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Changes in Electrolyte Status and Neuronal Cell Distortions in Rats Fed with Dichlorvos Treated Beans Diets.

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ABSTRACT

In recent times, there have been research works on health hazards associated with the use of chemicals for preservation of food meant for human and animal consumption. This work assessed changes in electrolyte status and neuronal cell distortions in Wistar rats fed with dichlorvos treated beans diets after six months of application. Forty kilograms of freshly harvested beans samples were grouped into four; I, II, III and IV. Group I is the control while groups II – IV were divided into three sub-groups each and labeled as IIa, IIb, IIc, IIId,IIIe IIIf, IVg, IVh and IVi and treated with different doses of dichlorvos as follows; IIa, IIId and IVg (-high dose/8mL/4kg), IIb, IIIe and IVf (medium dose 4mL/4kg) and IIc,IIIf and IVi (low dose 2mL/4kg). Group III samples were parboiled while group IV contained a ratio of 1:1 mixture of both parboiled and un-parboiled beans. All the samples were then stored for six months thereafter ground to powdered form using a blender. The rats were euthanized by mild chloroform inhalation. Blood sample was collected by cardiac puncture and analyzed for serum potassium (K^+) sodium (Na^+)and chloride (Cl^-) ions using standard methods. The harvested brain tissue was subjected to histological studies. The result showed significant increases in values of K^+, Na^+, Cl^- in groups II and IV. Photomicrographs of the brain tissue revealed various degrees of dose dependent neuronal cell distortions such as moderate neuronal cell atrophy, increased cytoplasmic eosinophilia, shrinkage and loss of nuclear features. Findings in group III revealed no histological derangement in the brain tissue except for non - significant increase ($p > 0.05$) in the serum electrolytes levels.

KeyWords: Atrophy, dichlorvos, electrolyte, eosinophilia, neuronal cell distortion, parboiled, un-parboiled.

1. INTRODUCTION

The prevalence of insecticide infested food toxicity has raised serious public health concerns especially in developing countries where patronage is most predominant (Okoroiwu and Iwara, 2018). Though pesticides are of immense use in agriculture, forestry/horticulture and industry, most pesticides cause severe health challenges to humans, fishes and other animals (Kandasamy, *et al*., 2023). In most developing countries, regulatory policies on the indiscriminate use of pesticide are poor or not effectively enforced. Consequently toxic and persistent pesticides that have been banned in developed countries are still commonly being used in developing countries. Pesticide residues make food commodities hazardous for human consumption, export and also pollute the environment (WHO 2022). Food substances containing low level of pesticide residues consumed by humans over time may lead to serious health hazards such as, cancer, genetic damage and suppression of immune system (Okoroiwu and Iwara, 2018), congenital malformations, neurotoxic disorders, infertility, blood dyscrasias, and many others (Ogah *et al.*, 2012).

Food sources such as cowpea) have been studied to show high content of pesticide residues (Yusuf *et al* 2018) Beans are a major source of protein especially in developing world where other sources of protein are generally too expensive for the larger population. In Africa, it is amongst the topmost consumed food. In Eastern and Southern Africa it is the second most important source of dietary protein (Graham *et al.*, 2003*)* while in Nigeria it is ranked as the fourth most patronized food after cassava, yam and rice (Allen *et al*., 2023). It is consumed in different forms as beans porridge,*´akara´,´moi-moi´* etc. for its richness in amino acids such as lysine, tryptophan, minerals such as iron, calcium, manganese, copper and zinc, beneficial phytochemicals and antioxidants (Kris, 2023). The leaves and tendrils are also used for the production of various animal feeds (Sousa, 2019).

Beans kept for seeds can be treated with insecticides and then kept safely for longer period of time. In Nigeria one of the insecticides currently being used massively for preservation of beans is dimethyl 2,2-dichlorovinyl phosphate –DDVP- or dichlorvos and locally known as sniper or *otapia-pia* (Karigidi 2018). Its commercial availability spans over six decades being traded under different trade names such as: Sniper 100EC, DDVP, Vapona, Nuvan 76% EC, Lava 77% EC, Nogos, Phosvit (Mohammed, 2020).

Several studies have been conducted to assess toxic effects of dichlorvos on organisms in the environment using indices including mortality, immobilization and growth inhibition. In humans and experimental animals, dichlorvos is rapidly absorbed through the gastrointestinal and respiratory tracts and skin, and metabolized by esterase that exist in most tissues (Okoroiwu and Iwara, 2018). Dichlorvos exposed to rats is distributed in the kidney and adipose tissue at relatively high concentrations while dichlorvos orally administered to pregnant rabbits transfers to fetuses in a short time (CERI, 2007). Dichlorvos is mainly metabolized by esterase to dimethyl phosphate and dichloroacetaldehyde which are excreted in the urine. Dichloroacetaldehyde is rapidly metabolized via two pathways to dichloroethanol glucuronide, hippuric acid, urea and carbon dioxide, and excreted in the urine and by expiration (CERI, 2007).

Exposure to dichlorvos have been shown by different authors to be neurotoxic, causes inhibition of neuropathy target esterase (NTF) and affect the electrolyte balance in humans and animals alike with severe consequences (Izah *et al*., 2020, Owunari *et al*., 2021).

