Journal of Anatomical Sciences

Email:anatomicaljournal@gmail.com

J Anat Sci 12 (1)

Effect of Tocopherol Administration on Aluminium Phosphide- Induced Structural and Functional Changes in the Cerebellar Cortex of Adult Wistar Rats

Babatunde A.O*, Musa S.A, Hamman W. O, Akinyemi A. O
Department of Human Anatomy, faculty of Basic Medical Sciences, College of Health Science, Ahmadu Bello University Zaria, Nigeria.

Correspondence Author: Babatunde A.O
E-mail- babatundeolaniyi2016@gmail.com. Phone: 08034263732

ABSTRACT
Aluminium phosphide is an inorganic phosphide used to control insects and rodents in a variety of settings. It is soluble in most organic solvents emit a colourless gas and is odourless when pure. Tocopherol is a lipid-soluble antioxidant that can attenuate the effects of peroxide and protect against lipid peroxidation in cell membranes. The effect of tocopherol on AluminiumPhosphide induced histological, stereological and neurobehavioral changes in adult Wistar rats were studied. Twenty-five adult Wistar rats were divided into five groups with five rats each. Group 1 was the control group and was given normal saline. Group 2 received only the medium (Peanut oil). Group 3 was administered Aluminium phosphide (2.3 mg/kg) only. Group 4 received Aluminium phosphide (2.3 mg/kg) and 800 mg/kg of Tocopherol. Group 5 received Aluminium phosphide (2.3 mg/kg) and 1200 mg/kg of Tocopherol. The rats were administered Aluminium Phosphide and Tocopherol orally for the period of 7 days. Beam walking test was done to access the motor coordination function after which the rats were sacrificed and the tissues were collected for analysis and processed for histological and stereological studies. The result of motor coordination revealed significant differences in the time taken to walk across the beam before and after administration in the experimental animal group exposed to aluminium phosphide only.Histological study of the Cerebellar cortex demonstrated pathological changes in the animals administered Aluminium phosphide when compared to control animals. The stereology evaluation shows non- significant decrease in the cerebellar volume of experimental rats. Aluminium phosphide exert nerotoxicity in adult Wistar rats. Tocopherol administration has been demonstrated to have some level of protection on Aluminium phosphide induced structural and functional toxicity in the cerebellar cortex of adult Wistar rats.

Key words: Cerebellar cortex, Aluminium phosphide, Tocopherol, Wistar rats.

INTRODUCTION
Aluminium phosphide is an inorganic phosphide used to control insects and rodents in a variety of settings. It is mainly used as an indoor fumigant during crop transport, storage or in processing facilities for both food and non-food crops. It may also be used as an outdoor fumigant for burrowing rodent and in mole control or in bait for rodent control in crops [1 2].

Aluminium phosphide is available in pallet and tablet form, and also available in porous blister packs, sachets or as dusts. Aluminium phosphide products emit a colourless gas and is odourless when pure [3]. The technical product has a foul, garlic or rotten fish smell [3]. It is soluble in most organic solvents and supplied in cylinders either as pure phosphide or diluted with nitrogen [4]. It may be formulated as 55% active ingredient along with aluminiumcarbamate and inert ingredients [5 6]. Aluminium phosphide is readily soluble in water and reacts both, with water as well as moisture present in the air, to yield the highly toxic phosphine gas.

Breathing-in low level of phosphine gas can cause headache and medium level of exposure can cause nausea, dizziness and tightness of the chest [7]. While higher exposure levels can cause diarrhoea, abdominal pain, vomiting, chest pain, pulmonary oedema, irregular heartbeat, shock, convulsions, coma and death [6 7 8].

Antioxidants protect the body cells against the effects of free radicals and free radicals are produced when the body breaks down food or exposed to substances like tobacco smoke and radiation [9]. Tocopherol is a lipid-soluble antioxidant that can attenuate the effects of peroxide and protect against lipid peroxidation in cell membranes [10 11]. It has been reported that systemic complications and multi-organ failure are associated with aluminum phosphide exposure and may invariably result to death [6 8 12].

The aim of the present study was to evaluate the effects of antioxidant (tocopherol) on Aluminium phosphide induced histological, histochemical and neurobehavioral changes in the Hippocampus of adult Wistar rats.

