Asian Journal of Research in Medical and Pharmaceutical Sciences

1(1): 1-7, 2017; Article no.AJRIMPS.34825

Adverse Potentials of Herbal Administration of Ethanolic Root Bark and Leaf Extracts of *Rauwolfia vomitoria* on Cerebellar Cytoarchitecture of Wistar Rats

Akaninyene M. Okon[1], Clementina F. Iniodu[2], Gabriel D. Edem[2*], Ekemini I. Johnson[2] and Mokutima A. Eluwa[1]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Nigeria.
[2]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AMO designed the study, performed the statistical analysis, wrote the protocol. Author CFI wrote the first draft of the manuscript. Authors GDE and EIJ managed the analyses of the study. Author MAE managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2017/34825
Editor(s):
(1) Bulang Gao, Shijiazhuang First Hospital, Hebei Medical University, China.
Reviewers:
(1) Mostafa Abbas Shalaby, Cairo University, Egypt.
(2) Mosad Ahmed Ghareeb, Theodor Bilharz Research Institute, Egypt.
(3) Daniela Hanganu, University of Medicine and Pharmacy, Romania.
Complete Peer review History: http://www.sciencedomain.org/review-history/19996

Original Research Article

Received 14th June 2017
Accepted 29th June 2017
Published 11th July 2017

ABSTRACT

The use of herbal medicine in Nigeria has become increasingly abused since there is no standardization in measurement and dosages. People consume these herbal products at will not minding the adverse impact it creates to the body. This study was aimed at investigating the adverse effect posed by administration of *Rauwolfia vomitoria* (root bark and leaf extracts) on cerebellar cytoarchitecture. 30 adult Wistar rats were used (n=5). Histological studies of the cerebellar cortex using H &E method showed a dose-dependent distortion of the cerebellar cytoarchitecture characterized by reduction in size and number of Purkinje cells, distortion of the

**Corresponding author: E-mail: Profgabe4sure@gmail.com;*

Purkinje cell layer as well as reduction in cell population in both the molecular and granular layers in the experimental groups (200/300 mg/kg body weight of root-bark and leaf extracts). However, these changes were more prominent in groups that were treated with ethanolic root-bark extract of *Rauwolfia vomitoria*. It may be concluded that precautions should be imbibed while taking this herb.

Keywords: Rauwolfia vomitoria; herbal; cerebellar; root bark; leaf extract; H&E.

1. INTRODUCTION

Rauwolfia vomitoria is one of the many herbal products consumed in Nigeria for its medicinal values, especially for its antipsychotic values. Many consumers believe that herbal medicines are safe because they are natural [1]. Phytochemical analysis of *Rauvolfia vomitoria* revealed the presence of alkaloids, saponins and cardiac glycosides with the absence of tannins, cyanogenetic glycosides and anthraquinones; and these biogenic chemicals include ajmaline, aricine, corynanthine, deserpiden lankanescine rauwolscine, rescinnamine, reserpine, reserpiline, isoreserpine, isoreserpine, and yohimbine [1].

Other chemical constituents of *Rauwolfia Vomitoria* include: Canembine, Corynanthine, Seredine, Yohimbine, Mitoridine, Purpeline, Pelirine, Semperflorine, Ajmaline, Raunticine, Raujemedine, Samatine, Deserpidine, Ajmalidine, Rauwolfine, Obscuridine, Obscurine, Rauvoxinine, Mitoridine, Vomilenine, Seredamine, Tetraphyllicine, Ajmalicine, Reserpiline, Reserpinine, Sarpagine, Vincamajine, Neoreserpiline, Aricine, Picrinine, Sandwichine, Rauwolfinine, Rauvomitine, Raucaffridine, Raucaffriline, Raunamine etc [2,3].

Reserpine is the most important alkaloid found in this plant. It is mostly derived from the root back of the plant. In traditional herbal medicine, the root was brewed as a tea and consumed to treat hypertension, insanity, snakebites, schizophrenia and cholera. Pure reserpine was first isolated in 1952 and is considered the first modern drug for the treatment of hypertension [4]. It had drastic psychological side effects and has been replaced as the first-line antihypertensive drug by other compounds that lack such adverse effects, although a combination drugs that include it are still available in some countries as second-line antihypertensive drugs. Reserpine irreversibly binds to the storage vesicles of neurotransmitters, particularly norepinephrine, serotonin and dopamine. Eventually, catecholamine depletion occurs because of the body's inability to store these neurotransmitters. It has also been observed to cause extrapyramidal side effects such as orofacial dyskinesia and tremor [5].