This study was therefore aimed at evaluating the electrolyte status and neuronal cell distortion in rats fed with dichlorvos treated beans diets after six months of application.

2. MATERIALS AND METHODS

2.1 Collection and Treatment of Beans

Forty kilograms (40kg) of dry beans samples (Phaseolus vulgaris) was obtained directly from farmers and used for the research. The sample was properly cleaned by carefully picking out the stones and other farm debris. The cleaned sample was then packaged into ten different air tight containers of 4kg each and labelled: Group I (control); Groups IIa, IIb, IIc, IIId, IIIe, IIIf, IVg, IVh and IVi.

2.2 Procurement of Dichlorvos

Dichlorvos was procured from a reputable agrochemical store in Makurdi, Benue State and mixed with the beans according to the specification below.

Group **I-** control group; no treatment with dichorvos

Groups IIa ,IIId and IVg –high dose (8mL of dichlorvos/4kg of beans)

Group IIb,IIIe and IVh –medium dose (4mL dichlorvos/4kg of beans)

Group IIc, IIIe and IVi- low dose (2mL of dichlorvos/4kg of beans)

All group **II** samples were un-parboiled, group **III** samples were parboiled while group **IV** samples consisted of an equal mixture of un-parboiled and parboiled beans samples**.**

2.3 Albino Wistar Rats

Fifty (50) albino Wistar rats were purchased from and housed in the Animal House Laboratory, College of Health Sciences, Benue State University Makurdi. They were acclimatized for 2 weeks prior to the experiments. The animals were fed with normal rat feed and water *ad 'libitum* in a properly ventilated room under standard laboratory conditions.

The rats were grouped into ten different groups and fed with different feed preparations as indicated in table 1. All the groups contained five rats each.

Table 1. Rats allocation per feed treatment regimen

Group	No. of rats	Feed Treatment Plan	Dichlorvos dose level*
I	5	Normal feed + untreated beans diet	None
II		Normal rat feed + treated un-parboiled beans	
IIa	5	NRF+ TUBD	- High
IIb		NRF + TUBD	- Medium

IIc	5	NRF+TUBD	- Low
III		Normal rat feed + dichlorvos treated parboiled beans diet	
IIId	5	NRF + TPBD-	-High
IIIe	5	NRF+TPBD	- Medium
IIIf	5	NRF +TPBD	-Low
IV		Normal rat feed + treated un-parboiled beans diet + dichlorvos treated parboiled beans diet	
IVg	5	NRF+ TUBD + TPBD	-High
IVh	5	NRF+ TUBD + TPBD	-Medium
IVi	5	NRF+ TUBD + TPBD	-Low

**** Key*:**

High dose =	8mL sniper/4kg beans
Medium dose =	4mL sniper/4kg beans
Low dose =	2mL sniper/4kg beans
NRF-	**Normal rat feed**
TUBD-	**Treated un-parboiled beans diet**
TUBD-	**Treated parboiled beans diet**

After four (4) weeks of treatment with the various feed compositions, the rats were euthanized with a little quantity of chloroform and sacrificed. Blood sample (5mL) was collected by cardiac puncture using a Vacotena, a sterile, pyrogen free non-toxic Maxicom Multiple Sample Needle. One(1mL) out of the 5mL collected was quickly transferred to sterile EDTA-K3 Vacuum tube and used for haematological analysis while the remaining 4mL was transferred into heparinized bottles and centrifuged at 4000rpm for 5 minutes using Centrifuge Model 80-2, Lenfield Medical England. The supernatant containing the plasma was carefully siphoned into plain bottles using a 5mL syringe and kept for biochemical analysis. The brain was harvested, preserved in 10% formalin, kept in a refrigerator and used for evaluation of any neuronal cell distortions.

2.4 Statistical analysis.

Data was presented as mean value ± standard deviation. Analysis of variance (ANOVA) was used for multiple factor analysis. Duncan's multiple range test at $P < 0.05$ was adopted for analysis of differences between means that are statistically significant.

3. RESULTS

3.1 Serum electrolytes

The results of effects of dichlorvos treated beans diet on some serum electrolytes (K, Na, Cl, iCa and TCa) of albino Wistar rats presented in Figures 1 – 5