MATERIALS AND METHODS

Chemicals: Aluminium Phosphide manufactured by Agricore chemical industry Co., LTD. Zhujia Gang Road, Shanghai,China with batch number ACI20160930 was purchased from reputable Agro allied store in Zaria, Kaduna State, Nigeria. The Aluminium phosphide was dissolved in a medium (Peanut oil) which served as the vehicle through which Aluminium phosphide was administered. Vitamin E manufactured by Bactolac pharmaceutical inc. New york, USA with batch number 1707477 was purchased from reputable Pharmaceutical Store in Zaria Kaduna State, Nigeria. It was soft gelatin capsules containing 100 mg of vitamin acetate.

Ethical Approval: Ethical approval was obtained from the Ahmadu Bello University Zaria animal use and care committee.
Application No: ABUCAUC/2020/HUMAN ANATOMY/62.
Approval No: ABUCAUC/2020/62.

Experimental Animals: Twenty-five Wistar rats were obtained and acclimatized for 2 weeks in the animal house of Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University,Zaria. The animals were separated into five groups of five animals each and were fed with standard pellets and water was provided *ad-libitum.*

Experimental Protocol: The animals in group 1 serve as the control and were given distilled water. Group 2 received the medium (Peanut oil). Group 3 receivedAluminium phosphide 2.3 mg/kg. Group 4 received Aluminium phosphide 2.3 mg/kg and 800 mg/kg of Tocopherol. Group 5 received Aluminium phosphide 2.3 mg/kg and 1200 mg/kg of Tocopherol. The administration was done orally, once per day and it lasted for the period of seven days.

Neurobehavioral Studies: In the beam walking test for motor coordination assessment, the animals were trained to traverse an elevated narrow beam to reach an enclosed escape platform. A smooth wood of 120 cm long and 28 mm wide was used as the elevated beam on which the animals traverse. Two narrow support stands of 50 cm high and 3 cm cross section each was used as support at each end of the beam. A goal box of 30 cm on each side with 5x7cm entrance hole was mounted on one side of the beam which served as the enclosed escape platform for the animals. The rats were placed on the beam one after the other at the starting point and the time latency to traverse the beam to the escape platform was taken and recorded for all the animals[13].

Animal Sacrifice: At the completion of administration, the animals were anaesthetized by injection of ketamine (75mg/kg IP)[14]and were sacrificed. The brains were removed, rinsed in ice-cold saline and dissected immediately. The cerebellum was removed and fixed in Boin's fluid, processed and stained for stereological study and histological analysis using haematoxylin and eosin (H & E) for general tissue architecture.

Stereological Evaluation: The absolute volume of the cerebellum was estimated using the Cavalieri estimator of volume according to the method of Gundersen*et al.,*[15] to provide 10 sections (10 µm thick) after To achieve this, the cerebellum of the Wistar rat per group were isolated, processed and sectioned serially using a microtome, as shown by Gundersen*et al.,*pilot study on how many slices could be derived. Tissue sections of the cerebellum were then selected at every third using the systematic universal random sampling method. The sections derived were stained using H and E. A transparent counting grid was placed randomly over the cut surface of every cerebellum slice at a magnification of (X10). The number of points hitting the cerebellum was counted. The volume was estimated using the Cavalieri's principle [15] as follows:

$$V := T\,(a/p)\,\Sigma p$$

where **":="** indicates that the result is the estimated value rather than the true value, "V" is the total volume of cerebellum, "T" = 0.03mm is the average slice thickness, "a/p" ¼ is the area associated with each point inthe counting grid (4mm²), and "µp" is the total number ofpoints cerebellum.
Coefficient of error (CE) was calculated as reported by Gundersen et al [15] as follows:

$$CE = \frac{\sqrt{\text{Total variance}}}{\mu p}$$

Statistical Analysis: Data obtained were expressed as Mean ± SEM (Standard error of mean). One – way analysis of variance (ANOVA) was employed followed by LSD posthoc tests using SPSS v20 for window to compare the mean differences between and within the groups. A $p \leq 0.05$ was considered to be significant.

RESULTS

Neurobehavioral Studies: The result from the motor coordination assessment revealed significant increase in the time taken to cross the beam by the animals in group exposed to aluminium phosphide only before (2.03±0.19s) the administration compared to after (4.10±0.36s) the administration. The animals that received tocopherol as supplement following aluminium phosphide shown non-significant differences ($p>0.05$) in the time taken to cross the beam before and after administration.