Another indole alkaloid indentified in *Rauwolfia vomitoria* is alstonine. It could also be found in various other plant species including *Alstoniaboonei*, *C. roseus*, *Picralima nitida*, *Rauwolfia caffra*. Laboratory analysis of a crude ethanol extract from powdered root material showed psychopharmacological activity, particularly antipsychotic properties. It also possess anticancer and anxiolytic properties, and interferes with the glutamate system [6]. Alstonine is believed to be especially effective against hormone-related cancers, including breast and prostate cancer, and a natural blend of Pao Pereira (which contains flavopereirine) and *Rauwolfia vomitoria* (which contains alsonine) have been shown to be effective against cancer cell lines, including brain, breast, ovarian, prostate, kidney, thyroid, pancreatic, colon, liver and skin cancer cells [7].

2. MATERIALS AND METHODS

2.1 Animal Care

Thirty (30) adult female Wistar rats weighing 150 g – 250 g were obtained from the Department of Biochemistry, University of Calabar. They were housed in the animal house of the Department of Anatomy under standard conditions. The animals were fed with standard diet and allowed access to drinking water *ad libitum*. They were randomly divided into 6 groups (n=5).

2.2 Preparation of Extracts

The root-bark and leaves of *Rauwolfia vomitoria* were obtained from the University of Calabar farm, Calabar on the 7th day of April. They were identified and authenticated by a botanist in the Department of Botany, University of Calabar. The roots and the leaves were washed in water and the root-bark was defoliated and dried. The dried root-bark and leaves were blended into

powdered form using a Binatone kitchen blender. The blended sample was soaked in ethanol for 24 hours and the extract was filtered and evaporated to obtain the crude extract.

2.3 Experimental Procedure

The animals were randormly divided into 6 groups of 5 animals each labelled A, B, C, D, E, F. Groups A and B were the normal control and olive oil control respectively. Groups C, D, E, and F served as the experimental. Group A animals received 0.5 ml/200 g of normal saline while group B animals received 0.5 ml/200 g of olive oil for 7 days respectively. The ethanolic extracts of *Rauwolfia vomitoria* root-bark and leaf were administered orally to the animals with the aid of orogastric tube. After the last dose schedule, the animals were sacrificed using chloroform. The cerebellum was removed and fixed in 10% formol saline solution. The cerebellar sections were stained using H&E.

3. RESULTS

Histological study of the cerebellum using Haematoxylin and Eosin staining method showed normal cerebellar cortex architecture defined by the molecular layer which is sparsely populated by neurons, and Purkinje layer comprising of a single layer of large pear-shaped Purkinje cell bodies with numerous dendrites projecting into the molecular layer in control group A. Also seen in this layer are smaller somata of epithelial (Bergmann) glial cells. The granular layer is very densely populated. The cerebellum of the olive control group B also showed normal cerebellar cortex architecture.

In the cerebellar cortex of the group C rats which were given 200 mg/kg body weight of ethanolic root-bark extract of *Rauwolfia vomitoria*, the Purkinje cell layer shows irregularly-shaped Purkinje cell bodies. There was also a reduction in the number and size of Purkinje cells and slight reduction in cell population in the granular layer.

The cerebellar cortex of group D rats which received 300 mg/kg body weight of the ethanolic extract of *Rauwolfia vomitoria* root-bark showed distorted cerebellar cortex. The cells of the molecular layer showed hypertrophy and hypoplasia. The Purkinje cell layer was distorted and sparsely populated with irregularly-shaped cells and were greatly shrunken. The granular layer was also distorted and the cell population greatly reduced.

The cerebellar cortex of the group E rats which were treated with 200 mg/kg body weight of the ethanolic extract of *Rauwolfia vomitoria* leaf showed a slight reduction in cell population in the molecular layer. The Purkinje cell layer was deeply stained compared to both control groups and the group that received 200 mg/kg of root-bark extract. The Purkinje cells were pear-shaped with dendrites projecting into the molecular layer.

The group F rats which were treated with 300 mg/kg body weight of ethanolic extract of *Rauwolfia vomitoria* leaf showed a distorted cytoarchitecture of the cerebellar cortex. The cellular population of the molecular layer was reduced. The Purkinje cell layer showed greatly distorted architecture. The Purkinje cells were reduced in number, shrunken in size, spindle-shaped and more lightly-stained compared to the group that received 300 mg/kg of root-bark extract. The granular layer is slightly reduced in cell population. The cell population in the granule layer was slightly reduced.

