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Study of Acetyl cholinesterase (Ache) Gene Expression And its Relation with RNA Content in Brain of Five Different Vertebrate Species

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Abstract : *Inhibition of acetylcholinesterase (AChE), the metabolizing enzyme of acetylcholine is evolving as the most important therapeutic target for development of cognitive enhancers. However, AChE activity in brain has not been properly evaluated on the basis of sex. In the present study, AChE expression was investigated in different brain areas of cerebrum and cerebellum in male and female of five different vertebrate species. On comparing male and female genders, increased AChE activity was seen in cerebrum and cerebellum of female of five different vertebrate species. However, no significant change in AChE activity was found between cerebrum and cerebellum within the same male and female. Thus it appears that sex alters AChE activity in different brain regions (G4 isoform) that may vary in male and female. Sequence analysis revealed that highest divergence was found in between male cerebrum and female cerebrum and least divergence was found in between male cerebellum and female cerebellum with control AChE of five different vertebrate species*
Keywords *- Acetylcholinesterase; Physiology; Invertebrates; Vertebrates; Cerebrum*

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I. Introduction

Acetylcholinesterase (AChE) is one of the most efficient enzymes of nervous system which is concentrated at the cholinergic synapses and at neuromuscular synapses where it rapidly hydrolyses the neurotransmitter acetylcholine (ACh) in to choline and acetate thus playing a vital role in cholinergic neurotransmission. The term acetylcholinesterase was introduced in 1949 by Augustintion and Nachmansohn for specific cholinesterase capable of hydrolyzing acetylcholine faster than other esterases. In 1964 the commission of enzymology recommended the name "Acetylcholinesterase" (Acetylcholine Acetyl hydrolase; 3.1.1.7) for a true and specific cholinesterase. The distribution of the enzyme in the central and peripheral nerve tissues of different vertebrates demonstrates a high range of variation (Aldridge WN et al, 1959; Gerebtzoff et al, 1959; Chacho et al, 1960; Boell J et al, 1996). It has been noted to be localized in non neuronal tissues and Glial cells also (Koelle et al, 1954 Brightman et al, 1959). The enzyme also exhibits molecular diversity with its six different molecular forms and structural dynamics which facilitates its affinity and action with various legends (Bon et al, 1982; Shen et al, 2002).

In addition, AChE is considered to play several non classical roles independent of its catalytic function i.e. hydrolysis of ACh. These classical and non classical roles of AChE illustrate adequacy about its wide occurrence in neuronal and non neuronal tissues (Soreq et al, 2001; Downes et al, 2004; Silman et al, 2005). AChE is widely distributed; it occurs in the central (CNS) and peripheral nervous systems (PNS), and the motor end-plates of the skeletal muscle and the electric organ, but it is also found in many other tissues and in erythrocytes. Therefore such a wide distribution and various functions, molecular forms, structural dynamics etc. of AChE provide adequate base to recall it a versatile enzyme, a detailed knowledge of which, might help to design specific drugs to combat various neurodegenerative diseases associated with this enzyme. In this context, we set up to study the distribution, structure of AChE gene and related RNA in neuronal tissues of in vertebrate animals.

II. Material and Methods

Study design

This prospective study was conducted in Anatomy department of a tertiary care teaching medical school in South India. The study was approved by institutional ethical committee. We ensured that study

complied with biomedical ethics guidelines for animal experimentation as laid down by Indian council of Medical Research (ICMR). All five vertebrate species of male and female weighing an average 108 ± 26 (90 - 180 grams) were purchased from a local supplier and transported live to the laboratory in aerated tanks. During the acclimatization period, the animals were fed daily (Safe feed 7711, Charoen Pokphand Foods PCL, Thailand) weighing about 1% of the body weight, and were then fasted for 24 hours before the experiment. They were sacrificed, the brain was rapidly removed, weighed, and dissected for RNA extraction and sequencing the brain was rapidly removed for RNA extraction followed by reverse transcription and fold induction of gene expression between AChE and 28S rRNA genes, and were then analysized by PCR and Gel analysis.

RNA isolation

Total RNA was extracted from the brain of house lizard using RNeasy Mini Kit (QIAGEN GmbH, Germany), according to the manufacturer‟s instructions. RNA was analyzed in 1% agarose gel, containing ethidiumbromide and visualized with UV light. The 1 Kb DNA ladder plus and 100 bp DNA ladder plus (Fermentas, USA) was used as molecular marker.

AChE cDNA synthesis and Sequence Analysis

Reverse transcription-polymerase chain reaction (RT-PCR): Complementary DNA (cDNA) was synthesized by using First Strand cDNA Synthesis kit for PCR thermo scientific, according to the manufacturer‟s instructions. PCR amplification used degenerate primers. Primers of AChE gene (F-5‟ GTCACCAAGAGGAGAAAGAGAC „3; R-5‟ GACAACGTCCACACCATACA „ 3) designed in conservedregion of chick from GENBANK using CODEHOP program.

For the PCR reaction, 4 Hl of cDNA from each synthesis were added to 7 Hl of „2X PCR master mix‟ containing 10X PCR buffer, 10 mM dNTP, 25 mM MgCl2, 5 U of Taq DNA polymerase (Fermentas, USA). Twenty HM of each pair of the primers was added, and the final volume was adjusted to 14 Hl with nuclease free water. The mixtures were denatured at 94oC for 3 min. Thirty five cycles of PCR were carried out, with denaturation at 94oC for 45 sec, annealing at 57oC for 30 sec, and extension at 72oC for 1 min, followed by a final extension period of 5 min. PCR products were analyzed by electrophoresis on 1% agarose gels stained with GelStar Nucleic Acid Gel Stain (Cambrex Bio Science Rockland, Inc.).

PCR products were cloned into the pGEMT plasmid vector (Promega) and sequenced using forward and reverse primers. Sequencing was performed with the Big DyeTM Terminator Cycle Sequencing Ready Kit, version 3.0 (ABI PrismTM, Perkin Elmer) and an ABI 3700 Applied Biosystems Model automated DNA sequencer. Nucleotide sequences of NWS were analyzed by BLASTN to search for similarities, and sequence alignments was performed with CLUSTAL W (Megalign program, DNASTAR Inc., Madison, WI).

Quantitative assessment of RNA by methyl green-pyronin staining

The tissues are fixed in Methacarn solution (methanol: chloroform: glacial acetic Acid = 6:3:1) fixatives for 4 hours for fixation, and then followed by routine histological processing. The 5 to 6 um sections are taken for all tissues and stained with MGP (methyl green pyronin). The Number of RNA granules are estimated by image analysis by used software IMAGE pro 6.2.

Statistical analysis

AChE gene Nucleotide sequences were analyzed by BLASTN to search for similarities, and sequence alignments was performed with CLUSTALW (Megalign program, DNASTAR Inc., Madison, WI). Also analysed methyl green-pyronin staining of RNA granules by image pro 6.2 software.

III. Results

AChE cDNA sequence of *Channa striata*

AChE cDNA sequence was used to investigate difference in male and female cerebrum and cerebellum. The ORF of AChE is comprised of 338 nucleotides (GenBank accession number JX190065.1), showing significant nucleotide similarity 88.2% and 83.4% respectively with *Channa striata* male cerebrum and cerebellum whereas 88.1 % with female cerebrum and cerebellum AChE (Fig.2). The highly divergent regions between the AChE sequences when compare with standard sequence are found in male cerebrum where as when compare with male cerebellum, female cerebrum, female cerebellum was less significant, But when compare with male and female cerebrum and cerebellum, the highest divergence was found in between male cerebrum and female cerebrum and least divergence was found in between male cerebellum and female cerebellum (Fig.4).

Expression of AChE gene

Expression of Acetylcholinesterase (AChE) was significantly more in female cerebrum and cerebellum when compared with male cerebrum and cerebellum (Fig.5). Also, it was found that methyl green-pyronin staining on Channa striata brain regions shows more RNA granules in female cerebrum and female cerebellum (Fig.6).

AChE cDNA sequence of *Duttaphrynus melanostictus*

AChE cDNA sequence was used to investigate difference in male and female cerebrum and cerebellum. The ORF of AChE is comprised of 221 nucleotides (GenBank accession number HM998937.1), showing significant nucleotide similarity 22.2% and 99.1% respectively with *Duttaphrynus melanostictus* male cerebrum and cerebellum whereas 14.9 % and 89.6 respectively with female cerebrum and cerebellum AChE (Fig 7). The highly divergent regions between the AChE sequences when compared with standard sequence are found in male and female cerebrum, where as when compared with male cerebellum, female cerebellum was less significant, But when compare with male and female cerebrum and cerebellum the highest divergence was found in between male cerebrum and female cerebrum and least divergence was found in between male cerebellum and female cerebellum (Fig.9).

Expression of AChE gene

Expression of Acetylcholinesterase (AChE) was significantly more in female cerebrum and cerebellum when compared with male cerebrum and cerebellum (Fig 10). Also, it was found that methyl green-pyronin staining on *Duttaphrynus melanostictus* brain regions shows more RNA granules in female cerebrum and female cerebellum (Fig 11).

AChE cDNA sequence of *Hemidactylus frenatus*

AChE cDNA sequence was used to investigate difference in male and female cerebrum and cerebellum. The ORF of AChE is comprised of 256 nucleotides (GenBank accession number EF534897), showing significant nucleotide similarity 86.2% and 84.4% respectively with *Hemidactylus frenatus* male cerebrum and cerebellum whereas 87.1 % with female cerebrum and cerebellum AChE (Fig 12). The highly divergent regions between the AChE sequences when compare with standard sequence are found in male cerebrum where as when compare with male cerebellum, female cerebrum, female cerebellum was less significant, But when compare with male and female cerebrum and cerebellum, the highest divergence was found in between male cerebrum and female cerebrum and least divergence was found in between male cerebellum and female cerebellum (Fig 14).

Expression of AChE gene

Expression of Acetylcholinesterase (AChE) was significantly more in female cerebrum and cerebellum when compare with male cerebrum and cerebellum (Fig.15). Also, it was found that Methyl green-pyronin staining on Hemidactylus frenatus brain regions shows more RNA granules in female cerebrum and female cerebellum (Fig16).

AChE cDNA sequence of *Gallus gallus domesticus*

AChE cDNA sequence was used to investigate difference in male and female cerebrum and cerebellum. The ORF of AChE is comprised of 359 nucleotides (GenBank accession number NM_205418.1), showing significant nucleotide similarity 89.2% and 88.4% respectively with *Gallus gallus domesticus* male cerebrum and cerebellum whereas 87.1 % with female cerebrum and cerebellum AChE (Fig 17). The highly divergent regions between the AChE sequences when compare with standard sequence are found in male cerebrum where as when compare with male cerebellum, female cerebrum, female cerebellum was less significant, But when compare with male and female cerebrum and cerebellum, the highest divergence was found in between male cerebrum and female cerebrum and least divergence was found in between male cerebellum and female cerebellum (Fig19).

Expression of AChE gene

Expression of Acetylcholinesterase (AChE) was significantly more in female cerebrum and cerebellum when compared with male cerebrum and cerebellum (Fig 20). Also, it was found that methyl green-pyronin staining on Gallus gallus domesticus brain regions shows more RNA granules in female cerebrum and female cerebellum (Fig 21).