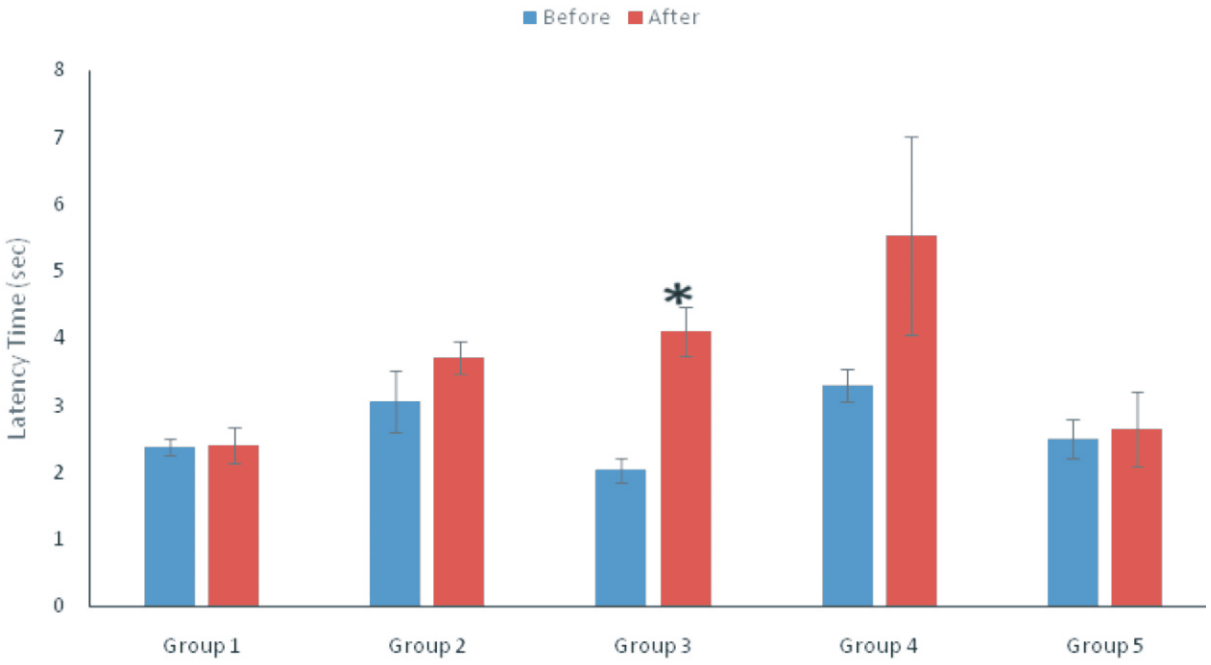

Figure 1: Result for motor coordination assessment of the rats administered AlP and AlP treated with tocopherol. Bars carrying the superscripts (*) are significantly (p<0.005) different.

Group 1= Control (distilled water 0.2ml/kg body weight), Group 2= Peanut Oil Control group (0.2ml/kg), Group 3= 2.3mg/kg of Aluminium Phosphide, Group4 =2.3mg/kg of Aluminium Phosphide + 800mg/kg Tocopherol, Group 5= 2.3mg/kg of Aluminium Phosphide + 1200mg/kg Tocopherol,

Cerebellum volume estimation

The stereological study shows no significant differences in the cerebellum volume across the groups.