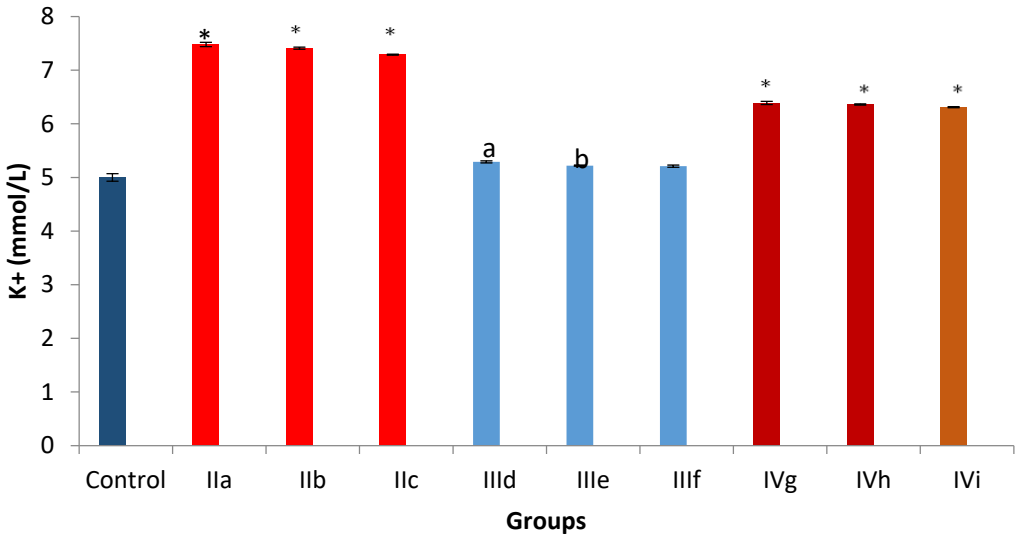

Figure 1: Effect of dichlorvos treated beans diets on plasma K^+ in albino Wistar rats

N = 5, *= significant relative to control at $P < 0.05$, a = significant relative to a at $P < 0.05$, b = significant relative to b at $P < 0.05$.

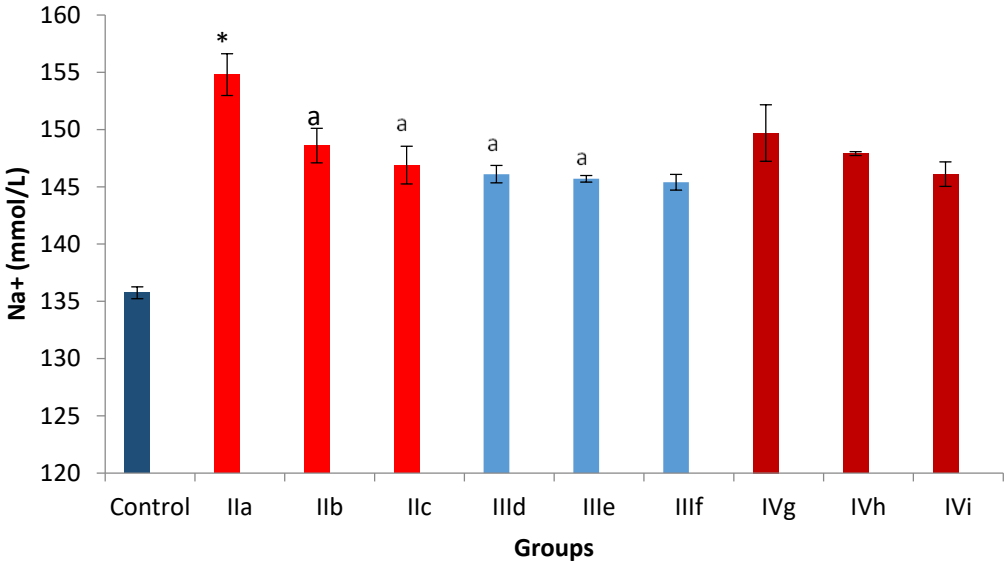

Figure 2: Effect of dichlorvos treated beans diets on plasma Na^+ in albino Wistar rats

N = 5, *= significant relative to control at P < 0.05, a = significant relative to a at P < 0.05, b = significant relative to b at P < 0.05.

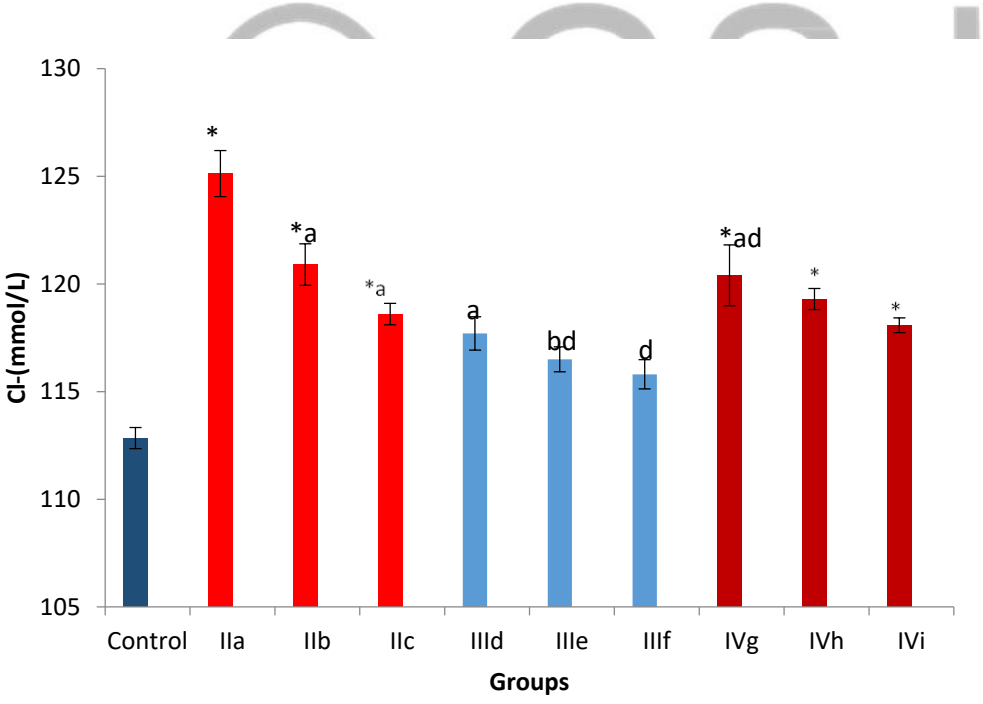

Figure 3: Effect of dichlorvos treated beans diets on plasma Cl^- in albino Wistar rats