AChE cDNA sequence of *Rattus norvegicus*

AChE cDNA sequence was used to investigate difference in male and female cerebrum and cerebellum. The ORF of AChE is comprised of 543 nucleotides (GenBank accession number NM_172009.1), showing significant nucleotide similarity 82.2% and 81.4% respectively with *Rattus norvegicus* male cerebrum and cerebellum whereas 84.1 % with female cerebrum and cerebellum AChE (Fig 22). The highly divergent regions between the AChE sequences when compare with standard sequence are found in male cerebrum where as when compare with male cerebellum, female cerebrum, female cerebellum was less significant, But when compare with male and female cerebrum and cerebellum, the highest divergence was found in between male cerebrum and female cerebrum and least divergence was found in between male cerebellum and female cerebellum (Fig 24).

Expression of AChE gene

Expression of Acetylcholinesterase (AChE) was significantly more in female cerebrum and cerebellum when compared with male cerebrum and cerebellum (Fig 25). Also, it was found that methyl green-pyronin staining on Rattus norvegicus brain regions shows more RNA granules in female cerebrum and female cerebellum (Fig 26).

IV. Discussion

Acetylcholinesterase (AChE) terminates the neurotransmission at cholinergic synapses by splitting the neurotransmitter acetylcholine. The nature and distribution of the enzyme has extensively been studied in many invertebrates and vertebrates including human, histochemically and biochemically. The distribution of the enzyme in the central and peripheral nerve tissues of different vertebrates demonstrates a high range of variation (Tripathi et al, 2007). It has been noted to be localized in non neuronal tissues and Glial cells also. The enzyme also exhibits molecular diversity with its six different molecular forms and structural dynamics which facilitates its affinity and action with various legends (Shen et al, 2002). In addition, AChE is considered to play several non classical roles independent of its catalytic function i.e. hydrolysis of Ach. These classical and non classical roles of AChE illustrate adequacy about its wide occurrence in neuronal and non neuronal tissues (Silman et al, 2005).The importance of AChE in body homeostasis is underscored by the fact that they are the targets of some of the most potent toxins including insecticides, snake venom and chemical weapons (Silman et al, 2000).

AChEs have been so far identified in different tissues of most vertebrates and more than 20 invertebrate animals (Talesa et al, 1999; Jones et al, 2002; Zhao et al, 2010). For instance, AChE activity has been detected in erythroid cells (Keyhani et al, 1981), brain (Boudinot et al, 2009), muscle and liver (Askar et al, 2011), kidney (McKenna et al, 1968) and lungs (El-Bermani et al, 1978) of vertebrates, And it was also detectable in different tissues of invertebrates (Anguiano et al, 2010; Zaitseva et al., 2008; Hornstein et al, 1994; Arpagaus et al., 1998; Kang et al, 2011), such as in the gills, mantle and haemolymph of mollusc (Anguiano et al, 2010; Zaitseva et al, 2008, von Wachtendonk et al, 1978), the eye and brain of arthropod (von Wachtendonk et al., 1979), and the head of nematode (Arpagaus et al, 1998; Kang et al, 2011). There is a great difference in the amino acid sequence of AChEs from different animal, and it even varies greatly among the different tissues of the same organism (Arpagaus et al, 1998 Shen et al, 2002). All the AChEs share some conserved structural features responsible for their catalysis function. For example, an active site triad (Ser, Glu and His) exist in all the reported AChEs, and the three residues form a plannar array at the bottom of a deep and narrow gorge, which closely resembles the catalytic triad of other a/b hydrolase fold family proteins (Steitz et al, 1982).

Acetylcholinesterase (AChE; EC 3.1.1.7) in vertebrates was involved in cell development and maturation (Monnet-Tschudi et al, 2002), neuronal development and nerve regeneration (Oron et al, 1984) and inflammation modulation (Das et al, 2007). AChE had also been identified in most invertebrates, including mollusc [Zaitseva et al, 2008], arthropoda (Cymborowski et al, 1970), platyhelminthes (Rybicka et al, 1967], annelida [Seravin et al, 1965) and nematoda (Rand et al, 2007). And AChE was also reported to be involved in many behaviors in these invertebrates, including locomotion (Rand et al,2007, Xuereb et al, 2009; Azevedo-Pereira et al, 2011), feeding [Rand et al, 2007, Xuereb et al, 2009), egg laying, male mating (Rand et al, 2007), embryo development (Gibson et al, 1981) and digestive activity (Zaitseva et al, 2008). However, the immunomodulation of AChE is still unclear in invertebrates.

In the present study, an AChE gene was studied in male and female cerebrum and cerebellum of five vertebrates (*Ratus norvegicus, Gallus-gallusdomesticus, Hemidactylus frenatus, Duttaphrynus melanostictus, Channa striata*). An AChE gene was studied in male and female *Ratus norvegicus* cerebrum and cerebellum. The deduced protein of *Ratus norvegicus* AChE (NM_172009.1) was comprised of 160 amino acids,and it shares 49.4% and 11% identity with other AChEs of cerebrum of male and female cerebrum respectively, whereas if we see male and female cerebellum we found very less identity 8.1 and 10% respectively (Fig 23).The ORF of AChE is comprised of 112 aminoacids (GenBank accession number NM_205418.1), showing

significant aminoacids similarity 90.2% and 92.0% respectively with *Gallus-gallus domesticus* male cerebrum and cerebellum whereas 92% and 94% with female cerebrum and cerebellum AChE (Fig 18). Other vertebrate *Hemidactylus frenatus* was also studied and deduced protein of its AChE (EF534897) was composed of 203 amino acids and it share 20% and 87% with male and female respectively where as in cerebellum its show significant match 92.1% and 93.6% with male and female respectively (Fig 13).The ORF of AChE is comprised of 73 aminoacids (GenBank accession number HM998937.1), showing significant nucleotide similarity 5.5% and 4.1% respectively with *Duttaphrynus melanostictus* male cerebrum and cerebellum whereas 2.7% and 90.4%respectively with female cerebrum and cerebellum AChE (Fig.8).

Channa striata gene was studied in same line and deduced protein of AChE (JX190065.1) was composed of 253 amino acids, and it shared 4.3 and 8.7 %with male and female cerebrum respectively and cerebellum shows low identity 6.7 and 4.3 with male and female cerebellum respectively (Fig 3).

In the present study, AChE activity was investigated in different brain areas of cerebrum and cerebellum in male and female of different vertebrates. Females had a significant increase in AChE activity in cerebrum and cerebellum in comparison with male cerebrum and cerebellum. We also found that RNA granules are more in female cerebrum and cerebellum and it may be one of the reason that affects the expression of AChE gene. On the basis of this new understanding of AChE brain organization and its evolutionary relationships, we found that female had more AChE activity or more expression than male but the exact reason was not clear. Therefore more studies are required to know why AChE activity is more in female counter part.

Restraint of acetylcholinesterase (AChE)- metabolizing compound of acetylcholine, is in a matter of seconds the most imperative restorative focus for improvement of psychological enhancers. Nonetheless, AChE movement in cerebrum has not been legitimately assessed on the premise of sex. It is unrealistic, at present, to dole out a positive component to clarify the example of deficiency saw in the chemical movement in male. Among the conceivable outcomes, diminished blood stream in cerebrum bringing about hypoxia has been proposed for decrement in AChE turnover in entire mind of male (Reiner et al, 1995). Absence of consistency in profile of AChE movement might be an impression of useful heterogeneity in focal cholinergic framework saw by a few specialists on different parameter.

References

[1] J. Boell, D. Nechmansohn, Electron microscopic localization of cholinesterase, by a copperleadthiocholine technique, J Neurochem, 3, 1966, pp. 1345.

[2] WN. Aldridge, MK. Johnson, Cholinesterase, succinic dehydrogenase, nucleic acids, esterase and glutathione reductase in subcellular fractions from rat brain, J Biochem, 73, 1959, pp. 270.

[3] [M A.Gerebtzoff, Cholinesterases: A histochemical Contribution to the solution of some functional problems, (Pergemon press, London), 1, 1959.

[4] LW. Chacho, JA .Cerf, Histochemical localization of cholinesterase in the amphibian spinal cord and alterations following ventral root section, J Anat 94, 1960, pp. 74.

[5] C .Hebb, Cholinergic neurons in vertebrates, Nature (London) 192, 1961, pp. 527.

[6] E. De Robertis, A. Pellegraino De Iraldi, AG. Rodriguez De Lores, L .Salganicoff, Cholinergic and noncholinergie nerve endings in rat brain. Isolation and subcellular distribution of acetylcholine and acetylcholinesterase, J Neurochem 9, 1962, pp. 23.

[7] PR. Lewis,CD. Shute,Confirmation from choline acetylase analyses of a massive cholinergic innervation to the hippocampus, J Physiol 172, 1964,pp. 9.

[8] CCD.Shute, PR. Lewis, The fine localization of cholinesterase in the hippocampal formation, J Anat 99, 1965, pp. 938.

[9] M. Papp, G. Bozsik, Comparison of the cholinesterase activity in the reticular formation of the lower brain stem of cat & rabbit, J Neurochem 12, 1966, pp. 697.

[10] EL. Bennett, MC. Diamond, H. Morimoto, M. Herbert, Acetylcholinesterase activity and weight measures in fifteen brain areas from six lines of rats, J Neurochem 3, 1966,pp. 563.

[11] JW.Phillis , Acetylcholinesterase in the feline cerebellum, J Neurochem 15, 1968 , pp. 611.

[12] A.Tripathi , UC.Srivastva , Histoenzymological distribution of acetylcholinesterase in the cerebral hemispheres of Indian wall lizard, Hemidactylus sflaviviridis, Ann Neuro Sci 14, 2007,pp. 64.

[13] KS.Bhasker,KP.Joy, Acetylcholinesterase positive intrapineal neuronal system in the palm squirrel Funambulus pennati, Biol struct Morphog 2, 1989, pp. 7.

[14] MW.Brightman , RW.Alberr , Species differences in the distribution of extra neuronal cholinesterase within the vertebrate central nervous system, J Neurochem 3, 1959,pp. 244.

[15] GB. Koelle , The histochemical localization of cholinesterases in the central nervous system of the rat, J Comp Neurol 100, 1954, pp. 211.

[16] S. Bon , Molecular forms of acetylcholinesterase in developing Torpedo embryo, Neuro Chem 4, 1982, pp. 577.

[17] T. Shen , K. Tai , RH. Henchman,HA. Mc Cammon , Molecular dynamics of Acetylcholinesterase, Acc Chem Res 35, 2002, pp. 332.

[18] H. Soreq , S. Seidman , Acetylcholinesterase – new roles for an old actor, Nat Rev Neurosci 2, 2001,pp. 294.

[19] GB. Downes , M. Granto , Acetylcholinesterase function is dispensable for sensary neurite growth but is critical for neuromuscular synapse stability, Dev Biol 270, 2004, pp. 232.

[20] I. Silman , JL. Sussman , Acetylcholinesterase: Classical and nonclassical functions and Pharmacology, Curr opin pharmacol 5, 2005, pp. 293.

