have asses to water. The animals were carefully conditioned. Handling and care of the animals conform to the animal right committee of the University of Ilorin, Nigeria and the rule guiding Good Laboratory Practice was also adhered to. This research was conducted in the Department of Anatomy, College of Health of Sciences, University of Ilorin, Ilorin, Nigeria, for period of three months, between January and March, 2011.

Excision of the cerebral cortex: After sacrifice by chloroform inhalation, the brains were excised, using the bone forceps, from the animals and were blotted using filter paper and there in weight was recorded, using Gallenkomp electric weighing balance (Model FA2104A). The brains were quickly transferred into bottle containing 10% formol calcium for 48 h.

Histological procedures: The frontal cortex were carefully excised and processed routinely for paraffin embedding. Serial sections were obtained at 5 μ thickness from a rotary microtome and subjected to the Haematoxylin and Eosin (H and E) and Cresyl Fast Violent (CFV) stains (Adeniyi *et al.*, 2009; Bancroft and Stevens, 1990). The sections were mounted and examined with the light microscope and the photomicrograph of each slide was taken for further analysis.

Biochemical evaluation: The tissues for biochemical assay were weighed using a sensitive balance (Model FA2104A) and they were placed in 0.25 M sucrose and homogenized in a cold mortar with pestle. The homogenate was poured into a test-tube and centrifuged at 5000 rpm for 5 min using a centrifuge (Model 90-1). The supernatants were collected, using Pasteur pipettes, were immediately stored in the deep freezer (GC-B207WVQ) at -20°C and thereafter assayed.

Using RANDOX Laboratories Ltd (UK) biochemical kits, the activities of Lactate Dehydrogenase (LDH) in the homogenate and Glucose-6-dehydrogenase (G-6-PDH) enzyme in the homogenate were determined through spectrometry (colorimetric method) (Ofusori *et al.*, 2008; Abo-Kassem, 2005).

Statistical analysis: The data were expressed as Mean±SEM. The means were compared using paired samples t-test. A p-value less than 0.05 were considered statistically significant, using SPSS software version 16.0.

RESULTS

Morphological analysis

Sulci and gyri: The sulci and gyri in the brain of pangolin were prominent and shallower than those of the hedgehog.

Brain weight: There was significant difference ($p < 0.05$) in the brain weight between the two mammals. The Pangolin has higher brain weight (10.56 ± 0.48 g) compare to hedgehog (1.42 ± 1.00 g) and this correlates with their body weight (Table 1).

Cerebral weight: There was significant difference ($p < 0.05$) in the cerebral weight between the two mammals. The Pangolin has their higher cerebral weight (7.60 ± 0.21 g) compare to hedgehog (1.1640 ± 0.13 g) and this correlates with their body weight (Table 1).

Relative Brain Weight (RBW): Using the students' test ($p < 0.05$), there was significant different in the RBW between the two mammals. RBW (0.78%) of the hedgehog is significant higher than that of Pangolin (0.68%) (Table 1).

Histological observations: The microscopic observations made from histological preparations with Haematoxylin (H) and Eosin (E) and Cresyl Fast Violent (CFV) revealed the following;

Blood vessels: The pangolin brain is well vasculated than that of hedgehog (Fig. 1).

Stain intensity: The intensity of the soma of the cells in the hedgehog brain appears to be more deeply stained compared to those of pangolin (Fig. 1).

Cell size: the cells in the hedgehog cortex appears to be larger compared to those of pangolin (Fig. 1).