N = 5, *= significant relative to control at P < 0.05, a = significant relative to a at P < 0.05, b = significant relative to b at P < 0.05, d = significant relative to d at P < 0.05.

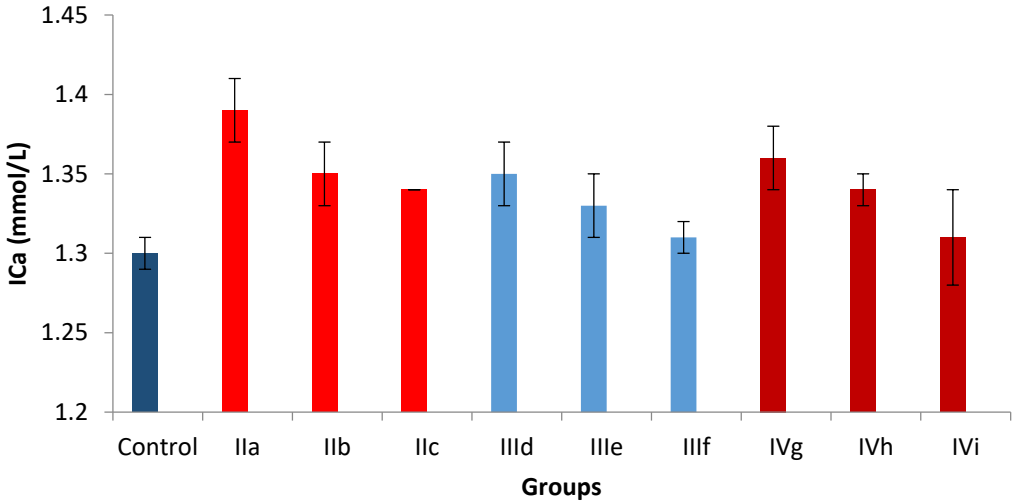

Figure 4: Effect of dichlorvos treated beans diets on ionized calcium (iCa^{2+}) in albino Wistar rats

N = 5, *= significant relative to control at P < 0.05, a = significant relative to a at P < 0.05, b = significant relative to b at P < 0.05, d = significant relative to d at P < 0.05.

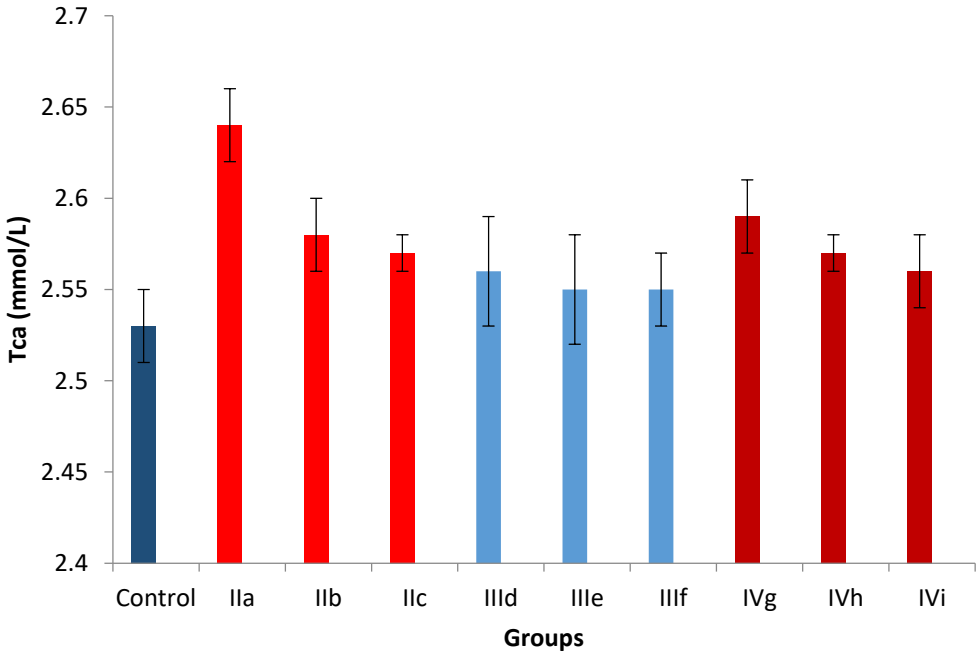

Figure 5: Effect of dichlorvos treated beans diets on TCa^{2+} in albino Wistar rats

N = 5, *= significant relative to control at P < 0.05, a = significant relative to a at P < 0.05, b = significant relative to b at P < 0.05, d = significant relative to d at P < 0.05.