[21] V. Talesa , M. Grauso , M. Arpagaus , E. Giovannini , R. Romani , Molecular cloning and expression of a full-length cDNA encoding acetylcholinesterase in optic lobes of the squid Loligo opalescens: a new member of the cholinesterase family resistant to diisopropyl fluorophosphates, J Neurochem 72, 1999, pp. 1250.

[22] AK. Jones,GN. Bentley,WG. Oliveros Parra,A. Agnew , Molecular characterization of an acetylcholinesterase implicated in the regulation of glucose scavenging by the parasite Schistosoma, FASEB J 16, 2002, pp. 441.
[23] P.Zhao , KY. Zhu K, H. Iang , Heterologous expression, purification, and biochemical characterization of a greenbug (Schizaphis graminum) acetylcholinesterase encoded by a paralogous gene (ace-1), J Biochem Mol Toxicol 24, 2010, pp. 51.
[24] E. Keyhani E, J. Maigne , Acetylcholinesterase in mammalian erythroid cells. J Cell Sci 52, 1981, pp. 327.
[25] E. Boudinot, V. Bernard, S.Camp, P.Taylor, J. Champagnat, Influence of differential expression of acetylcholinesterase in brain and muscle on respiration, Respir Physiol Neurobiol 165, 2009, pp. 40.
[26] KA. Askar , AC. Kudi , AJ. Moody , Purification of Soluble Acetylcholinesterase from Sheep Liver by Affinity Chromatography, Appl Biochem Biotechnol 165, 2011, pp. 336.
[27] OC. McKenna,ET. Angelakos , Acetylcholinesterase-containing nerve fibers in the canine kidney, Circ Res 23, 1968, pp. 645.
[28] AW. El-Bermani , EI. Bloomquist , Acetylcholinesterase- and norepinephrine- containing nerves in developing rat lung, J Embryol Exp Morphol 48, 1978 , pp. 177.
[29] GA. Anguiano , A. Amador , M. Moreno-Legorreta , F. Arcos-Ortega , C. Vazquez- Boucard , Effects of exposure to oxamyl, carbofuran, dichlorvos, and lindane on acetylcholinesterase activity in the gills of the Pacific oyster Crassostrea gigas, Environ Toxicol 25, 2010, pp. 327.
[30] OV.Zaitseva , TV. Kuznetsova , [Distribution of acetylcholinesterase activity in the digestive system of the gastropod molluscs Littorina littorea and Achatina fulica], Morfologiia 133, 2008, pp. 55.
[31] EP. Hornstein , DL. Sambursky, SC. Chamberlain, Histochemical localization of acetylcholinesterase in the lateral eye and brain of Limulus polyphemus: might acetylcholine be a neurotransmitter for lateral inhibition in the lateral eye ? Vis Neurosci 11, 1994, pp. 989.
[32] M. Arpagaus , D. Combes, E. Culetto , M. Grauso , Y. Fedon , Four acetylcholinesterase genes in the nematode Caenorhabditis elegans, J Physiol 92, 1998,363.
[33] JS. Kang , DW. Lee, YH. Koh, SH. Lee, A soluble acetylcholinesterase provides chemical defense against xenobiotics in the pinewood nematode, PLoS One 6, 2001, e19063.
[34] D. von Wachtendonk , J. Neef , Isolation, purification and molecular properties of an acetylcholinesterase (e.c. 3.1.1.7) from the haemolymph of the sea mussel Mytilus edulis, Comp Biochem Physiol 63, 1978, pp. 279.
[35] TA. Steitz, RG. Shulman, Crystallographic and NMR studies of the serine proteases, Annu Rev Biophys Bioeng 11, 1982, pp. 419.
[36] F. Monnet-Tschudi , MG. Zurich , B. Schilter, LG. Costa , P. Honegger, Maturation-dependent effects of chlorpyrifos and parathion and their oxygen analogs on acetylcholinesterase and neuronal and glial markers in aggregating brain cell cultures, Toxicol Appl Pharmacol 165, 2001, pp. 175.
[37] U. Oron , Acetylcholinesterase and nerve axon formation during muscle regeneration in rats, Cell Mol Biol 30, 1984, pp. 411.
[38] [38]. UN. Das, Acetylcholinesterase and butyrylcholinesterase as possible markers of low-grade systemic inflammation, Med Sci Monit 13, 2007, pp. 214.
[39] B. Cymborowski, J. Skangiel-Kramska , A. Dutkowski , Circadian changes of acetylcholinesterase activity in the brain of house-crickets (Acheta domesticus L), Comp Biochem Physiol 32, 1970, pp. 367.
[40] K. Rybicka , Emryogenesis in Hymenolepis diminuta V. Acetylcholinesterase in embryos, Exp Parasitol 20, 1967, pp. 263.
[41] LN. Seravin, [Role of Acetylcholinesterase in Water Metabolism of the „„Dorsal Muscle"" in Leeches], Dokl Akad Nauk SSSR 160, 1965, pp. 486.
[42] JB. Rand , Acetylcholine, WormBook 1, 2007.
[43] B. Xuereb, E. Lefevre, J. Garric, O. Geffard, Acetylcholinesterase activity in Gammarus fossarum (Crustacea Amphipoda): linking AChE inhibition and behavioural alteration, Aquat Toxicol 94, 2009, pp. 114.
[44] HM. Azevedo-Pereira, MF. Lemos, AM. Soares , Effects of imidacloprid exposure on Chironomus riparius Meigen larvae: linking acetylcholinesterase activity to behaviour, Ecotoxicol Environ Saf 74, 2001, pp. 1210.
[45] GE. Gibson, C. Peterson, DJ. Jenden, Brain acetylcholine synthesis declines with senescence, Science 213, 1981, pp. 674.
[46] I. Silman, JL. Sussman , Structural studies on cholinesterases. In cholinesterases and cholinesterase inhibitors, (Ed) Giacobini E, (Mortin Dunitz, London), 9, 2000.
[47] PB. Reiner, HC.Fibiger, Functional heterogeneity of central cholinergic system, in Psychopharmacology: The Fourth Generation of Progress (Raven Press Ltd, New York), 147, 1995.

Table. 1: Primer sequences and their size

S No.	Organism		Primer
1	Rattus norvegicus	Normal pcr primers	
	Acetylcholinesterase	forward	F:GGGCTCCTACTTTCTGGTTTAC
	NM_172009.1	reverse	R:AGGTTCAGGCTCACGTATTG
2	Gallus gallus domesticus	Normal pcr primers	
	Acetylcholinesterase	forward	F:GGGTTCCTATTTCCTGGTCTATG
	NM_205418.1	reverse	R:ACCTCCTCCCTGGTGTAAT
3	Hemidactylus frenatus	Normal pcr primers	
	Acetylcholinesterase	forward	F:GTCACCAAGAGGAGAAAGAGAC
	EF534897.1	reverse	R:CGGCTGTTGGACAAGGTAAT
4	Dttaphrynus melanosticus	Normal pcr primers	
	Acetylcholinesterase	forward	F:GACTTCCATCCCTGACAGATAC
	HM.998937.1	reverse	R: CAGTCACCCACTCGCTAATAC
5	Chana striata	Normal pcr primers	
	Acetylcholinesterase	forward	F: GGAAACCTCCTGATCTCCATTC
	JX 190065,1	reverse	R: GACAACGTCCACACCATACA

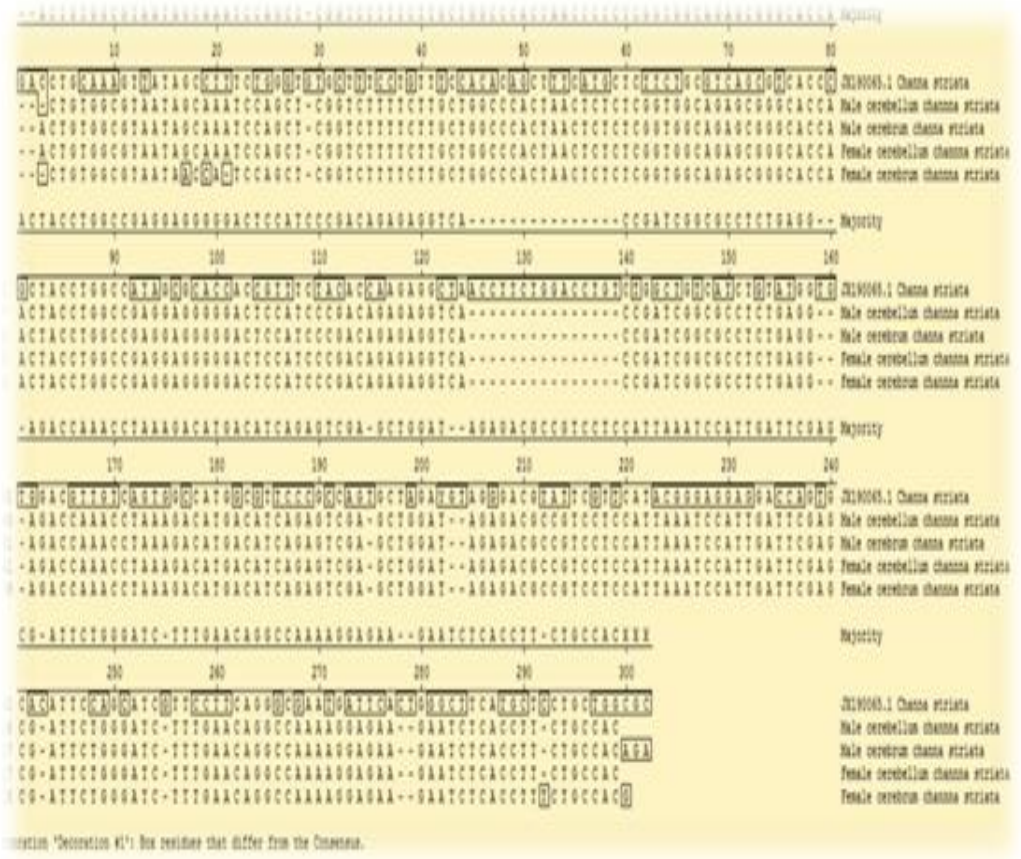

Figure 2: Alignment of nucleotide sequences of *Channa striata* from male and female cerebrum and cerebellum with ACHE JX 190065.1

Boxes residue differ from the consensus.