Table 1: Showing the results of morphology in the two mammals

Weight	Hedgehogs (n = 6)	Pangolins (n = 5)
Body weight (g)	181.50±23.79	1558.40±234.72*
Body length (cm)	15.80±0.09	115.75±6.75*
Brain weight (g)	1.4200±1.00	10.56±0.48
Cerebral weight (g)	1.1640±0.13	7.60±0.21*
Cerebral volume (mL)	1.27±0.32	8.52±0.23*
RBW (%)	0.78±0.00	0.68±0.00

The values were expressed as Mean±Standard error of mean. *Significantly different from hedgehog ($p < 0.05$)

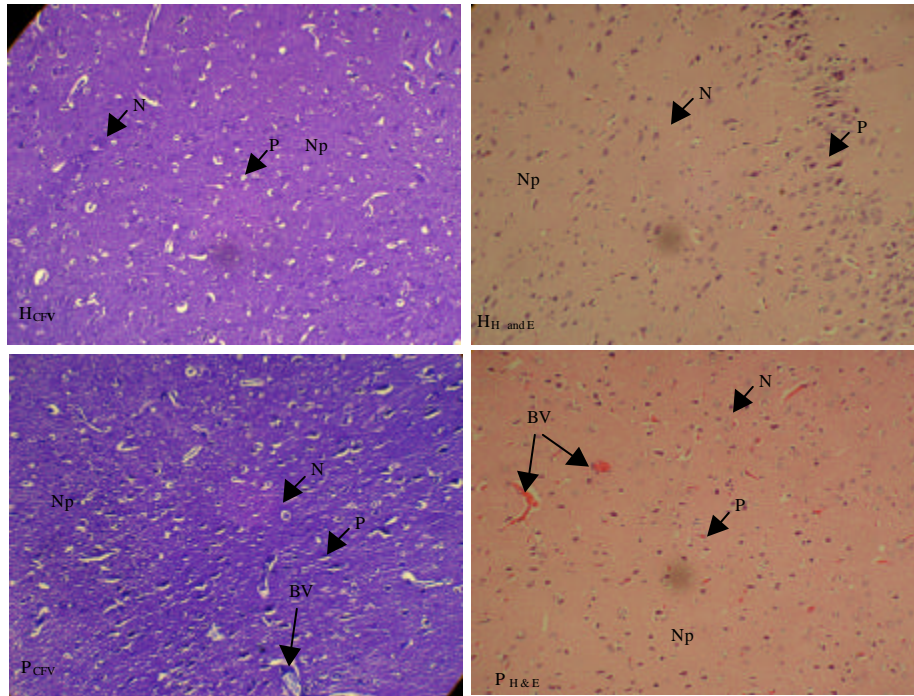


Fig. 1: Frontal Cortex (H and E AND CFV) of Hedgehog (H) and Pangolin (P): Mg X 320 Np: Neuropil, N: Neural cell; V: Vacuole; BV: Blood Vessel

Table 2: Showing the results of biochemical parameters in the two mammals

Biochemical parameters	Hedgehogs (n = 6)	Pangolins (n = 5)
LDH (U L ⁻¹)	12449.80±322.4	17090.75±0.00*
G6PDH (U L ⁻¹)	11728.60±1011.2	14937.00±0.00*
LDH: G6PDH	1.0615	1.1442*

The values were expressed as Mean±Standard error of mean. *Significantly different from hedgehog (p<0.05)

Biochemical evaluation

Lactate Dehydrogenase (LDH): Table 2 shows that the LDH in pangolin frontal cortex is more than that in the hedgehog significantly (p<0.05).

Glucose-6-phosphate dehydrogenase (G6PDH): Table 2 shows that the G6PDH in frontal cortex is more than that in the hedgehog significantly (p<0.05).

LDH: G6PDH: Table 2 shows that ratio of LDH-G6PDH in hedgehogs and pangolins were 1.0615 and 1.1442, respectively, though not statistically significance (p>0.05).