3.2 Histopathological analysis

Photomicrographs of the brain obtained from albino Wistar rats across ten different experimental groups were examined for histological changes and the results presented in plates 1-10.

Group 1-Control

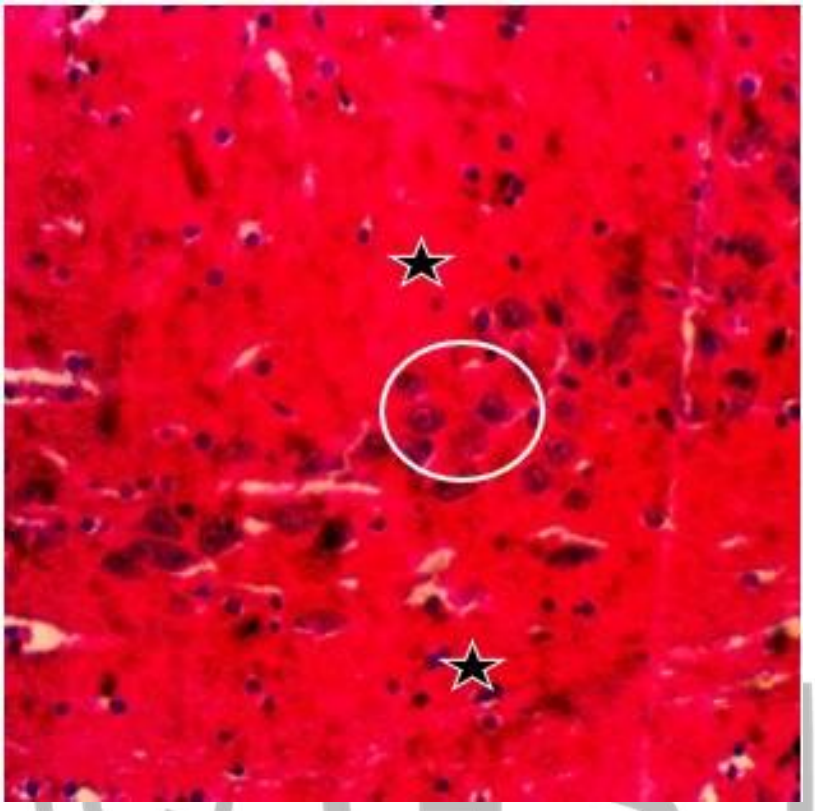

Plate 1: Brain photomicrograph shows sections of the cerebrum with presence of neuroglia cells (black star) and neuronal cell bodies of the grey matter (white circle). There is no observable lesion.

Group IIa: High dose of dichlorvos

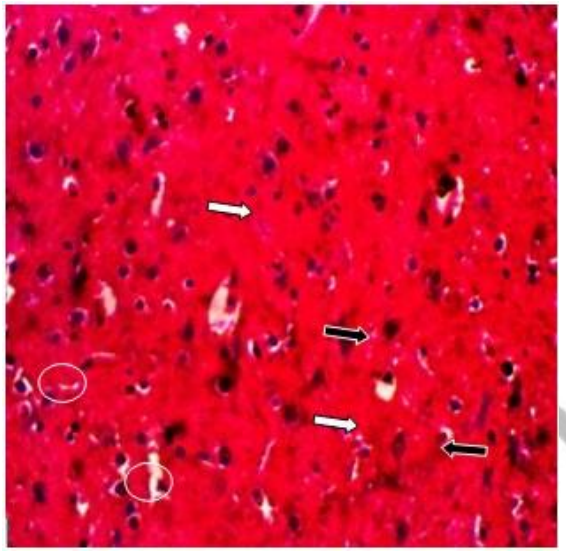

Plate 2: Brain photomicrograph shows moderate neuronal atrophy (black arrow) and microglia cell proliferation (white arrows) and oligodendroglia cells with larger nuclei and perinuclear hallow (white circle). *Hematoxylin & eosin stain x400*

Group IIb: Medium dose of dichlorvos

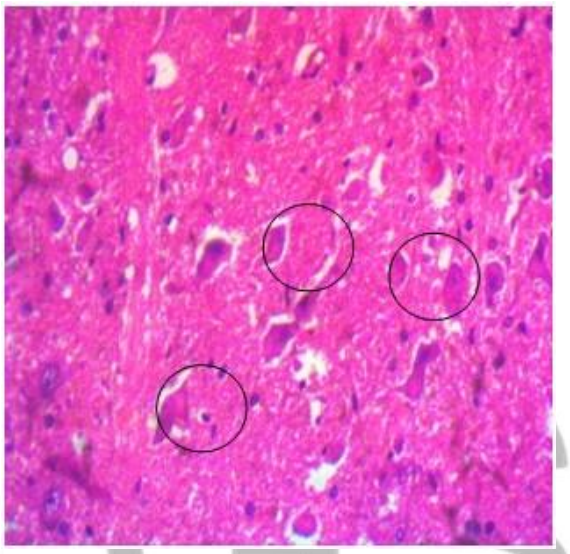

Plate 3: Brain photomicrograph shows cerebral neuron cell bodies acute injury changes (red neuron) with increased cytoplasmic eosinophilia, shrinkage and loss of nuclear features (black circle). *Hematoxylin & eosin stain, x 400*