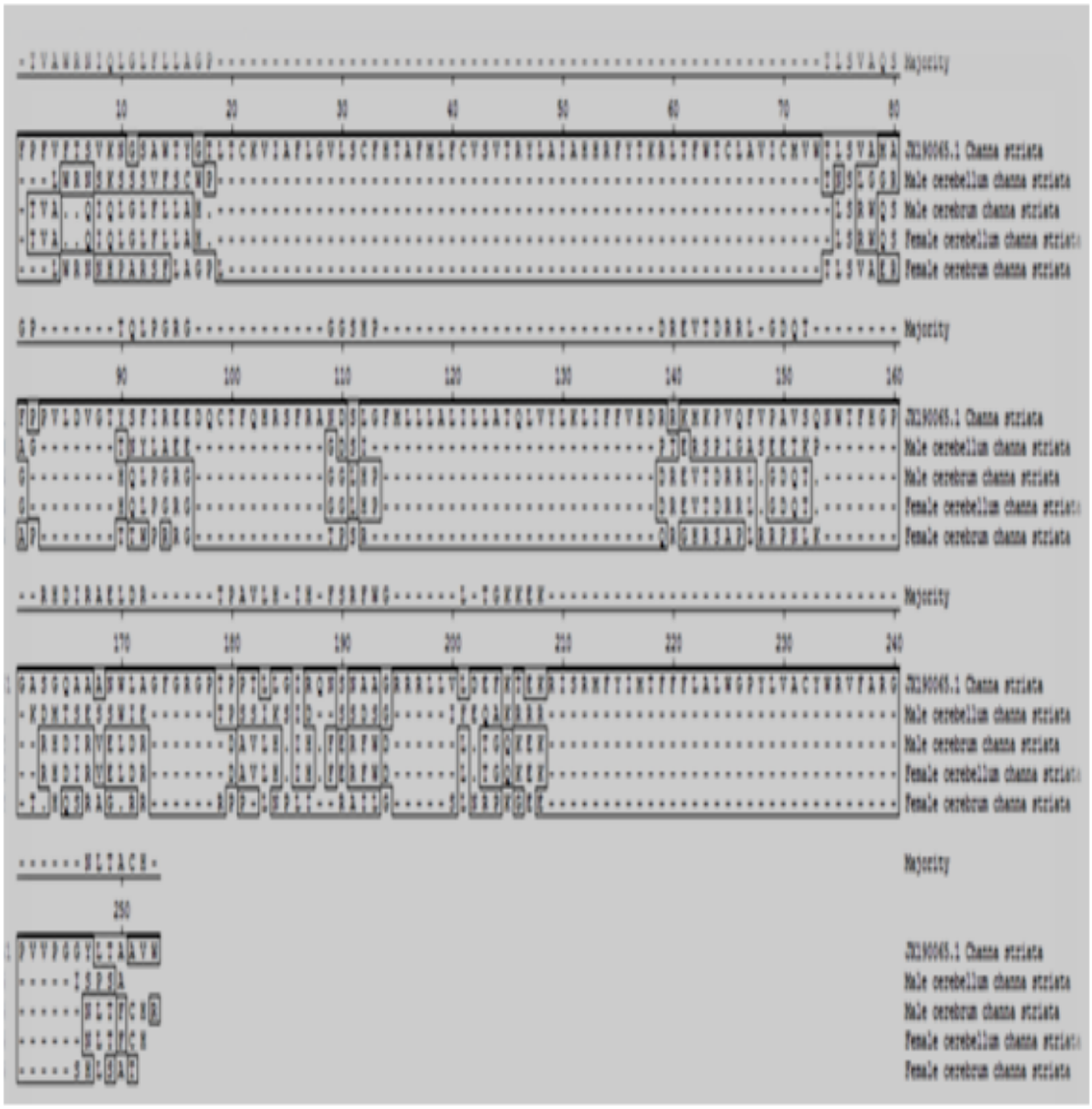

Figure 3: Alignment of peptide sequences of *Channa striata* AChE from male and female cerebrum and cerebellum with AChE JX190065.1 Boxes residue differ from the consensus

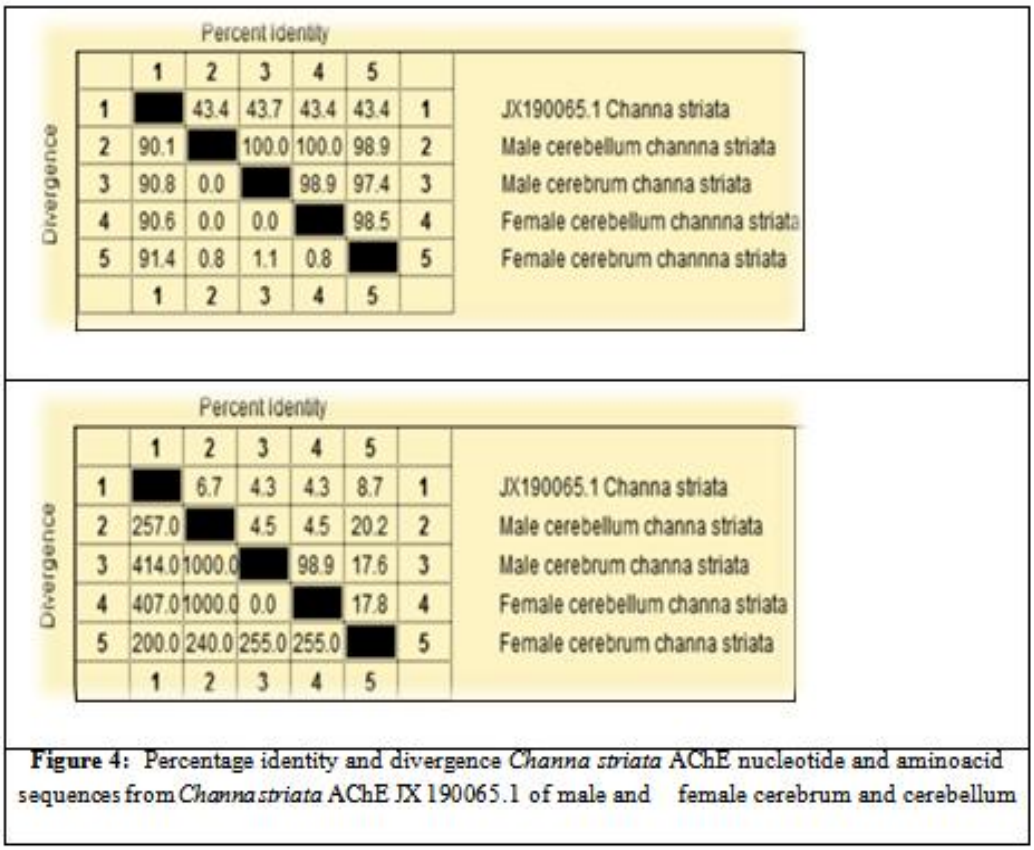

Figure 4: Percentage identity and divergence *Channa striata* AChE nucleotide and aminoacid sequences from *Channa striata* AChE JX 190065.1 of male and female cerebrum and cerebellum

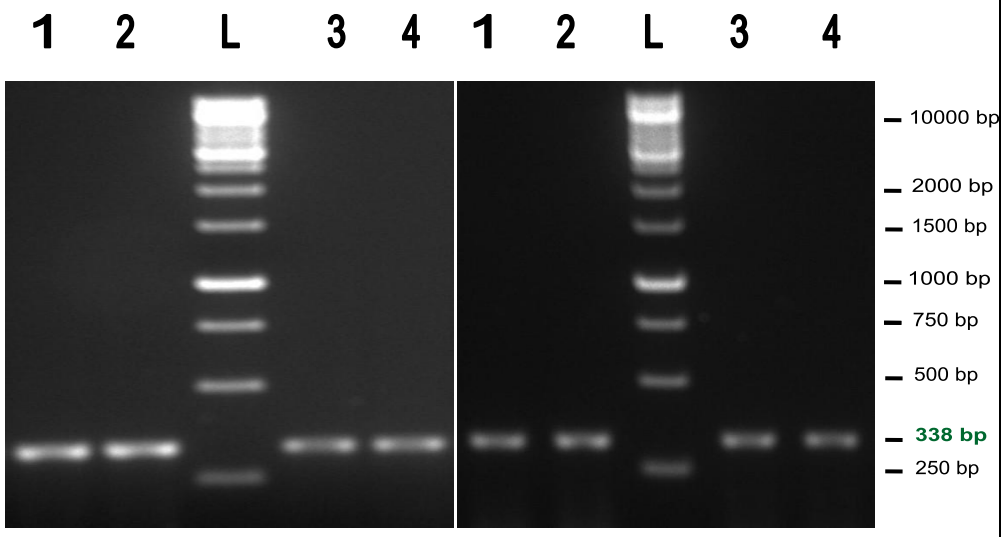

Figure 5: Electrophoretogram of Acetylcholinesterase (AChE) (A) Lane 1,2 Female cerebrum; Lane 3,4 Male cerebrum (B) Lane 1,2 Female Cerebellum; Lane 3,4 Male Cerebellum (1 kb ladder marker shown in middle lane). The PCR product size is 338 bp.

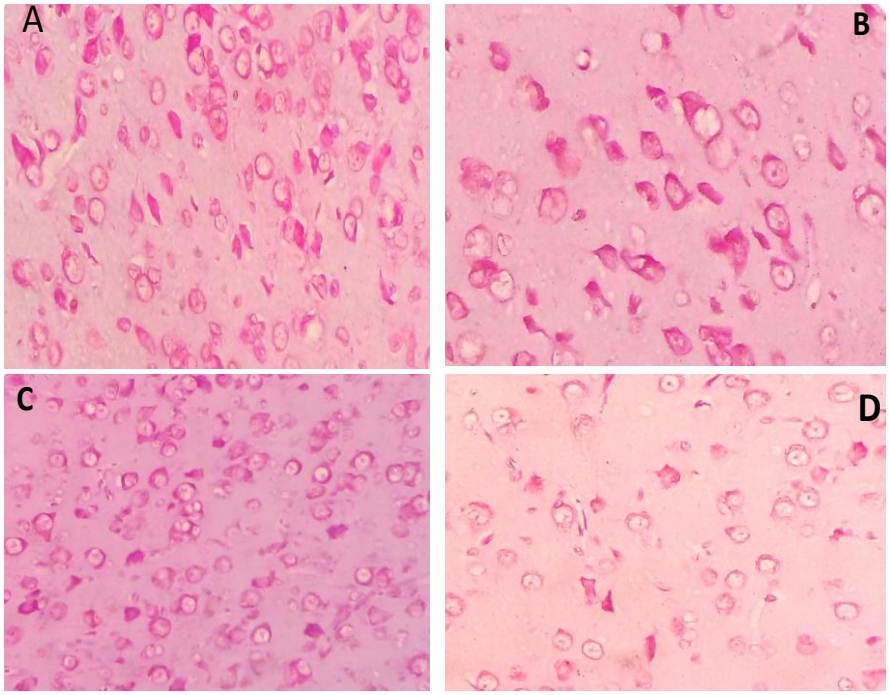

Figure 6: Methyl green-pyronin staining on *Channa striata* brain regions (A) Female Cerebrum showing maximum numbers of RNA granules in comparison with male cerebrum (B) where as again RNA granules are more in female cerebellum (C) than that seen with male cerebellum(D).

