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EFFECT OF DICHLORVOS ON HISTOARCHITECTURE OF THE CEREBRAL BLOOD VESSELS IN ADULT WISTAR RATS

YETİŞKİN WISTAR SIÇANLARINDA SEREBRAL KAN DAMARLARININ Histomimarisi üzerindeki diklorvos etkisi

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Abstract

Cerebral blood vessels are vital in supplying brain in both human and animals. Any anomaly by rupture or interruption of blood flow may lead to fatal consequences. Dichlorvos is a volatile organophosphate that forms the active ingredient of locally formulated insecticide and pesticide known as Otapiapia or Madararpiapia. It is an anti-acetylcholinestrase that binds irreversibly to acetylcholinesterase and leads to its inhibition. The study aims to determine the effects of dichlorvos on the histology of the cerebral vessels in adult wistar rats. Twenty five apparently healthy adult wistar rats were randomly selected and divided into five groups. The first two groups were used as control while the last three groups were exposed to graded doses of dichlorvos in ethanol solution and experimented for twenty eight days. Twenty four hours after the last exposure the animals were sacrificed and the brain tissues were collected for routine histological technique. The relative brain weights of all the animals were determined and one – way ANOVA was conducted to compare the mean of the control with the treated groups. The H&E stain of the treated groups showed variable degrees of perivascular oedema, pyknosis and apoptosis. Prolong use of dichlorvos could cause cerebral vascular changes in the histoarchitecture such as perivascular oedema and apoptosis, may not affect the brain weight.

Keywords: Dichlorvos, Histoarchitecture, Cerebral blood vessels

Özet

Serebral kan damarları insanların ve hayvanların beyinleri için çok önemlidir. Kan akışının kesilmesi ya da durması nedeniyle oluşan herhangi bir anomali ölümcül sonuçlara sebep olabilir. Diklorvis, Otapiapia ya da Madarar piapia olarak bilinen bölgesel olarak formüle edilmiş sinek ve böcek ilaçlarının aktif maddeleriyle oluşan uçucu bir organofosfattır. Diklorvis, geri dönülemez bir şekilde asetilkolinesteraza bağlanan ve inhibisyonuna sebep olan bir anti- asetilkolinesterazdır. Bu çalışma yetişkin wistar sıçanlarının serebral damar histolojisindeki diklorvos etkilerini saptamayı amaçlar. Sağlıklı görünen 25 adet wistar sıçanı rastgele seçilmiş ve beş gruba ayrılmıştır. İlk iki grup kontrol grubu olarak kullanılırken son üç grup etanol çözeltisinde aşırı dozda diklorvosa maruz bırakılmış ve 28 gün boyunca deney uygulanmıştır. Son dozdan 24 saat sonra hayvanlar öldürülmüş ve beyin dokuları rutin histolojik teknikler için alınmıştır. Bütün hayvanların nisbi beyin ağırlıkları saptanmış ve tedavi edilen gruplarla kontrol grubunun ortalamasını kıyaslamak için ANOVA uygulanmıştır. Kontrol gruplarıyla tedavi edilen grupların ortalama beyin ağırlıklarında istatiksel olarak önemli bir fark H&E kimyasal maddeleri(kalıntıları) perivasküler ödem, piknoz ve apoptozun farklı derecelerini göstermiştir. Diklorvosun aşırı kullanımı perivasküler ödem ve apoptoz gibi histomimaride serebral vasküler değişikliklere sebep olabilir fakat beyin ağırlığını etkilemeyebilir.

Anahtar Kelimeler: Diklorvos, Histomimari, Serebral kan damarları

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1. Introduction

Blood vascular networks constitute a major component of the structure of the brain in addition to neurons, glia, and the extracellular milieu. The neurovascular unit is comprised of the endothelial cells which make up the vessels as well as several other associated cell-types including astrocytes and perivascular cells such as pericytes and smooth muscle cells (Ramos et al., 2008). Pericytes wrap around vessels and are in direct contact with endothelial cells via gap junctions (Bergers and Song, 2005). Blood vessels in the brain are also surrounded by the endfeet of astrocytes. Thus, astrocytes constitute a cellular bridge between neurons and blood vessels. Astrocyte endfeet located on vessels interact directly with endothelial cells and are capable of up-take and/or release of a number of molecules such as amino acids, growth factors (Abbott, 2002; Abbot et al., 2006).