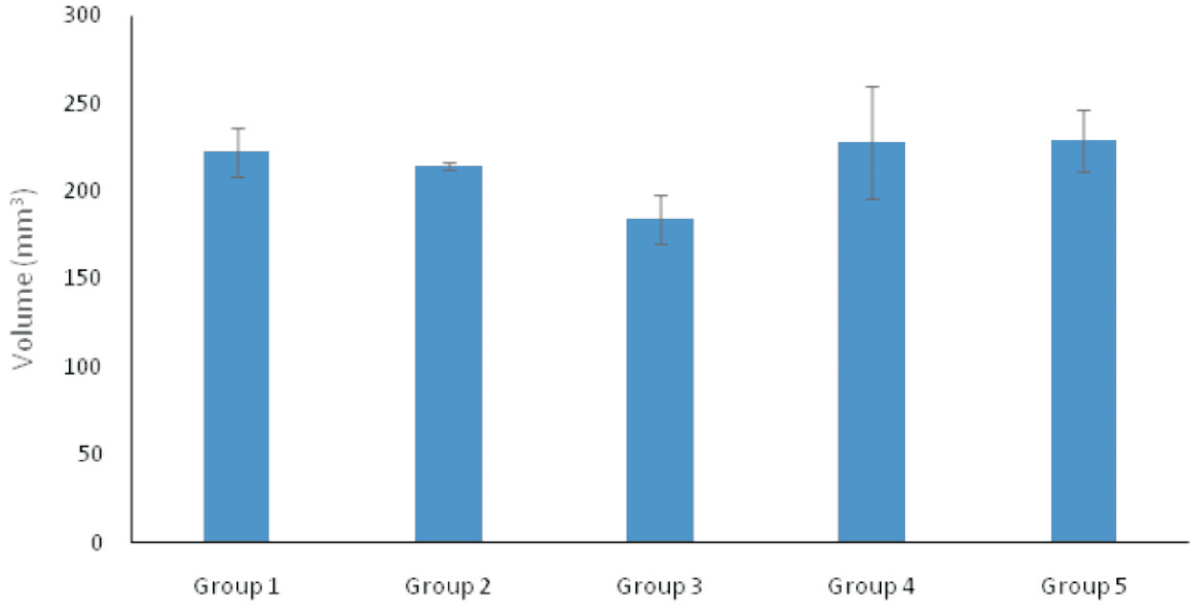

Figure 2: Result of Cerebellum volume estimation of rats administered AlP and AlP treated with tocopherol. Group 1= Control (distilled water 0.2ml/kg body weight), Group 2= Peanut Oil Control group (0.2ml/kg), Group 3= 2.3mg/kg of Aluminium Phosphide, Group 4 =2.3mg/kg of Aluminium Phosphide + 800mg/kg Tocopherol, Group 5= 2.3mg/kg of Aluminium Phosphide + 1200mg/kg Tocopherol.

Histological Observation: The result of histological study from the transverse section of the Cerebellar cortex of the experimental animals showed normal histo-architecture in the group 1 (Normal saline) and 2 (Peanut oil), while the groups that were administered Aluminium phosphide alone and Aluminium phosphide with tocopherol presented different levels of structural and cellular changes. The Aluminium phosphide treated group (group 3) showed more severe pathological changes compared to the group that received tocopherol as a supplement (group 4 and 5).The transverse section of cerebellar cortex in group 1 and 2 animals showing normal cellular layers, structure and arrangement. Group 3 showed degenerated neurons, areas of degenerative changes, and necrotic patches, while group 4 and 5 animals showed less degenerated neuron, infiltration of the normal cells into the molecular layer and little or non- disappearance of the pr.

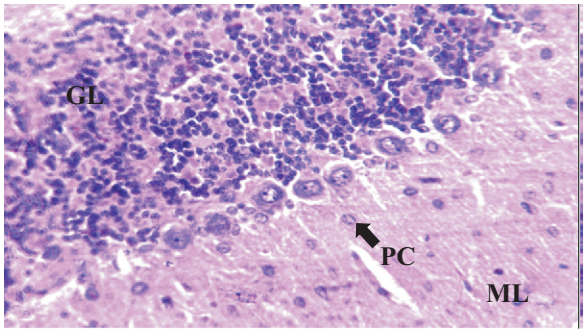

Plate 1: Sections of the Cerebellum. (H&E stain; x 250). Control (distilled water 0.2ml/kg body weight). ML= Molecular layer, GL= Granular layer. PC = Purkinje cell.

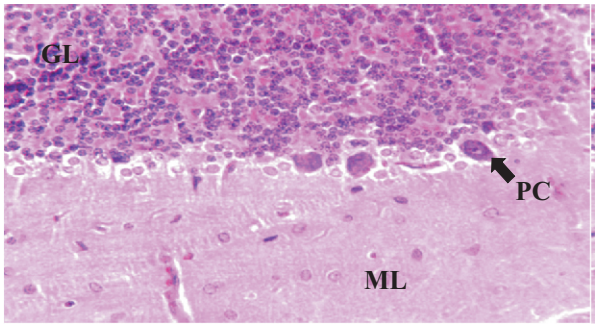

Plate 2: Sections of the Cerebellum . (H&E stain; x 250). G2= Peanut Oil Control group (0.2ml/kg). ML= Molecular layer, GL= Granular layer. PC = Purkinje cell.