Group IIc: Low dose of dichlorvos

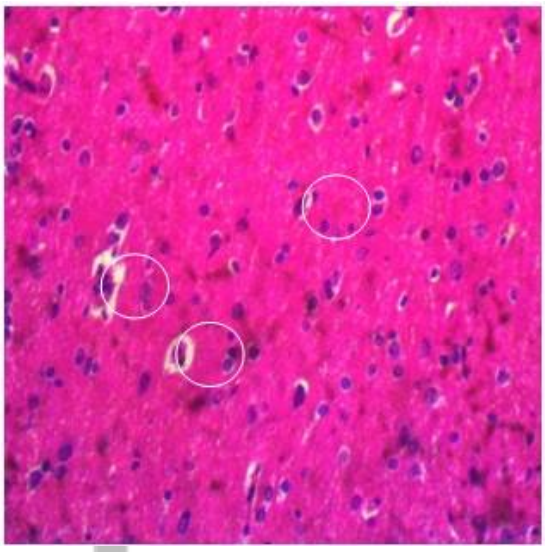

Plate 4: Brain photomicrograph show cerebral neuron cell bodies exhibiting features of degenerative changes with nuclear shrinkage and increased cytoplasmic eosinophilia (white circle). *Hematoxylin & eosin stain, x 400*

Group IIIe – Medium dose of dichlorvos

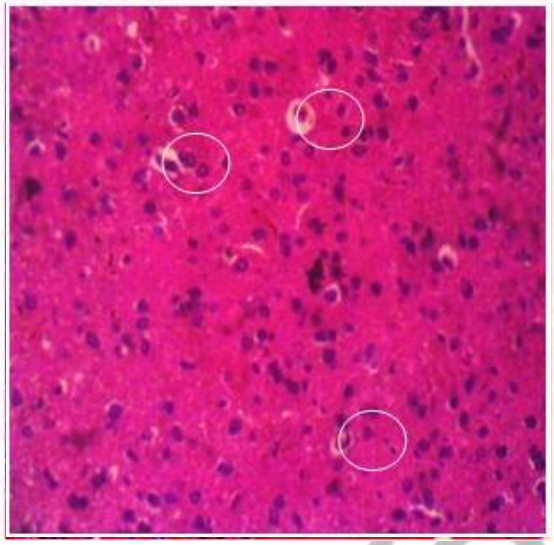

Plate 5: Purkinje fibres of the cerebellum are seen mid-field to the right of the granule cell layer with small dark nuclei (white star). There is no observable lesion. *Hematoxylin & eosin stain x400*

Group IIIe – Medium dose of dichlorvos

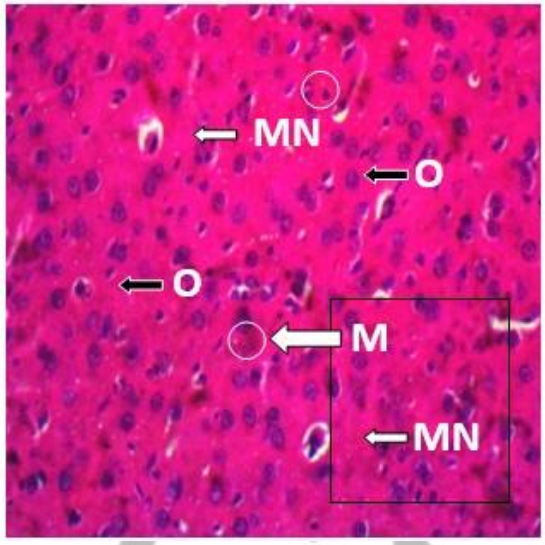

Plate 6: Brain photomicrograph shows the grey mater consisting of the pinkish neutrophils and a combination of cells. These include large cell bodies of motor neurons (white arrows), oligodendroglia (black arrows) and microglia nuclei (enclosed in white circles). MN = motor neuron cell body, O = oligodendroglia, M = microglia nuclei. There is no observable lesion.

Group IIIf– Low dose of dichlorvos

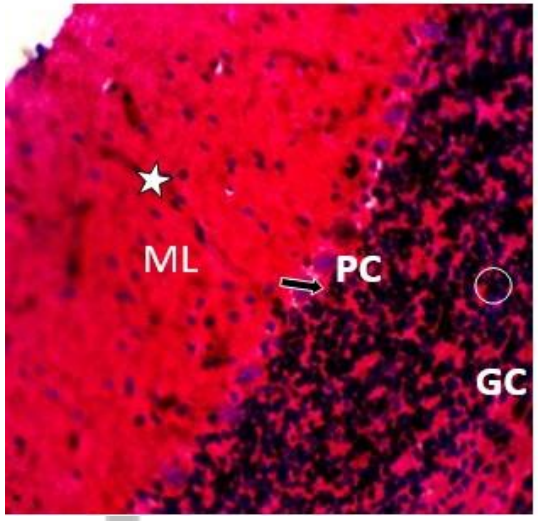

Plate 7: Cerebellum photomicrograph shows the three layers of a normal cerebellar cortex which include the outer molecular layer (white star), middle layer of Purkinje cells which have large nuclei (black arrow) and inner granular cell layer of densely packed neurons with small dark nuclei (white circle). ML = Molecular layer, PC = Purkinje cells, GC = Granule cells. There is no observable lesion present. *Hematoxylin & eosin stain x400*