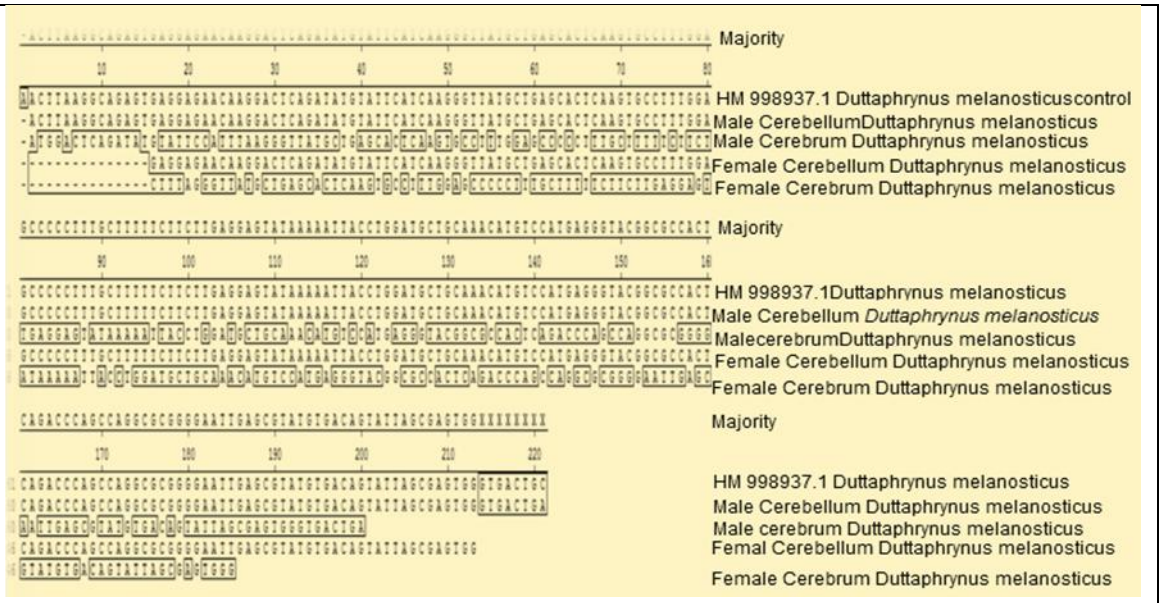

Figure 7: Alignment of nucleotide sequence of *Duttaphrynus melanosticus* ACHE from male and female cerebrum and cerebellum with AChE HM 998937.1 Boxes differ from the consensus.

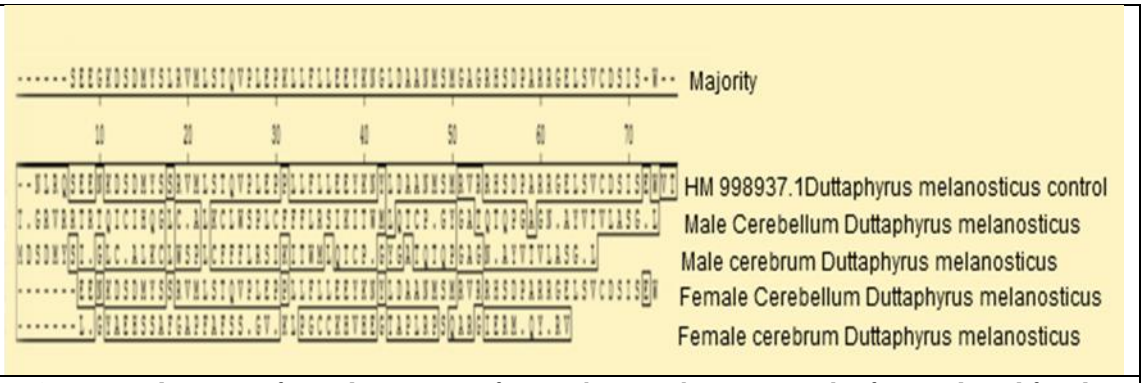

Figure 8: Alignment of peptide sequence of *Duttaphyrus melanosticus* AChE from male and female cerebrum and cerebellum with AChE HM 998937.1 Boxes residue from the consensus.

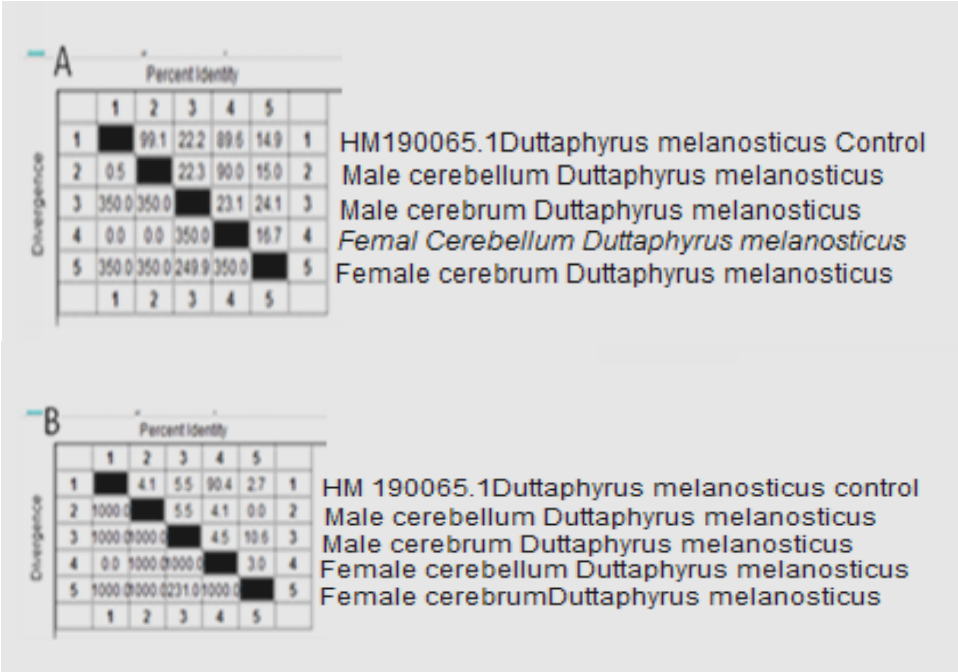

Figure 9: Percentage identity and divergence *Duttaphyrus melanosticus* AChE nucleotide and aminoacid sequences from *Duttaphyrus melanosticus* AChE 190065.1 of male and female cerebrum and cerebellum.

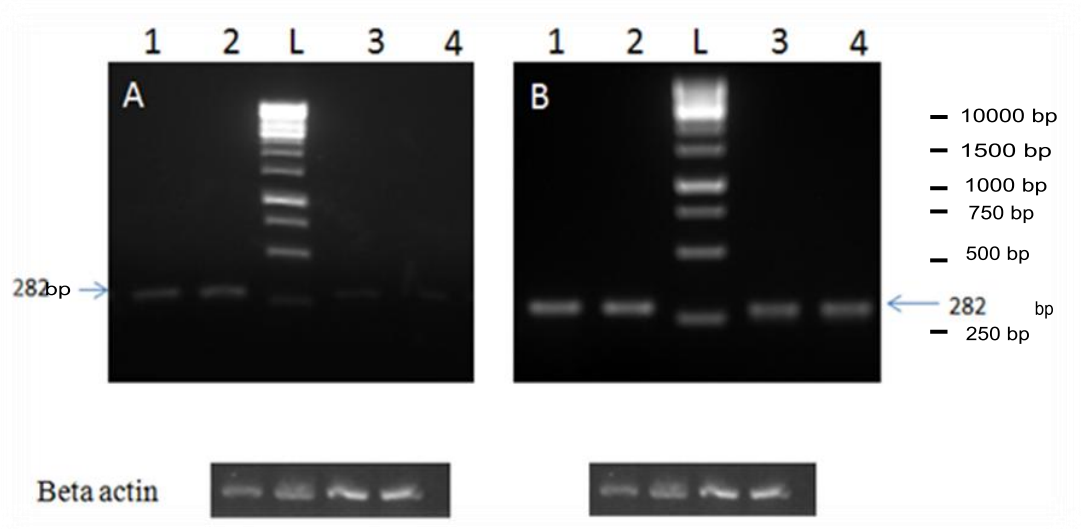

Figure 10: Electrophoretogram of Acetylcholinesterase (ACHE) (A) Lane 1,2 Female cerebrum: Lane 3,4 male cerebrum (B) Lane 1,2 Female cerebellum: Lane 3,4 Male cerebellum. The house keeping gene Beta actin is depicted in the lower panel. The PCR product size is 282 bp.

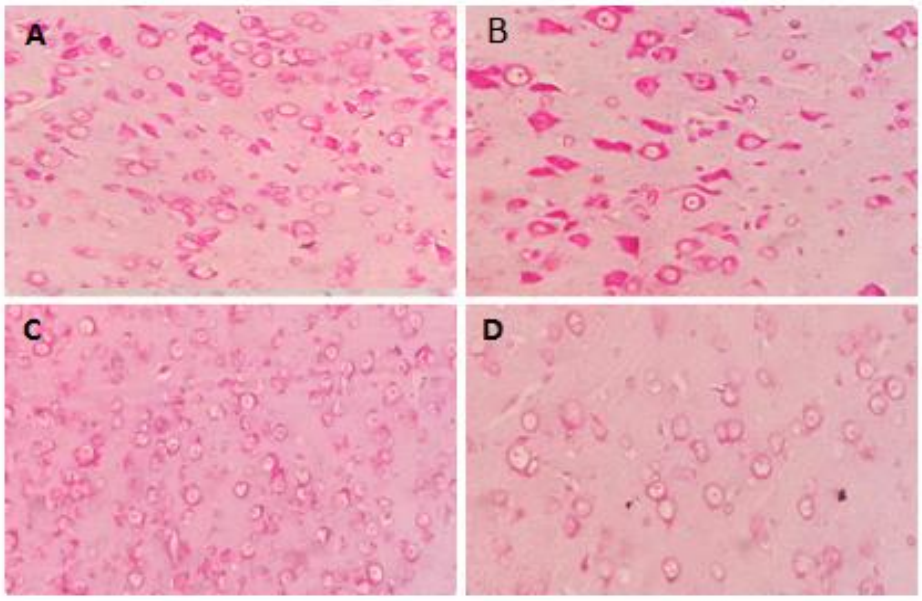

Figure 11: Methyl green-pyronin staining on *Duttaphyrus melanosticus* brain regions (A) Female Cerebrum showing maximum numbers of RNA granules in comparison with male cerebrum (B) whereas again RNA granules are more in female cerebellum (C) than that seen with male cerebellum(D).