4. DISCUSSION

Following the administration of *Rauwolfia vomitoria* (RV) root-bark and leaf extracts for seven (7) days on adult Wistar rats, the cerebellum was stained and studied for cytoarchetectural changes using haematoxyline and eosin.

Sections of the cerebellum in the root-bark extract treated groups C and D showed dose-dependent distortions in the cerebellar cortex defined by the presence of irregularly-shaped Purkinje cells in the Purkinje cell layer, reduction in the number and size of the Purkinje cells and reduction in cell population in the granular layer. The group D rats which were treated with 300 mg/kg body weight of the ethanolic extract of *Rauwolfia vomitoria* root-bark also showed hypertrophy and hypoplasia of cells in the molecular layer and a sparsely-populated Purkinje cell layer with vacuolous spaces and greatly shrunken Purkinje cells when compared with the group F that received 300 mg/kg of the leaf extract. The cells were also more deeply stained. It is therefore possible that *Rauwolfia vomitoria* might cause cell loss in the cerebellum, and serve as indicator of pathologic processes, thus, suggesting possible early stages of

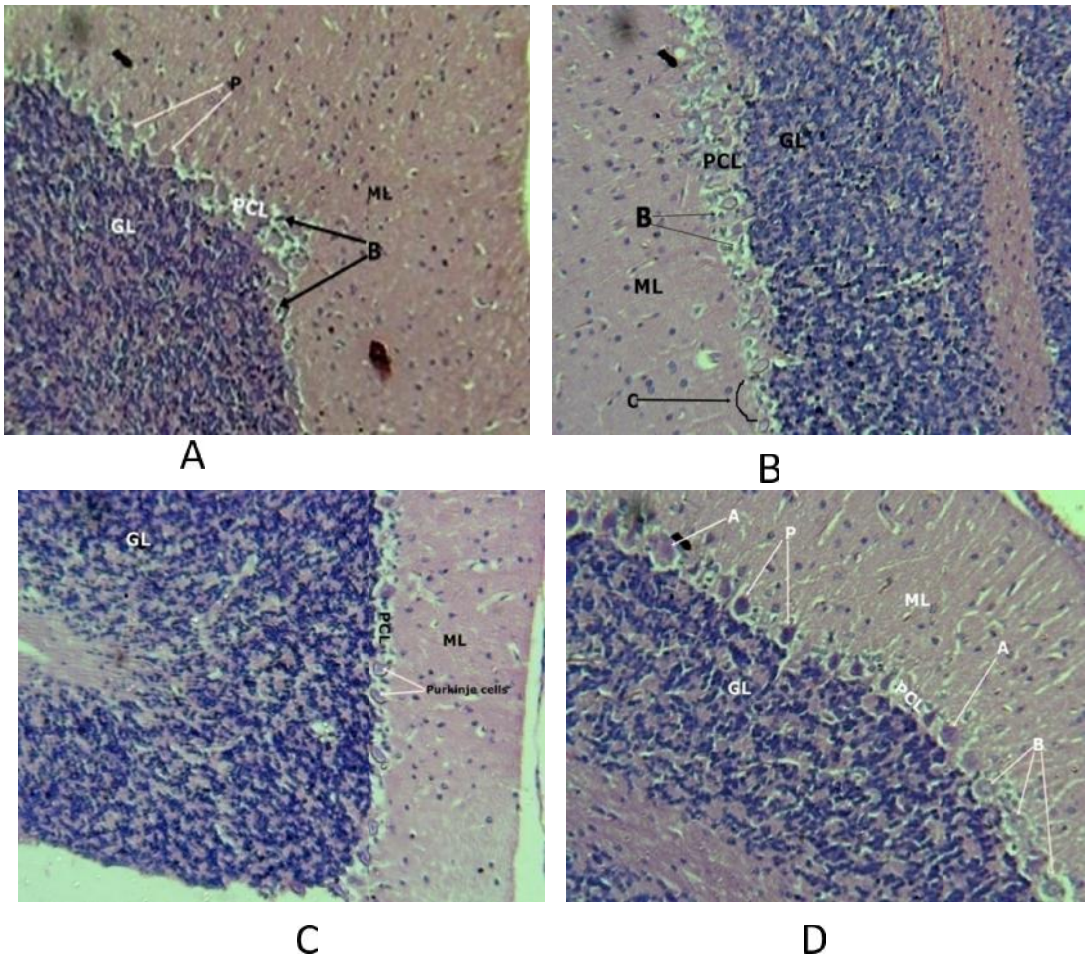

Fig. 1. Photomicrographs of cerebellum of normal control, olive oil control and treated group which received 200 mg/kg root bark and leaf extracts of *Rauwolfia vomitoria* (H&E; x 100)