Group IVg – High dose of dichlorvos

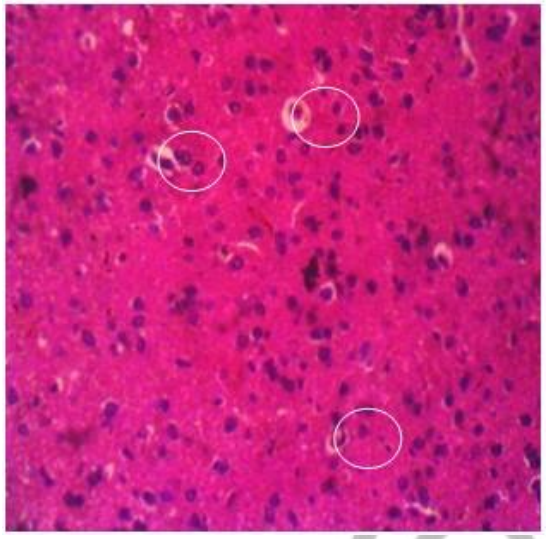

Plate 8: Brain photomicrograph shows cerebral neuron cell bodies exhibiting features of degenerative changes with nuclear shrinkage and increased cytoplasmic eosinophilia (white circle). *Hematoxylin & eosin stain, x 400*

Group IVg – High dose of dichlorvos

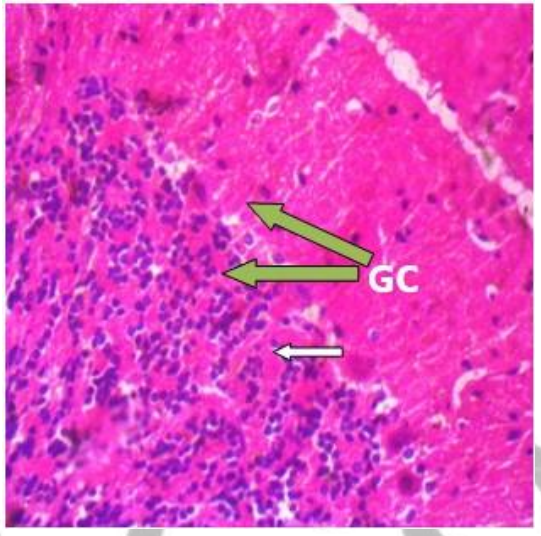

Plate 9: The cerebellum shows relative loss in the population of the granule cells. The intervening spaces are filled with homogeneous eosinophilic material (White arrow). GC = Granule cell. *Hematoxylin & eosin stain, x 400*

Group IVg – High dose of dichlorvos

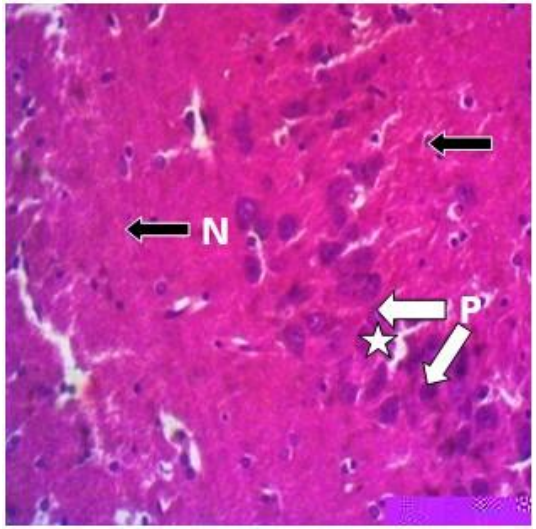

Plate 10: Brain photomicrographs show a band of large pyramidal cells (white star), surrounding neuroglia cells (black arrows). N = Neuroglia, P = Pyramidal cell. There is no observable lesion. *Hematoxylin & eosin stain, x 400*

4. DISCUSSION

Evaluation of serum electrolyte profile has been an important tool in the assessment of the patho-physiological state of patients. Electrolytes are found in tissues such as blood, urine and other body fluids. They function mainly for the maintenance of water balance, acid/base (pH) level and movement of nutrients and wastes in and out of cells (Gbakon *et al.*, 2018).