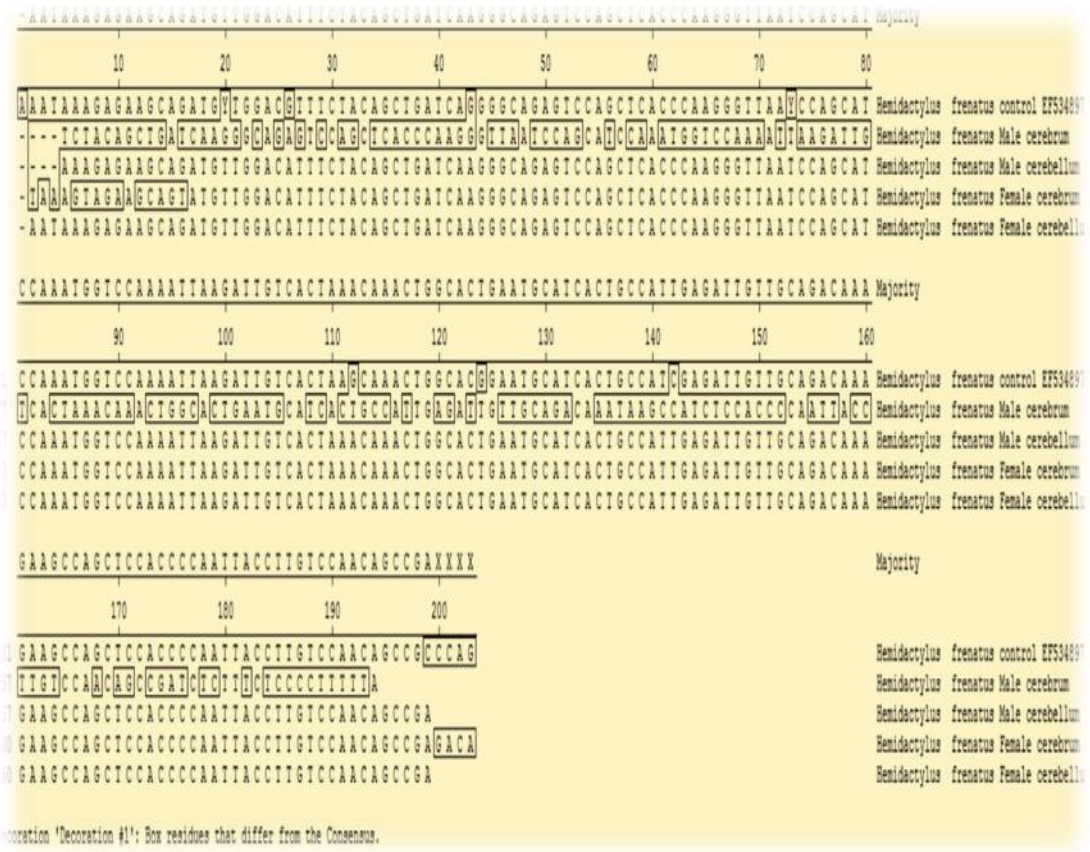

Figure 12: Alignment of nucleotide sequences of *Hemidactylus frenatus* from ACHE male and female cerebrum and cerebellum with AChE EF534897 Boxes residue differ from the consensus

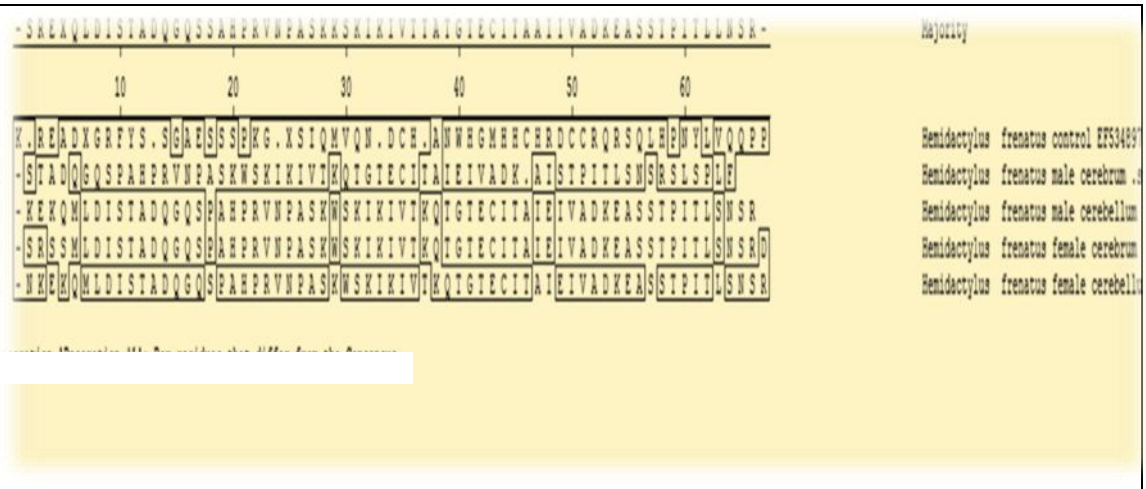

Figure 13: Alignment of peptide sequences of *Hemidactylus frenatus* AChE from male and female cerebrum and cerebellum with ACHE EF534897.Boxes residue differ from the consensus.

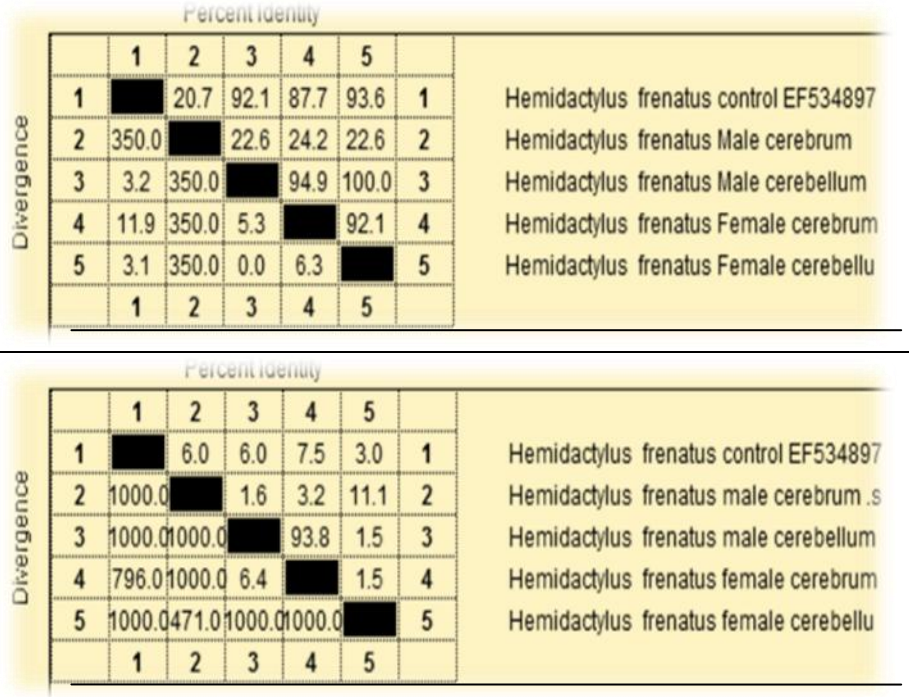

Figure 14: Percentage identity and divergence of *Hemidactylus frenatus* AChE nucleotide and amino acid sequences from and *Hemidactylus frentus* AChE EF534897 of male and female cerebrum and cerebellum.

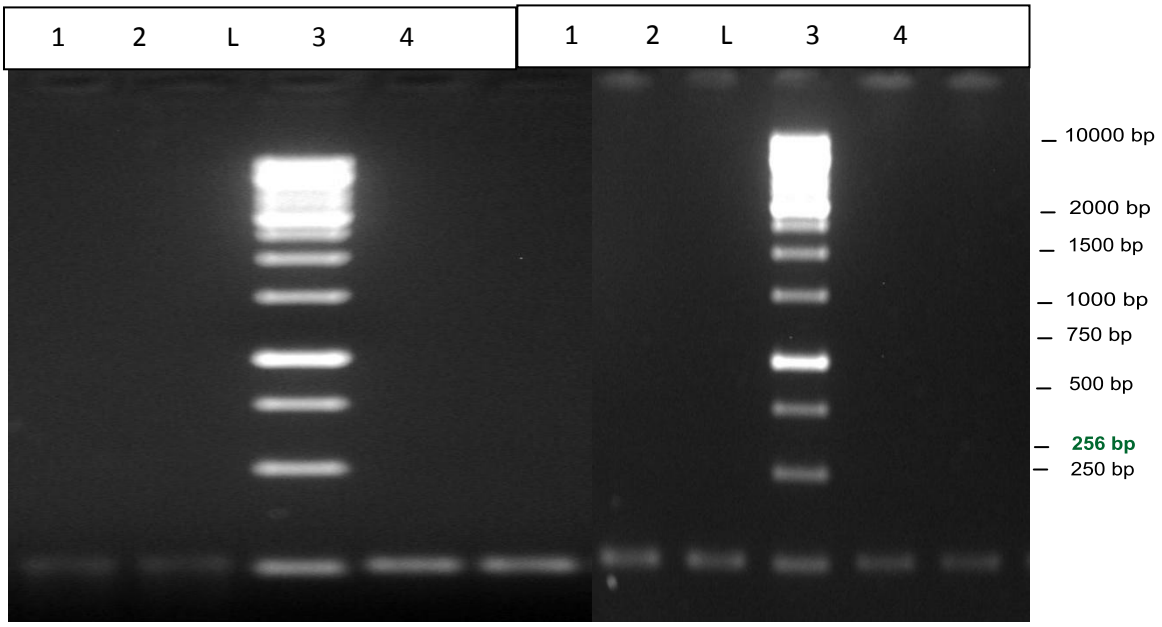

Figure 15: Electrophoretogram of Acetylcholinesterase (AChE) (A) Lane 1,2 Female cerebrum; Lane 3,4 Male cerebrum (B) Lane 1,2 Female Cerebellum; Lane 3,4 Male Cerebellum.(1 kb ladder marker shown in middle lane). The PCR product size is 256 bp.

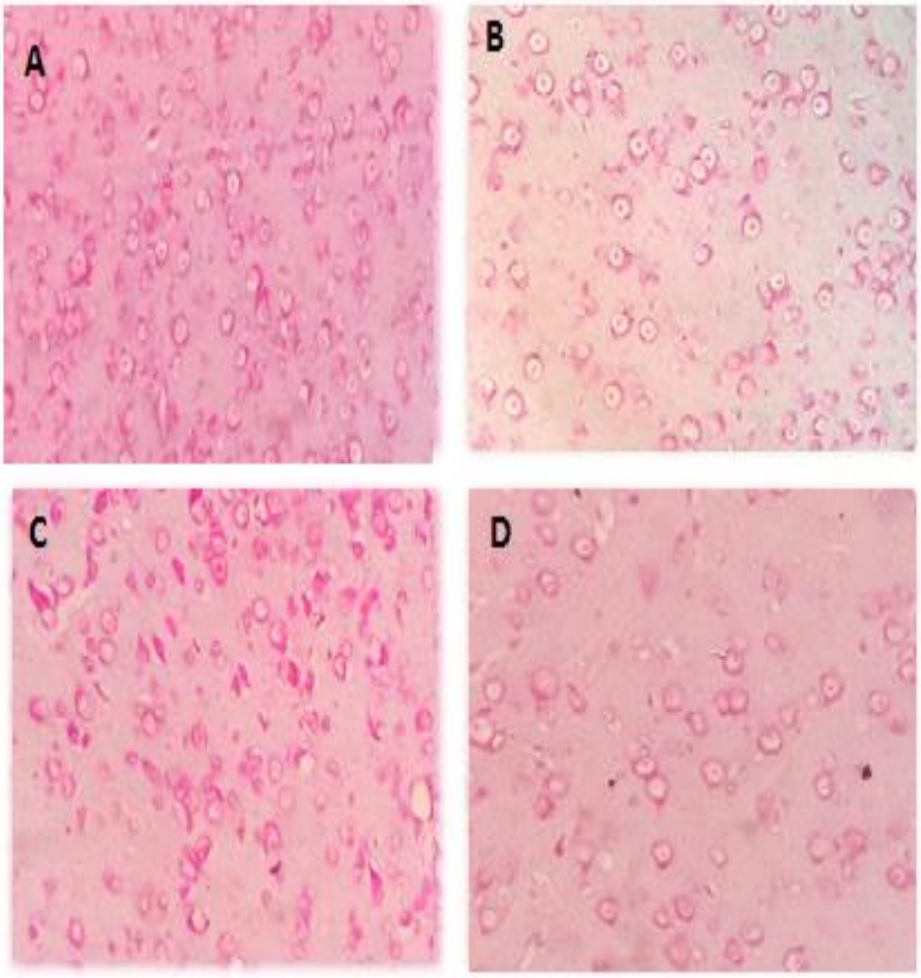

Figure 16: Methyl green-pyronin staining on *Hemidactylus frenatus* brain regions (A) Female Cerebrum showing maximum numbers of RNA granules in comparison with male cerebellum (B) whereas again RNA granules are more in female cerebellum (C) than that seen with male cerebellum (D).