The use of dichlorvos has long been in practice. In addition to its use as control for insects on crops, household, and stored products, dichlorvos is also use to treat external parasitic infections in farmed fish, livestock, and domestic animals (Erdogan et al., 2007). In Nigeria it is hawked around and used for agricultural and domestic purposes to kill various forms of insects and to protect stored products from insects. Dichlorvos is a volatile organophosphate which is preponderantly active pesticide ingredient in the locally formulated Ota-piapia (Musa et al., 2010). This is because of solely to its cheap production, efficacy, accessibility, and affordability (Essiet, 2009). Dichlorvos is rapidly absorbed through the gastrointestinal and respiratory tracts and skin, it enters human system via inhalation, dermal or oral routes, and it is metabolized by the liver and excreted by the kidney (Durkin & Follansbee, 2004; CERI, 2007).

The mechanism of action for the dichlorvos is mainly by blocking of acetylcholinesterase - an enzyme which in turn decomposes acetylcholine (Lewalter&Korallus, 1986; Harlin& Dellinger, 1993). Overdose of this OP leads to symptoms which include weakness, headache, tightness in chest, blurred vision, salivation, sweating, nausea, vomiting, diarrhea, respiratory failure, and abdominal cramps being an acetylcholinesterase inhibitor, (CEPA, 1996). It is mainly metabolized by esterase to dimethylphosphate and dichloroacetaldehyde. Dimethylphosphate is excreted in the urine, while dichloroacetaldehyde is rapidly metabolized via two pathways to dichloroethanolglucuronide, hippuric acid, urea and carbon dioxide, and excreted in the urine and expiration (CERI, 2007). The mechanisms underlying these effects are not known, and the role of acetylcholinestrase (AChE) inhibition is controversial (Kamel and Hoppin, 2004; Abou-Donia, 2003) and may vary depending on the exposure parameters. Chronic neurotoxicity subsequent to a single acute exposure to dichlorvos

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may be triggered by AChE inhibition. Acute and sub lethal doses of dichlorvos were shown to have longterm effects in humans (Ohbuet al., 1997; Proctor et al., 2006). Oral administration of dichlorvos to rat (70 mg/kg) inhibited not only AChE but also hexokinase, phosphofructokinase, lactate dehydrogenase and glutamate dehydrogenase. Dichlorvos administration also caused significant depletion in the brain glycogen content along with increased glycogen phosphorylaseactivitity (Sarin and Gill, 1998). Repeated administration of 50% of LD50 (i.e., 40 mg/kg body wt per day for 10 - 21 days) of dichlorvos caused myelin pallor and microvacuolation of the white matter. This may also leads to primary degeneration of axons and secondary myelin sheath abnormalities caused the spongy tissue loosening when observed under the electron microscope (Zelman, 1977; Zelman and Majdeckt, 1979).

Dichlorvosor Madararpiapia in Hausa parlance is one of the organophosphates that are used for eradicating pests and insects especially among low income countries. This was because of its low price and availability. Because of the quest for greater efficiency, dichlorvos is often used beyond the recommended quantity not mindful about the health consequences. Many researches were conducted regarding the use of dichlorvos, however less attentions was paid to its effects on the cerebral vasculature, therefore this study aims at determining the effects of this chemical on histoarchitecture of the cerebral blood vessels in adult wistar rats.