The result shown in figures 1-5 revealed increased levels of all the electrolytes investigated in all the Wistar rats exposed to dichlorvos as compared to the control (group 1). This result is in consonance with the work of Owunari and Chika (2021) where administration of 4mg/kg of dichlorvos was reported to have provoked increased electrolyte concentrations in Wistar rats. Desai and Desai (2008) reported a dose dependent elevation in renal content of Na^{+}, Ca^{++}, K^{+} and Cl^{-} in rats while Almalihi (2016) demonstrated increased Ca^{++} levels in rabbits exposed to dichlorvos. The kidney is the organ responsible for the maintenance of electrolyte stability or balance in man and other animals. A defective kidney will therefore lose its ability to properly maintain the electrolyte balance. A body of compiling scientific evidence demonstrates that dichlorvos has toxic effect on the functional status of the kidney (Nwaichi *et al*., 2018, Hart *et al*., 2022). The results of this study showed that there was significant increase in electrolyte levels in rats exposed to doses of dichlorvos with consequent impairment in renal functions. Electrolyte imbalance can also be caused by other factors such as fluid loss, medications such as diuretics, heart and lung disorders, cancer, hyperthyroidism, severe dehydration, muscle contraction, alcohol use, malnutrition, tumor lysis syndrome among many others. The result obtained in this study showed a dose dependent behaviour with the groups exposed to higher levels of dichlorvos having more elevated electrolyte values. Almalihi (2016) reported the work of other researchers where hypercalcemia (Ca^{++}) was recorded at a later stage of exposure of Wistar rats to dichlorvos. Dichlorvos was also reported to have caused a significant elevation in the intra-synaptosomal calcium level, altered the transcript abundance of calcium storage and signaling protein pathways, thus interfering with calcium homeostasis. The hyperkalemia (K^{+}) observed in this work as presented in figure 1 was significant ($p<0.05$) in groups IIa, IIb, IVg and IVh while hypernatremia (Na^{+}) shown in figure 2 was significant only in group IIa relative to control group. Significant hyperchloremia (Cl^{-}) was noted in groups II and group IV (figure 3) while the hypercalcemia (Ca^{++}) as represented by ionized and total calcium (iCa and TCa) was not significant in all the groups relative to the control group. Elevated electrolyte values as recorded in this study can lead to illnesses such as myocardial dysfunction, fatique, muscle cramps, vomiting, oedema, dizziness, constipation, general collapse of normal body functions which may ultimately be life threatening etc (Shrimanker and Bhattarai, 2023).

No histopathological changes nor observable neuronal cell lesions were observed in the brain cells of the control group as shown in the photomicrographs (plate 1). This is however not the same for group II and sub-groups IVg and IVh where some degree of distortions in the brain cell architecture was observed due to exposure to dichlorvos. Abnormalities such as neuronal cell atrophy, shrinkage and loss of nuclear features, degenerative and increased cytoplasmic eosinophilia, relative loss in population of granule cells and acute injury changes were noticed in the photomicrographs of brain cells of groups II and IV. This finding is consistent with the work of Xiao, *et al.*, (2020) where pathological examination of brain cells of chicken exposed to

dichlorvos showed nuclear disintegration of neurons (at low dose), brain cell apoptosis (at high dose) and general destruction of the mitochondrial architecture at both dose levels. Huang *et al.*, (2021) revealed the inducement of edema, abnormal expression of glial fibrillary acidic protein (GFAP) and neuronal mitochondrial damage in broilers exposed to dichlorvos. Their findings provided useful information on the molecular mechanism of brain tissue responses to DDVP and neurodegenerative diseases caused by acute DDVP exposure. Histological findings from brain tissues of rats exposed to dichlorvos showed abnormal ultra-structural changes, nuclear vacuolization and lymphatic inflammatory changes (Huang *et al.*, 2021).

The neurodegenerative abnormalities observed in this work may be associated with a possible increase in free radicals in the brain cells occasioned by the exposure of the experimental animals to dichlorvos. Free radicals are potent pro-oxidants (Chen *et al.*, 2008). Through the process of lipid peroxidation, DDVP with high affinity for fatty acid compositions oxidatively damages polyunsaturated fatty acid (PUFA) rich cells thus leading to pathogenesis (Mittal and Flora, 2006). The result obtained here showed a dose dependent distortion of neuronal cells by dichlorvos. Group II which contained treated but un-parboiled beans diet displayed more severe brain cell damages than groups III and IV. Group III which contained the least dichlorvos dose as a result of parboiling, showed the least severity in the brain cell damage. The observation above is in line with the work of Xiao *et al.*, (2020).

The functions of the brain is closely related to electrolyte status. Electrolyte imbalance can lead to neurologic manifestations such as swelling, shrinkage and seizures (Diringer, 2017). This is demonstrated in this work, where there is a correlation between the level of electrolytes and corresponding neuronal cell distortion.

5 Conclusion

The results obtained for changes in electrolytes status and neuronal cell compositions in rats exposed to dichlorvos showed that six months after application, DDVP residues in the beans diets still posed serious health risks to the experimental animals. This is evidenced by the elevated electrolytes levels and various neuronal cell distortions recorded in this research. The results revealed a dose dependent behaviour to dichlorvos. Parboiling of the treated beans before storage showed an appreciable reduction in the deleterious effect of DDVP, however it was not sufficient to declare the diet safe for consumption. Information obtained from this work can be used to educate farmers, marketers and consumers about the dangers associated with dichlorvos treated beans diets.

6 Acknowledgment

We appreciate the Animal House and Biochemistry Laboratory, College of Health Sciences and the Teaching Hospital, Benue State University, Makurdi for providing the space and equipment used in carrying out this work. We equally acknowledge the technical expertise of all the technical staff and every other person involved in the successful completion of this work.

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