DISCUSSION

The average brain weight and volume of the hedgehog and pangolin were found to be 1.42 g, 1.27 mL and 10.56 g, 8.52 mL; with those of pangolin statistically higher (p<0.05). The average brain weight of pangolin (10.56 g) is higher than those of African Giant Rat (AGR) (4.94 g) and

Grasscutter (10.02 g) obtained in the work of Umosen *et al.* (2008a) and bat (3.4 g) in our earlier work (Adeniyi *et al.*, 2009). Also the average cerebral weight of pangolin (7.60 g) is also higher than that of Grasscutter (5.70 g) in the work of Umosen *et al.* (2008b). The Relative Brain Weight (RBW) of hedgehog and pangolin were found to be 0.0078 and 0.0068, respectively. These are smaller than those of Man (0.02) (Adeniyi, 2011) and Grasscutter (0.01) (Umosen *et al.*, 2008a) and bigger than that of Red Sokoto sheep (0.006) reported by Olopade and Onwuka (2002). A direct relationship of the body weight increase to the increase in the Relative Brain Weight (RBW) was not observed in this data. In this study it is important to note the change in activity of the enzymes of carbohydrate metabolism (Table 2), Glycogen utilization through glycolysis is one of the metabolic pathways which directly participate in generation of sufficient Adenosine Triphosphate (ATP) to meet energy demands of the brain and other vital organs of the body (Mohamed *et al.*, 2009). Glucose system of the brain depends on the stage of development of the animal, in the embryonic brain the cells are characterized by anaerobic metabolism while the adult neurons are characterized by aerobic metabolism (Standring, 2005). This study of the enzymes LDH which catalyses the conversion of Lactate to pyruvate; which is an end product of Pentose phosphate pathway (glycolysis) serves as a substrate which is then converted to Succinyl Co.A, a starting enzyme in the tricarboxylic (Krebs Cycle), this however explains the anaerobic system of the neurons which is believed to partly occur in the astrocytes and the substrate is then supplied to the neurons, G6PDH in another case is an important, although not the starting enzyme of the Hexose Monophosphate shunt (HMP) serves as an indicator of glycolysis preferred to Hexokinase as an indicator (Katzung, 2005; Mohamed *et al.*, 2009). Determination of LDH and G6PDH levels is to describe the shift model in carbohydrate metabolism in the study, otherwise energy production and consumption pattern adopted for each animal in this study. The levels of LDH and G6PDH in pangolin's frontal cortex were higher than those of hedgehogs', 17090.75, 14937.00 and 12449.80, 11728.60, respectively; however, the differences between their LDH: G6PDH were not significant statistically. Normal LDH activity is indicative of improved channeling of glucose (pyruvate) for mitochondrial oxidation and an abnormally high LDH activity showed a blockage in the glucose metabolism Glucose-6-phosphatase catalyses the final step of glucose production in cerebral cortex. Glucose-6-phosphatase and fructose-1,6-diphosphatase are regulatory enzymes of gluconeogenic pathway (Abo-Kassem, 2005; Mohamed and Metwally, 2009; Arulselvan and Subramanian, 2007). This indicates the proportional roles of these two enzymes of carbohydrate metabolism in the two insectivorous mammals were the same. Above all, the large size of cerebrum of the pangolin can offers advantages of experimental procedures. On the other hand it makes practicable for extensive neocortical ablation studies on the mammal. However, it will allow neocortical recording of bioelectrical activity by mere implant of screws into the skull over the neocortex. These findings are in agreement with our earlier work (Adeniyi *et al.*, 2009) and can be a research base line data for animal psychiatry and in pharmacology. It can also serve as control when working with pathological cases and in comparative neuroanatomy of other African mammals.

CONCLUSION

The result of this study showed that the average brain weight of pangolin is higher than those of African Giant Rat and Grasscutter and bat. Also the average cerebral weight of pangolin is also higher than that of Grasscutter; while the Relative Brain Weight (RBW) of hedgehog and pangolin were smaller than those of Man and Grasscutter and bigger than that of Red Sokoto sheep. It was

also established that an increase in body weight is not the same to an increase in the Relative Brain Weight (RBW) in these mammals. These data can be useful in comparative neuroanatomy for other insectivorous mammals and rodent.

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