2 Materials and Method

2.1 Animals

Twenty five apparently healthy adult wistar rats consisted of both sexes and weighed about 195 400g were purchased from the Pharmacology Department, Aminu Kano Teaching Hospital (AKTH) Kano, Nigeria and allowed to acclimatize for two weeks in laboratory condition before subjected to experimentation. The animals were housed in a well-ventilated rectangular aluminium cages (290 \times 320 \times 390 mm) bedded with soft saw dust and maintained under standard laboratory conditions with proper illumination of 12:12-h light/dark cycle in a temperature (210C \pm 20C) and humidity (55 \pm 5%) and humidity controlled room. The animals had free access to food (vital feed) and water ad libitum. The animals' sanitation and husbandry were in accordance with "Guide for the Care and Use of Laboratory Animals". The animals were then divided into five groups (i.e. I, II, III, IV& V) with each group containing at least one of the congeners.

2.2 Chemical and doses Preparation

A stock concentration of 1000 g/l of dichlorvos

(Delvap Super [®]) was purchased from the vendors of insecticides, pesticides and other Agro allied Chemicals at Sabongari market, Kano, Nigeria. The lethal concentration LC50 of dichlorvos which was reported as15mg/m3 (Lewis, 1996) was taken as a reference value. The present study was carried out with sublethal doses equivalent to 75% (11.25mg/m3), 50% (7.50mg/m3) and 25% (3.75mg/m3) of the reference LC50dose.

2.3 Experimental Design

Five poorly ventilated 1m x 1m cubed wooden boxes labelled A, B, C, D and E, each with a rectangular sliding glass pane measured about 0.2m x 0.1m for entrance at the top was constructed for the exposure of the animals. About 2 mls each of the graded solutions were drawn separately using 4 mls hypodermic syringes and then sprayed thoroughly into the boxes every day before the animals were exposed. The animals in groups I and II were exposed into ambient air and 2 mls of 2.5 mg/m3ethanol as positive and negative controls respectively whereas those in groups III, IV and V were exposed into the boxes sprayed with 11.25 mg/ m3, 7.50 mg/m3, and 3.75 mg/m3 concentrations of the standard solution in ethanol respectively. The exposure lasted for 2 hours in all the groups every day for twenty eight days. Twenty four hours after the last exposure, each of the animals was sacrificed by cervical dislocation.

2.4 Samples Collection and tissue *preparation*

Each of the animals was weighed on digital balance (AWS, USA) before brains were quickly dissected out and the tissues immediately collected also weighed on a digital scale and finally fixed in a Bouin's fluid. After overnight fixation, the tissues werethoroughly washed, dehydrated, cleared in xylol, and processed for paraffinembedding. Therefore relative brain weights were expressed and recorded as percentage of their body weights using the formula below.

Relative brain weight = $\frac{\text{Brain weight}}{\text{Body weight}} \times 100$

2.5 Histopathology of the Brain Tissues

Paraffin blocks of the brain tissues were cut at 2 - 3μ m thickness and stretched on glass slides. Afterdeparaffinization, the sections were stained with hematoxylin-eosin andobserved under light microscope.

2.6 Statistical Analysis

All values were represented as means \pm SEM.

Statistical significance between the control and experimental data was subjected to ANOVA together with Tukey's test (p < 0.05). All analyses were conducted using Minitab (version 16) statistical software.

2.7 Slides Preparation and Interpretation

The sections of the brain tissues were stained using hematoxylin and eosin and viewed underMotic photomicroscope to which fitted Celestron© digital microscope imager (USA) with an inbuilt 15x magnifying lens at 150 and 600 magnifications respectively.



Fig. 1: Mean Brain Weights of Control and Dichlorvos Treated Groups

Figure 1, shows bar chart of the ANOVA test comparison of the mean brain weights of the control and treated groups. The mean \pm SEM distribution showed 1.90 \pm 0.09, 1.87 \pm 0.03, 1.83 \pm 0.09, 1.76 \pm 0.04 and 1.76 \pm 0.05 for groups I, III, V, II&IV respectively. The inferential statistic showed there was no statistically significant difference in mean [F= 0.88, p = 0.49] between the control and treated groups.