A: *section of cerebellum of normal control showing normal cerebellar cytoarchitecture. Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P)*

B: section of cerebellum of normal control showing normal cerebellar cytoarchetecture. Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P)

C: Cerebellar cortex – 200 mg/Kg of root-bark extract showing reduction in number and irregularly shaped Purkinje cells (P) in thePurkinje Cell Layer (PCL) and slight cell population reduction in granular layer (GL) when compared with the controls.

D: Cerebellar cortex – 200 mg/kg of leaf extract showing large, round, lightly-stained Purkinje cells (A and B); Molecular Layer (ML); Purkinje cell layer (PCL); Granular layer (GL); deeply stained pear-shaped Purkinje cells (P).

neuronal deranged activity or loss. These changes may have been preceded, and/or accompanied by increase in nuclear activities as observed by the increase in basophilic staining intensities especially in the group D treated with 300 mg/kg of *Rauwolfia vomitoria* root-bark extract. This is in agreement with a study that was done to assess the effect of fluoride on rat cerebellar cortex which showed that the Purkinje cells appeared shrunken and deeply stained, with multilayer disposition [8]. Electron microscopic findings showed increased infolding of nuclear envelope, mitochondrial alterations, dilated rough endoplasmic reticulum cisternae and clusters of vesicles near the Golgi bodies.

This present study is also in line with the findings of Aktas et al. [9] who posited that inflammation within the central nervous system is a common phenomenon even in classic non-inflammatory brain diseases that are characterized by degeneration or trauma of