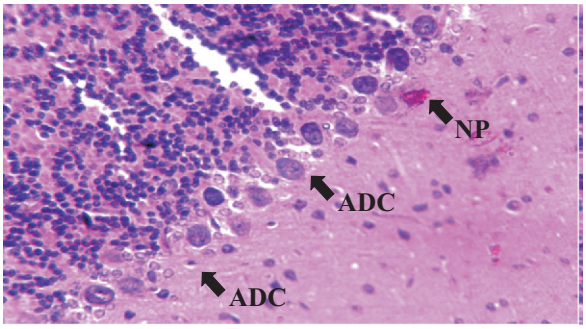

Plate 3: Sections of the Cerebellum. (H&E stain; x 250). G3= 2.3mg/kg of Aluminium Phosphide. , ADC= Area of degenerative changes, NP= Necrotic patch

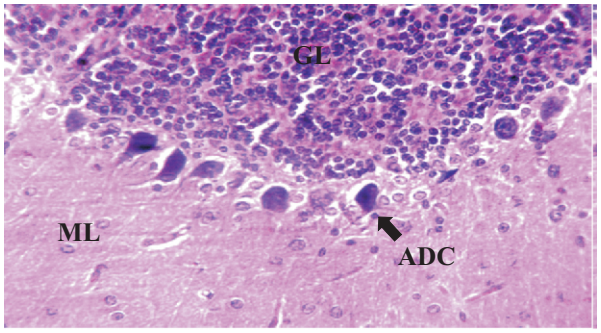

Plate 4: Sections of the Cerebellum. (H&E stain; x 250). G4= 2.3mg/kg of Aluminium Phosphide + 800 mg/kg Tocopherol. ML= Molecular layer,GL= Granular layer. ADC= Area of degenerative changes.

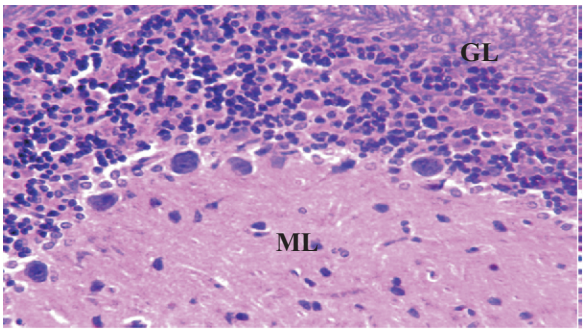

Plate 5: Sections of the Cerebellum. (H&E stain; x 250). G5= 2.3 mg/kg of Aluminium Phosphide + 1200 mg/kg Tocopherol. ML= Molecular layer, GL= Granular layer.

DISCUSSION

Neurobehavioral test was carried out to establish the effect of Aluminium phosphide on the functional status of the Cerebellum. From this study, result from the assessment of motor coordination test has shown that Aluminium phosphide exposure at a dose of 2.3mg/kg orally for the period of 7 days has detrimental effect on the motor coordination function in cerebellum of rats. The animals that received aluminium phosphide only demonstrated significant increase in the time taken to cross the beam before the administration when compared to after the administration which may be attributed to neurotoxic effect of aluminium phosphide on the cerebellum resulting to impairment of motor coordination functions. The other groups that were administered tocopherol following aluminium phosphide shows no significant differences in the time taken to cross the beam before and after the administration which can be related to the antioxidant effect of tocopherol negating the toxic effect of the aluminium phosphide on the nervous system.

The histological study in this present study has shown that aluminium phosphide exposure have some deleterious effect on the cerebellum of adult Wistar rats. A stained section of Cerebellar cortex of animals in control groups shows normal histological features, there is normal cellular arrangement and distribution in the cerebellum, while Aluminium phosphide exposed rats' shows areas of degenerative changes, degenerated neurons, appearance of necrotic patches and gliosis. This is in agreement with another study [16] that reported degenerated neurons, infiltration of round cells into the molecular layer, disappearance of the processes of purkinje cells, degeneration of nerve fibres and the appearance of neurotic patches in the Cerebellar cortex [16 17]. The histological changes in cerebellar cortex of tocopherol treated rats following aluminium phosphide exposure when compared to the rats exposed to aluminium phosphide only shows less deleterious features which may be due to the antioxidant and anti-inflammatory effect of tocopherol.