Plate IA&B: H&E normal photomicrograph of cerebral vascular architecture exposed to ambient air as control in Adult Wistar Rat at (A) x150 and (B) x600 magnifications respectively.
BC= Blood Clots; EN= Endotheliocytes; PVE= Perivascular oedema; pEN: Polymorphic Endotheliocytes; Lu= Lumen

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Plate IIA&B: H &E photomicrograph of cerebral vascular architecture exposed to 2.5 mg/m3 ethanol in Adult Wistar Rat at (A) x150 and (B) x600 magnifications respectively.
BC= Blood Clots; EN= Endotheliocytes; PVE= Perivascular oedema; pEN: Polymorphic Endotheliocytes; Lu= Lumen



Plate III A&B: H&E photomicrograph of cerebral vascular architecture exposed to 11.25mg/m3 in Adult Wistar Rat at x150 and x600 magnification respectively.

BV= Blood vessel PVE= Perivascular oedema;

EN= Endotheliocytes; APnu= Apoptotic nuclei; Lu= Lumen



Plate IV A&B: H&E photomicrographs cerebral vascular architecture exposed to 7.50mg/m3 dichlorvos at (A) x150 and (B) x600 magnifications respectively.

BV= Blood Vessel; pEN= Polymorphic Endotheliocytes; Lu= Lumen



Plate VA&B: H&E photomicrograph of cerebral vascular architecture exposed to 3.75 mg/m3 dichlorvos in Adult Wistar Rat at (A) x150 and (B) x600 magnifications respectively.
BM= Basement Membrane; BV= Blood Vessel; PVE= Perivascular oedema; EN= Endotheliocytes; APnu= Apoptotic nuclei; Lu= Lumen

The results in plate IA showed normal photomicrograph of cerebral vascular architecture exposed to ambient air as control. The cerebral blood vessel (BV) in this group featured squamous endotheliocytes (SE) at lower (150x) magnification lying peripherally on intact basement membrane (BM). At higher magnification (600x) in plate 1B, the normal blood vessels showed vascular lumen consisting of the remains of the blood clots (BC). The basement membrane (BM) was circumferentially lined with the squamous endotheliocytes (SE) providing lumen (Lu) studded with blood clots (BC). The photomicrograph of cerebral vessels in plate IIA was a histoarchitecture of the animals exposed to 2.5 mg/m3 ethanol as negative control group. The plate showed a highly circumscribed perivascular oedematous zone (PVE) around the blood vessels with the squamous endotheliocytes (EN) located peripherally on the circular basement membrane (BM) which formed the lumen (Lu) of the capillaries at lower magnification. At higher resolution (plate IIB), the photomicrograph showed the high circumferentially perivascular edematous (PVE) zone around the blood vessels. The lumen was however studded with the remnant of blood clots while basement membrane (BM) was lined by polymorphic endotheliocytes (pEN). In plate IIIA the photomicrograph of 11.25 mg/m3dichlorvos tested group at lower magnification showed mildly surrounded perivascular oedematous (PVE) zone with the endotheliocytes (EN) of some vessels bearing mild apoptotic (APnu) tendencies. The endotheliocytes were generally scanty. At higher magnification (plate IIIB), the blood vessels featured pronounced PVE with the basement membranes bearing very scanty or no endotheliocytes. The photomicrograph of the endotheliocytes exposed to 7.5 mg/m3dichlorvos at lower magnification was presented in plate IVA. The plate showed the vessels containing scanty endotheliocytes surrounded by mild perivascular edema (PVE) on the basement membrane (BM). At higher resolution (plate IVB), the endotheliocytes were virtually absent in the lining of the basement membrane. Plate VA was a photomicrograph of cerebral blood vessels of adult wistar rat exposed to 3.75 mg/ m3 at lower magnification. The endotheliocytes were absent in some of the blood vessels whereas others contain scanty and were surrounded by perivascular edematous zone (PVE). At higher magnification VB, the blood vessels still showed perivascular oedematous zone around the blood vessels with collapsed basement membrane (BM) bearing apoptotic endotheliocytes.