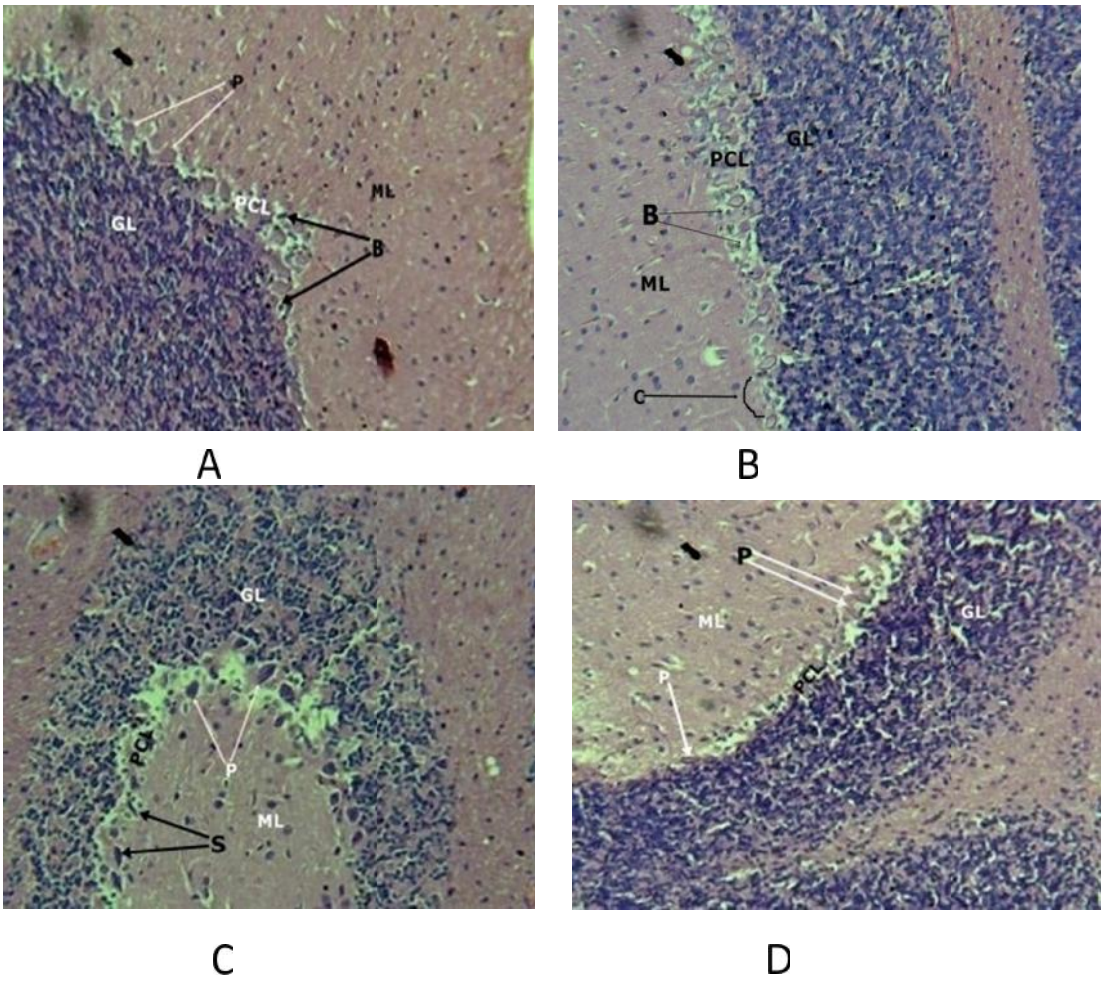

Fig. 2. photomicrographs of cerebellum of normal control, olive oil control and the groups which received 300 mg/kg root bark and leaf extract (H & E; x 100)

A: Cerebellum of normal control showing normal cerebellar architecture. Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P); Bergmann glial cells (B).

B: Cerebellum of olive oil control showing normal cerebellar architecture. Molecular layer (ML); Purkinje cell layer (PCL); Granular layer (GL); Purkinje cells (P); Bergmann glial cells (B).

C: Cerebellar cortex – 300 mg/mg root-bark extract showing hypertrophy and hypoplasia, distorted cerebellar archetecture. Molecular layer (ML); Purkinje cell Layer (PCL); Granular layer (GL); normal-sized Purkinje cells (P); Shrinked and spindle-shaped Purkinje cells (S).

D: Cerebellar cortex – 300 mg/kg of leaf extract showing distorted cytoarchitecture. Molecular layer (ML), Distorted purkinje cell layer (PCL), Granular cell layer (GL), lightly stained, spindle shaped purkinje cells (P)

neuronal structures, such as Alzheimer disease, Parkinson disease or stroke. This is also in line with the findings by Eluwa et al. [10] who reported some distortions in the experimental groups of rats which received 600 mg/kg and 500 mg/kg of root bark extract of *Rauwolfia vomitoria*. Significant reduction in Purkinje cell density and diameters in rats administered with artesunate was also reported by Ajibade et al. [11]. Young et al. [12] also reported loss of Purkinje cells in the cerebellum due to increased mercury contents in the cerebellum following oral administration of mercuric sulfide (HgS) and cinnabar.

Findings by Dare et al. [13] have also shown that grape fruit extract (*Citrus paradisi*) given to adult male Wistar rats causes histological alteration of the cerebellum characterized by a dose-dependent cellular degeneration and atrophy, thus leading to a decrease in number of cells in the granular and Purkinje layers respectively. Furthermore, a study of the histomorphologic alterations of the cerebellum of Wistar rats

following Amodiaquine and Artesunate administration showed various degree of destruction of the Purkinje cortical layers in the experimental groups compared to the control [14] and a study of the toxicological effects of *Teucrium stocksianum* after acute and chronic administration in rats showed focal loss of Purkinje cells in the cerebellum [15]. Significant decrease in the diameter of Purkinje cells and in the thickness of both molecular and granular layers, and cell death (necrosis) was reported amongst albino rats treated with morphine sulphate [16].

Megahed et al. [17] reported that combined administration of therapeutic doses of acetaminophen and phenytoin caused extension of the neuronal degeneration to more than one layer of the cerebellar cortex. Histological studies of the effects of Monosodium Glutamate on the Cerebellum of adult Wistar rats indicated that the treated sections of the cerebellum showed disruption of the Purkinje and granular layers, sparse granular cell distribution, cellular degenerative changes in the granular layer with the group that received a higher dose of Monosodium glutamate more severe [18]. The effect of *Astragaluslusi tanicuslam* in experimental subchronic neurotoxicity of lambs was studied and findings revealed that there were no clinical effects. This work is also in line with the findings of Soler et al. [19] who reported histological changes such as degeneration of neurons in the cerebrocortical grey matter, degeneration and loss of Purkinje cells in the cerebellum, satellitosis, neuronophagia, hyperemia and small hemorrhages evident throughout the Central Nervous System following the administration of *Astragaluslu sitanicus.*