Figure 17: Alignment of nucleotide sequences of *Gallus gallus domesticus* AChE from male and female cerebrum and cerebellum with AChE NM_205417.1 Boxes residue differ from the consensus.

Figure 18: Alignment of peptide sequences of *Gallus gallus domesticus* AChE from male and female cerebrum and cerebellum with AChE NM_205417.1 Boxes residue differ from the consensus.

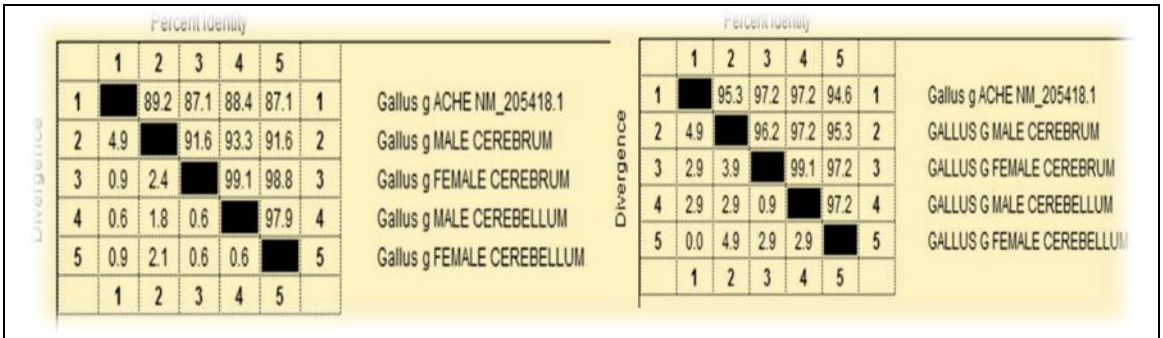

Figure 19: Percentage identity and divergence of *Gallus gallus domesticus* AChE NM_2054.18.1 nucleotide and amino acid sequences from and *Gallus gallus domesticus* AChE of male and female cerebrum and cerebellum

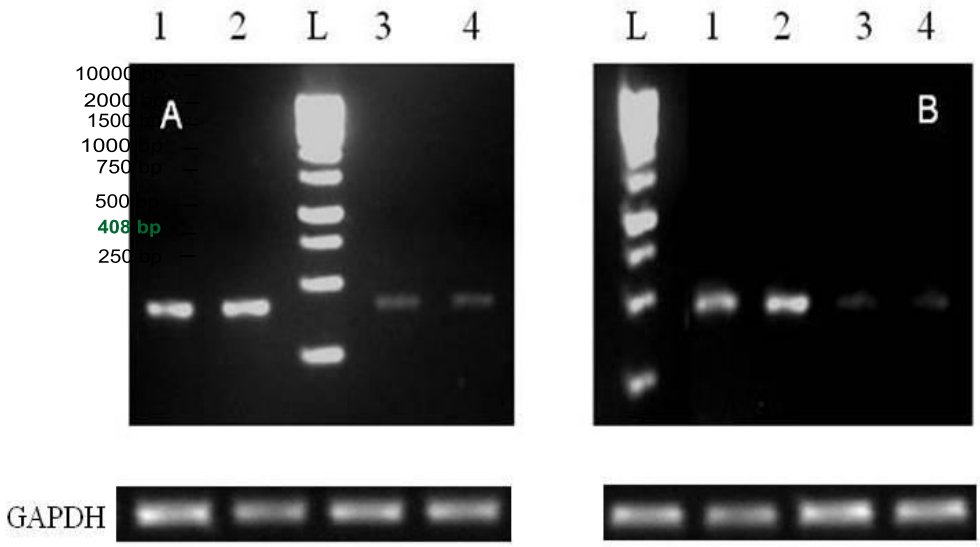

Figure 20: Electrophoretogram of Acetylcholinesterase (AChE) (A) Lane 1,2 Female cerebrum; Lane 3,4 Male cerebrum (B) Lane 1,2 Female Cerebellum; Lane 3,4 Male Cerebellum.(1 kb ladder marker shown in middle lane). The house keeping gene GAPDH is depicted in the lower pannel.the PCR product size is 408bp.

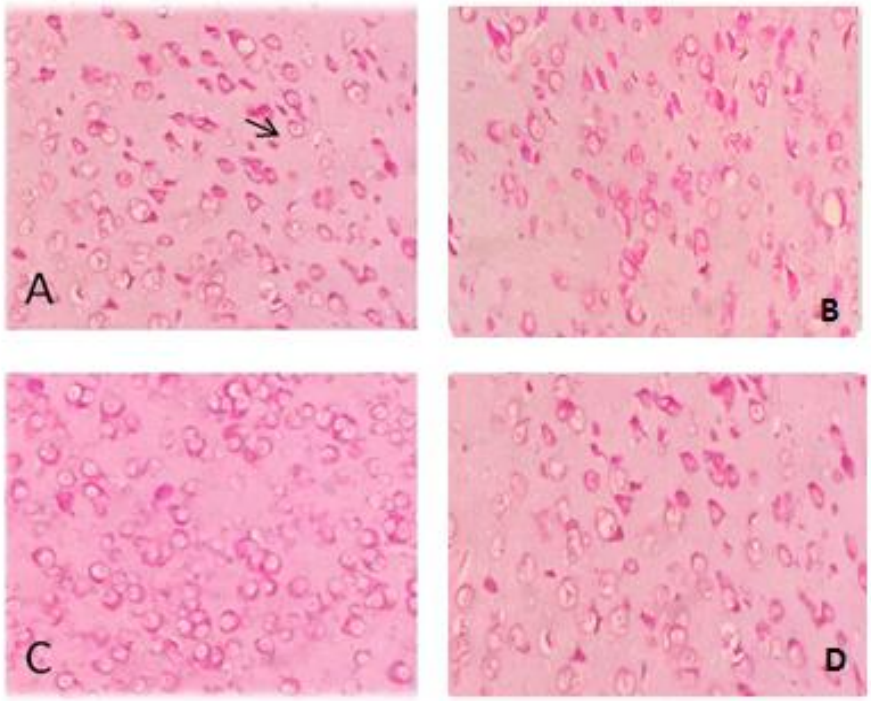

Figure 21: Methyl green-pyronin staining on *Gallus gallus domesticus* brain regions (A) Female Cerebrum showing maximum numbers of RNA granules in comparison with Male cerebrum (B)whereas again RNA granules are more in female cerebellum (C) than that seen with male cerebellum (D).

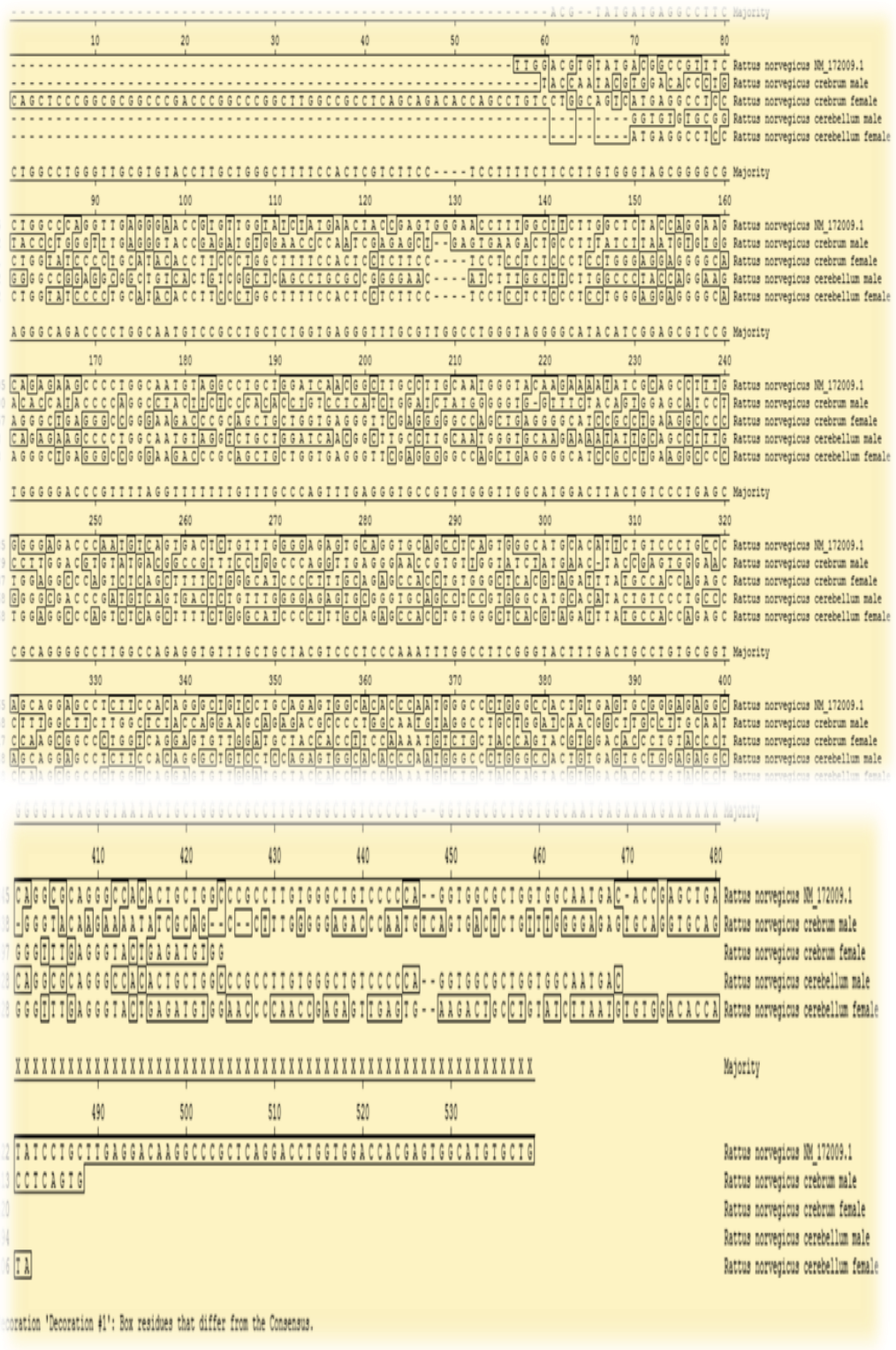

Figure 22: Alignment of nucleotide sequences of *Rattus norvegicus* ACHE from male and female cerebrum and cerebellum with AChE NM_172009.1 Boxes residue differ from the consensus.

