Original Research Article

HISTOLOGICAL AND HEMATOLOICAL ASSESSMENT OF THE EFFECT OF HEINSIA CRINATA EXTRACT ON DICHLORVOS (DDVP) INDUCED TOXICITY IN ADULT WISTAR RATS

Abstract

The aim of this study was to assess the "histological and hematological effect of *heinsia crinata* extract on Dichlorvos (DDVP) Induced Toxicity on liver of adult wistar rats,Thirty (30) adult male Wistar rats were randomly assigned into five groups with group A being the control. Each group contained 6 rats which was given food and water alongside the administration for a period of 21 days which was done orally after acclimatization. Group B was administered orally 35mg//kg DDVP only, group C was administered 35mg/kg DDVP and 500mg/kg *Heinsia crinita* extract, group D was administered 35mg/kg DDVP and 250mg/kg *Heinsia crinita* while group E was administered *Heinsia crinita* extract only. All rats were sacrificed at the end of the 21days and blood was collected by cardiac puncture for hematological analysis and their Livers harvest, processed and stained with Hematoxylin and Eosin for Histopathological studies. Result show PCV showed no significant change in group B, & E ($P > 0.05$) and was significantly changed in group D ($P < 0.05$) compared to the control. Hb values showed no significant change amongst the groups ($P > 0.05$) as compared to the control. No significant changes were observed for WBC in group C, D & E ($P > 0.05$) as compared to the control but group B was significantly changed ($P < 0.05$). MCH did not show any significant change across the groups as compared to the control however MCV and MCHC had a significant change in group D while MCHC also had a significant change in group C. Histologically Dichlorvos induce hepatoxicity and inflammation of liver, characterized by the presence of Kupffer's cells, congestion, necrosis and fatty infiltration. It also show that *heinsia crinata* has no effect on Dichlorvos induced toxicity.

Keywords

Heinsia crinata,Dichlorvos,Hepatotoxicity,Hematological, histological, toxixcity

Introducion

Dichlorvos which is popularly called "Otapiapia" in Nigerian vernacular is a locally formulated unspecified insecticide and/or pesticide that has the ability to cause paralysis, dizziness, ataxia, convulsions, hypotension (low blood pressure), cardiac arrhythmias and sometimes death (Umar *et al.*, 2010; FAO, 2001;Akunyi, 2007).

Otapiapia is a vernacular, which literally means "that which completely kills or consumes," as the local producers emphasized that it is highly potent and completely wipes out pests (Umar *et al*., 2010). It is applied indiscriminately as a household repellant, on crops, stored dry fish, foodstuffs, and in outdoor areas. It also serves as an acaricide, rodenticide and as anthelmintic in dogs, pigs and as a botacide in horses (USEPA, 1994). This practice is dangerous because the concentration and constituent of Otapiapia is unknown. However, it has been reported that Otapiapia contains dichlorvos (2, 2, dichlorovinyl dimethyl phosphate) and it is an organophosphate insecticide used in various insecticide formulation in Nigeria (FAO, 2001; PANN, 2007).

There have been several studies on how dichlorvos toxicity can be assessed. Example of such studies includes; a study on dichlorvos induced toxicity on haematology, clinical biochemistry and reproductive abnormalities in male albino rats (Onyinyechukwu *et al*., 2017)and a host of other studies.

A brief description of *Heinsia crinita* indicates that it has medicinal values. This plant (*Heinsia crinita)* is popularly called **'atama'** leaf in Southern Nigeria. It is a vegetable that can be found in Cross-Rivers and Akwa-Ibom state. The *Heinsia crinita* is a member of a plant family known as Rubiaceae. This plant family are basically tropical and are found in largely temperate regions. The physical look of the *Heinsia crinita* leave is recognized by the usually inferior ovary, regular corolla, separate anthers andstipulate simple entire leaves. The *Heinsia crinita* leave can be used for several purposes in Southern Nigeria but its major purpose is vegetable for food. The *Heinsia crinita* plant has a high level of alkaloid and cardiac glycosides which are the most essential and active ingredients of plants in West Africa. These components are associated with antimicrobial activity among the components of medicinal plants.