5. CONCLUSION

Rauwolfia vomitoria root-bark and leaf extracts has a neurotoxic and neurodegenerative impacts on the Cerebellum. Therefore, the use of this herb should not be arbitrary considering its effects as elucidated here. The public should also refrain from taking this herb on self medication as it could have possible long-term adverse effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sonibare MA, Lawal TO, Ayodeji OO. Antimicrobial evaluation of plants commonly used in the management of psychosis opportunistic infections. International Journal of Pharmacology. 2011;7(4):492-497.
2. Onike R. Uses of *Rauwolfia vomitoria* Afzel (asofeyeje) in African Traditional Medicine; 2010.
3. Sharma R. Agro-techniques of medicinal plants. India: Daya Publishing House; 2004.
4. Lopez-Munoz F, Bhatara VS, Alamo C, Cuenca E. Historical approach to reserpine discovery and its introduction in psychiatry. Actas Esp Psiquiatr. 2004;32(6):387–395.
5. Naidu PS, Singh A, Kulkarin SK. Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction, Phytother Res. 2006;20:140–146.
6. Elisabetsky E, Costa-Campos L. The alkaloid alstonine: A review of its pharmacological properties. Evidence-Based Complementary and Alternative Medicine. 2006;3(1):39-48.
7. Bemis DL, Capodice JL, Gorroochurn P, Kartz AE, Buttyan R. Anti-prostate cancer activity of a β-carboline alkaloid enriched extract from *Rauwolfia vomitoria*. International Journal of Oncology. 2006; 29(5):1065 –1073.
8. Saad El-Dien HM, El Gamal DA, Mubarak HA and Saleh SM. Effect of fluoride on Rat cerebellar Cortex: Light and Electron Microscopic Studies. Egyptian Journal of Istology. 2010;33(2):245–256.
9. Aktas O, Ullrich O, Infante-Duarte C, Nitsxh R, Zipp F. Neuronal damage in brain inflammation. Arch Neurology. 2007; 64(2):185-9.
10. Eluwa MA, Idumesaro NB, Ekong MB, Akpantah A O, Ekanem TB. Effect of

aqueous extract of *Rauwolfia vomitoria* root bark on the cytoarchetecture of the cerebellum and neurobehaviour of adult male Wister rats. The Internet Journal of Alternative Medicine. 2009;6,2.

11. Ajibade AJ, Fakunle PB, Shallie PD. Some histological observations and microstructural changes in the nissl substance of cerebellar cortex of adult wistar rats following artesunate administration. Current Research in Neuroscience. 2012;2(1):1–10.
12. Young YH, Chuu JJ, Liu SH, Lin-Shaiau SY. Neurotoxic mechanism of Cinnabar and mercuric sulfide on the vestibule-ocular reflex system of Guinuea pigs. Toxicology Science. 2007;67(2):256-63.
13. Dare BJ, Oyewopo AO, Saalu LC, Kadir RE, Osinubi AAA. Histological alteration of the cerebellum of adult male wistar rat treated with the grape fruit extract (*Citrus paradisi*). Anatomy and Physiology. 2012; 2:107.
14. Ekong, MB, Igiri AO, Egwu AO. Histomorphologic alterations of the cerebellum of wistar rats following amodiaquine plus artesunate administration. Internet Journal of Medical Update. 2009;4(2):15-18.
15. Tanira MO, Wasfi IA, Homsi MA, Bashir AK. Toxicological effects of Teucriumstocksianum after acute and chronic administration in rats. Journal of Pharmacology. 1996;48(10):1098–102.
16. Bekheet SH, Saker SA, Abdel-Kader AM, Younis AE. Histopathological and Biochemical changes of morphine sulphate administration on the cerebellum of albino rats. Tissue and Cell. 2010;42(3):165 – 175.
17. Megahed HMAS, Sherif HAH, Seif IA, El-Sehly WM, Mourad GM. Histological Assessment of the Safety of repeated administration of phenytoin combined to acetaminophen on the liver and cerebellum of mice. Alexandria Bulletin; 2006.
18. Eweka A, Om'Iniabohs F. Histoloical studies of the effects of monosodium glutamate on the cerebellum o adult wistar rats. The Internet Journal of Neurology. 2007;8.
19. Soler RF, Garcia RA, Moyano SMR, Infante MF. Effect of *Astragalus lusitanicu* lam in experimental subchronic neurotoxicity of lambs. Veterinary and Human Toxiccology. 1990;32(6):551– 554.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19996