Following aluminium phosphide exposure, stereology evaluation shows non- significant decrease in the cerebellar volume of experimental rats. The cerebellum volume of the animals administered with 2.3 mg/kg only was decrease although not significant when compared to the control group and the groups that were administered tocopherol following aluminium phosphide, which may be due to the toxic effect of aluminium phosphide on the cerebellum resulting to loss of it volume.

CONCLUSION

The results from this present study has demonstrated that Aluminium phosphide has toxic effects on the Hippocampus of adult Wistar rats and tocopherol through it antioxidant and anti-inflammatory properties has protective effects on the aluminium phosphide induced neuro toxicity in Adult wistar rats.

REFERENCES

1. Meister R.T., editor. Farm Chemicals Handbook. ‹92. Meister Publishing Company. Willoughby, OH. 1992; Pp.6-13.
2. O'Malley M., Fong H., Sánchez M.E., Roisman R., Nonato Y., Mehler L. Inhalation of phosphine gas following a fire associated with fumigation of processed pistachio nuts.*Journal of Agromedicine*.2013; 18(2):151-73.
3. Degesch, America Inc. Phostoxin Tablet and Pellet application manual. Degesch.2011.
4. Saheen D. Technical Vice President Degesch America Inc., Weyers Cave, USA via Oral Communication. 1996; Pp.5-7.
5. Gehring, P. J., Nolan, R. J., Watanabe, P. G., and Schumann, A.M. Solvents, fumigants, and related compounds. *in*: *Handbook of Pesticide Toxicology* (vol. 2: Classes of Pesticides), edited by W.J. Hayes, Jr. and E.R. Laws, Jr.). *Academic Press.* 1991; Pp. 637-730
6. Easter-wood, L., Chaffin, M. K., Marsh, P. S., Porter, B. and Barr, C. Phosphine intoxication following oral exposure of horses to aluminum phosphide-treated feed. *Journal of American Veterinary Medical Association*.2010; 236:446-50.
7. US Environment Protection Agency Office of Pesticides and Toxic Substances, (1992).Fact Sheet Number 118.
8. Proudfoot, A. T. Aluminium and zinc phosphide poisoning (2009). *Clinical Toxicology. (Phila)*; 47:89-100.
9. NIHODS. National Institute forHealth Office of Dietary Supplements Research Practicum.2012; p.1-5.
10. Gandhi, S, and Abramov, A. Y. Mechanism of oxidative stress in neurodegeneration. *Oxidative and Medical Cell Longevity,* 428010.2012
11. Dasuri, K., Zhang, L. and Keller, J. N. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. *Free Radical Biological Medicine*.2013; 62:170-185.
12. Abedini, M., Fatehi, F. and Tabrizi, N. Ischemic Stroke as a Rare Manifestation of Aluminum Phosphide Poisoning. A Case Report.*ActaMedicaIranica*.2014; 52(12):947-949.
13. Rebecca J. Carter, A. Jennifer Morton, and Stephen B. Dunnett. Motor Coordination and Balance in Rodents; Current Protocols in Neuroscience. *Behavioral Neuroscience*.2001; 8.12.1-8.12.14
14. Persson, J. Ketamine in pain management. *CNS*

Neuroscience and Therapeutics.2013; 19(3): 258-264.
15. Gundersen H.J, Bagger P, Bendtsen T.F, Evans S.M, Korbo L, Marcussen N, moller A, Nielsen K, and Nyengaard J.R. The new stereological tools: dissector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis.1988; 96(10): 857-81. PMID: 3056461.
16. Tripalti, S. K. and Pandey, S. K. The effect of aluminium phosphide on the human brain: A histological study. *Medical science law*.2007; 47(2): 141-146
17. Iyanda, A. A and Iheakanwa, C. I. Damaging effects of Phosphide-powder residue on Brain, Lung and Heart Histoarchitecture in Rats.*Asian journal of biomedical and pharmaceutical sciences*.2013; 3(17): 9-13