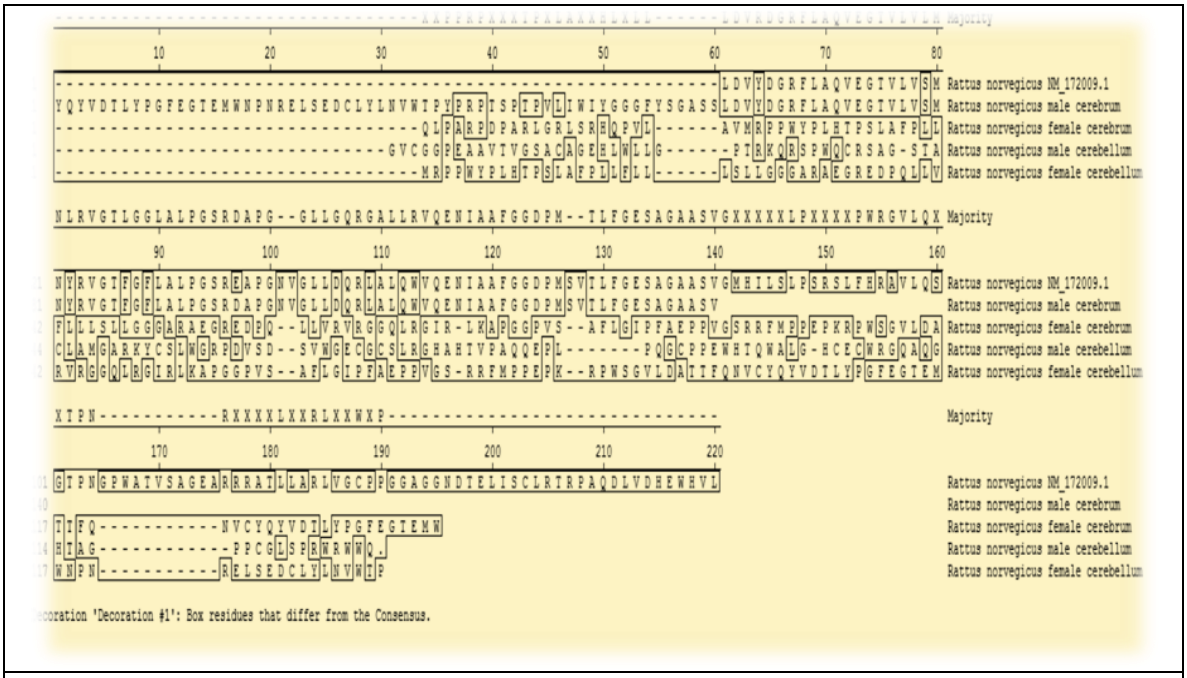

Figure 23: Alignment of peptide sequences of *Rattus norvegicus* AChE from male and female cerebrum and cerebellum with AChE NM_172009.1 Boxes residue differ from the consensus

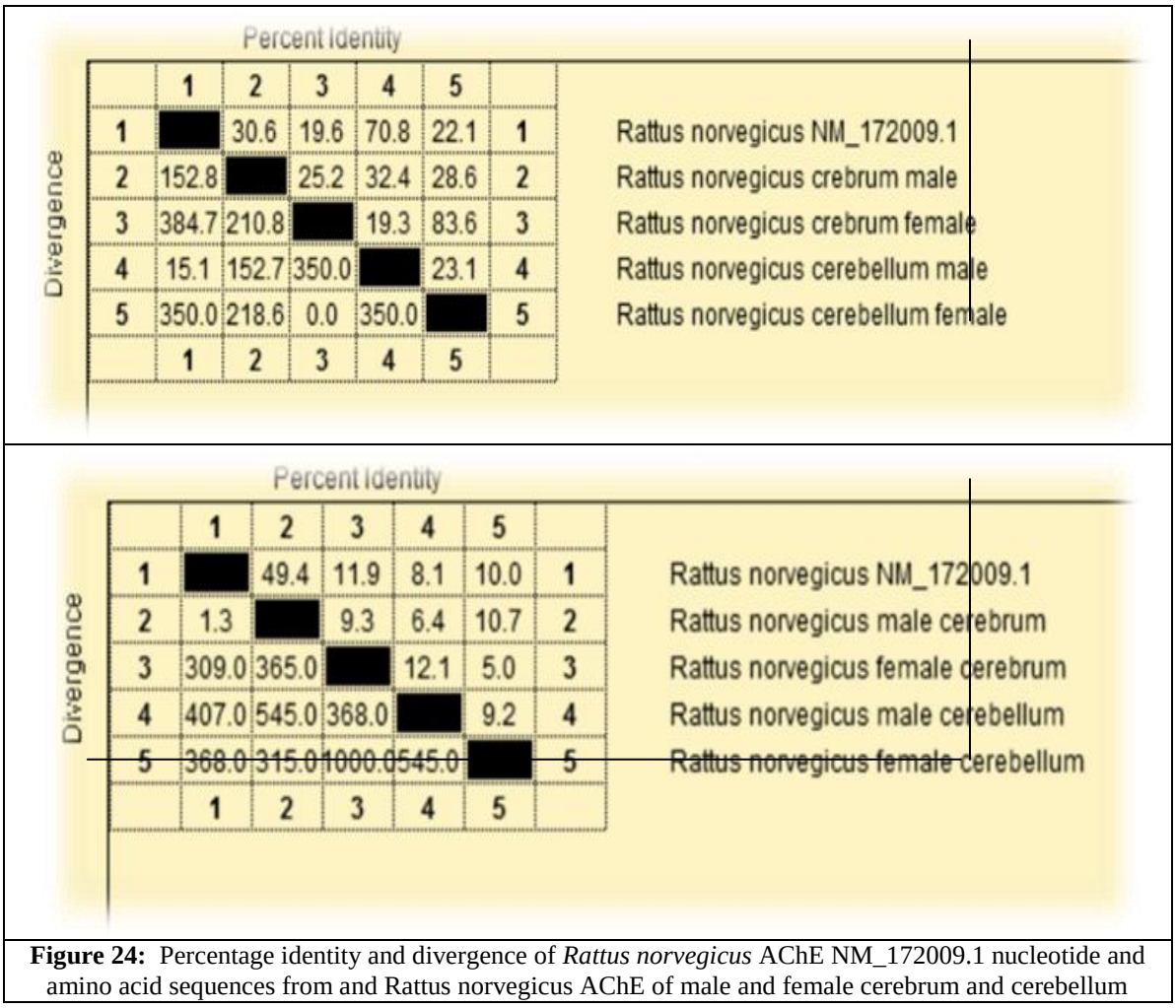

Figure 24: Percentage identity and divergence of *Rattus norvegicus* AChE NM_172009.1 nucleotide and amino acid sequences from and Rattus norvegicus AChE of male and female cerebrum and cerebellum

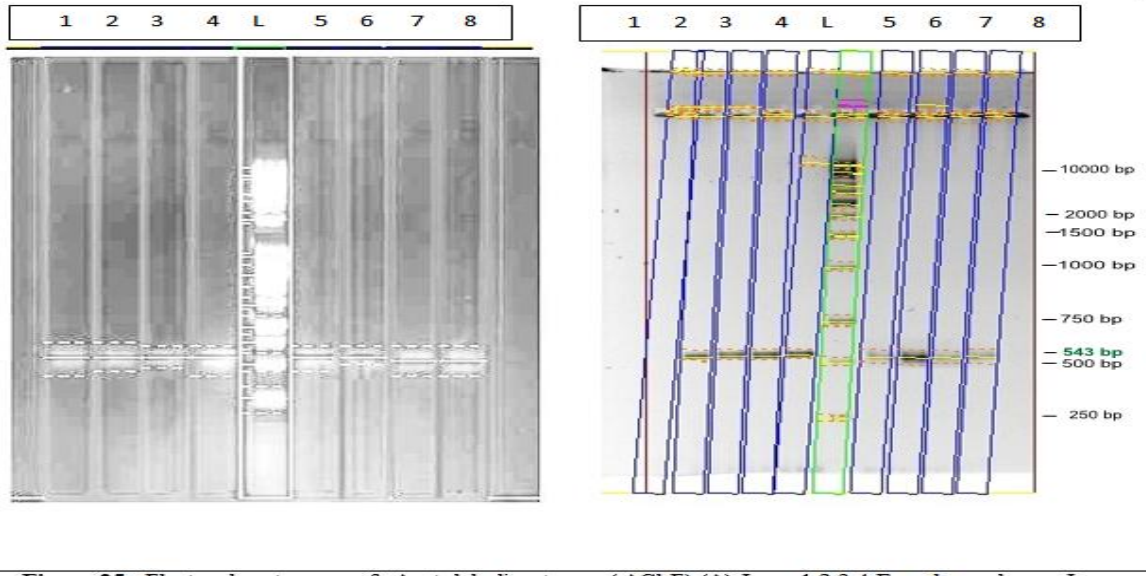

Figure 25: Electrophoretogram of Acetylcholinesterase (AChE) (A) Lane 1,2,3,4 Female cerebrum; Lane 5,6,7.8 Male cerebrum (B) Lane 1,2,3,4 Female Cerebellum; Lane 5,6,7,8 Male Cerebrum.(1 kb ladder marker shown in middle lane). The PCR product size is 338 bp.

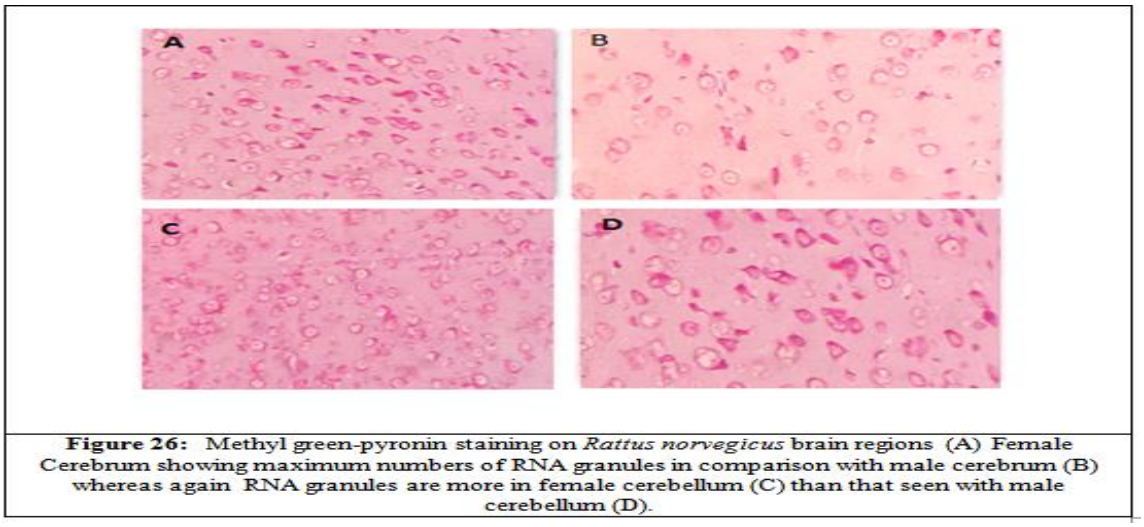

Figure 26: Methyl green-pyronin staining on *Rattus norvegicus* brain regions (A) Female Cerebrum showing maximum numbers of RNA granules in comparison with male cerebrum (B) whereas again RNA granules are more in female cerebellum (C) than that seen with male cerebellum (D).

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