4. Discussion

The results in table 1, showed variable degrees in the distribution of the brain weights following treatment with graded doses of dichlorvos solutions in exposure chambers. It is evident that the control groups had the highest mean±SEM of 1.90±0.09 while groups II & IV had the least distributions of 1.76±0.04 and 1.76±0.05 respectively. The ANOVA test however showed no statistically significant difference in the mean brain weights [F= 0.88, p = 0.49]. These findings contradicted Sarin and Gill (1998) and Zelman (1977) in related dichlorvos neurotoxicity study following oral treatment where it found significant inhibition of metabolic enzymes were such as hexokinase, phosphofructokinase, glutamate lactate dehydrogenase and dehydrogenase which depleted brain glycogen essential ingredient necessary for myelination and regeneration of axons. This variation could probably be due to difference in the route of exposure, concentration and setting of the experiments.

The photomicrograph of group I animals that were exposed to ambient air as control showed normal histology of cerebral blood vessels cerebral (BV) indicating squamous endothelicytes (EN) arranged peripherally on basement membrane (BM). It also showed a central vascular lumen containing the remains of the blood clots (BC). There were noticeable histologic changes of the vascular endotheliocytes in group II the ethanol exposed (negative control) group, in which the endotheliocytes on the blood vessels (BV) presented marked perivascular oedematous zone (PVE) around the blood vessels. The vascular endotheliocytes were also surrounded by polymorphic (pEN) endotheliocytes with some of them knocked out of the basement membrane (BM) lining thereby distorting the arrangements. This result contradicted the finding of Phillips (1986) which observed that the endothelial cells of brain blood vessels in rats breathing after continuous ethanol vapor during 3 weeks were normal, which in accordance to the present study, there was histopathological alterations in the endothelium when compared to controls. The photomicrograph of the VB in group III was presented in plate 3. The histoarchitechture showed perivascular edematous zone around the blood vessels (PVE). The basement membrane (BM) also collapsed with the lumen surrounded by haphazard squamous endotheliocytes that partly blocked it. The endotheliocytes also showed mild apoptotic tendencies. Similar results were obtained in group IV and V except that in group IV the lumen was circumferentially collapsed with partly ruptured basement membrane surrounded by scanty amorphous endotheliocytes in a perivascular However in group V the blood vessels edema. maintained the lumen of the blood vessel devoid of the endotheliocytes on the basement membrane surrounded by slight a perivascular edema. In a similar study conducted by Muthuviveganandavelet al., (2011) found that perivascular oedema and congestion of cerebral blood vessels in Albino rats following oral exposure to pyrethroids organophosphates. In a similar studies conducted by Owoeyeet al., (2014) and Sharma and Singh (2012) reported presence of apoptotic changes in pyramidal neurons of the CA1 and CA3 subfields and blood vessels caused by oxidative damage induced by dichlorvos. Similarly, Binukumar and Gill (2010) also indicated that decreased mitochondrial electron transfer activities of cytochrome oxidase (complex IV) along with altered mitochondrial complex I, and complex II activity, which might have resulted from elevated mitochondria calcium uptake might have caused an increase in malondialdehyde, protein carbonyl and 8-hydoxydeoxyguanosine formation which as a result enhances lipid peroxidation as well as protein and mitochondrial DNA oxidation that could lead to DNA fragmentation and cellular apoptosis.

5. Conclusion

Dichlorvos is the active ingredient of the locally formulated pesticide-cum-insecticide popularly known as Madararpiapia or Ota piapia. Prolong use of this chemical by constant exposure through inhalation caused change in the histoarchitecture of the cerebral blood vessels through the formation perivascular oedema and apoptosis of the endotheliocytes. Many diluents were available for use in preparation of local dichlorvos preparation; care has to be taken in the right choice of the diluents to avoid harmful agents that can affect the histology of the cerebral blood vessels. It is therefore recommendable that the National Agency for Food Drug Administration and Control (NAFDAC) should ensure strict obedience in the use of dichlorvos.

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