Other contents of the plant are; anthranoids, anthraquinones, saponins and tannins in trace concentration. Findings have also indicated that it contains an antidiabetic, antihypertensive and hypoglycemic active ingredient (Ajibesin *et al*., 2008; Okokon *et al*., 2009). As a result of this, its use has grown beyond been that of food source to that of medicinal substance. Indeed, its use by trado-medical personnel's in the treatment of various ailments is well known. Dichlorvos is one of the most used in the developing countries (Deka and Mahanta, 2015), and has been reported to be the cause of severe poisoning and death associated with most organophosphate products used in these nations (Musa *et al*., 2010; Brown *et al*., 2015). The search for a treatment and antidote for dichlorvos remains a challenge for scientists over the years. Experimentation on it with different medicinal and herbal substances is ongoing to determine which is more effective as a means of treatment or antidote.

The main aim of this study is evaluate the histologic effect of *Heinsia crinata* (Atama) extract on Dichlorvos induced toxicity in the Liver of adult Waster Rats and the effect *Heinsia crinata* (Atama) extract on Dichlorvos induced toxicity on haematoloical parameters.

MATERIALS AND METHODS

Experimental Design

The animals were weighed and divided into five groups. The duration of this study was for twenty-eight (21) days, the animals were allowed to acclimatize for two weeks. After the acclimatization period, thirty (30) young male rats were randomly divided into five groups A, B, C, D, E (6 rats in group A, B, C, D & E). Group A (Positive Control): Rats were administered orally with pelleted growers mash (feed) and water throughout the experiment (21days). Group B (Positive): Rats were administered orally with Dichlorvos (35mg/kg body weight) and given pelleted

growers mashes(feed) and water for 21days, half. Group C: Rats were administered orally with Dichlorvos (35mg/kg body weight) for 21days, and then given orally 200g/kg of *Heinsia crinita* extract 200g/kg body weight, pelleted growers mash (feed) and water throughout the experiment (21days). Group D: Rats were administered orally with 200g/kg of *Heinsia crinita* extract for 21days and administered orally with Dichlorvos (35mg/kg), and they were also given pelleted growers mash(feed) and water throughout the experiment (21days). Group E: Rats were administered orally with 200g/kg of *Heinsia crinita* extract and given pelleted growers mash and water for twenty-one days (21days).

Chart 1. Experimental Design

Group A (Control)	Group B	Group C	Group D	Group E
Feed and water only	Dichlorvos(35mg/kg) + feed and water	Dichlorvos(35mg/kg) + *Heinsia crinita* extract (500mg/kg) + feed and water	Dichlorvos(35mg/kg) + *Heinsia crinita* extract (250mg/kg) + feed and water	*Heinsia crinita* extract(500mg/kg) + feed and water

Experimental Design Area

This study was carried out in the Department of Medical Laboratory Science of Basic Medical Sciences, College of Health Sciences, Niger-Delta University, Wilberforce Island Amassoma, Bayelsa State of Nigeria.

Procurement of *Heinsia crinita* Leaf

Heinsia crinita leaf (200g) was purchased from Amassoma main market haven been harvested from it natural habitat, Amassoma, Bayelsa state used for this study.

Animal Housing

Thirty (30) adult wistar rats weighing between 120 to 150g were used for this study. These animals were obtained from the animal house of Pharmacology Department of Niger-Delta University, Bayelsa state, Nigeria. They were housed under standard condition of temperature (27± 20C) with twelve hours light and dark periodicity. These animals were housed in clean gauzed cages in groups and fed on standard feed pellets (Guinea feed Nigeria Plc) and clean water ad libitum throughout the duration of the study. ACC limitation was for two weeks. Animals were handled in the study according to institutions guidelines for experiments involving the use of animals.

Plant Material

Fresh leaves of *Heinsia crinita* (atama leaves) was bought from Amassoma market in Southern-Ijaw Local Government of Bayelsa-State and was identified by Dr Gideon Alade from Pharmacology department of Niger Delta University, Bayelsa State.

The leaves were air dried at room temperature for some days, when dried enough they were ground to powder form using a grinder and stored for extraction.

Plant Extraction

200grams of the ground leaves were weighed into 1000mls of distilled water. The mixture was left for 24hours for maximum extraction to take place. The soaked leaves were filtered using Cheesecloth and allowed to evaporate in a rotary evaporator.

Collection of Blood Sample

All experimental rats were sacrificed at the end of experiment, by anesthezing them with chloroform. Collection of blood samples were done using 5ml syringes with 21G

needles. The samples were collected from the animals through cardiac puncture into pre-labelled ethylenediaminetetracetate (EDTA) vials and gently agitated to ensure EDTA is spread uniformly after which samples were immediately used for measurement of haematological parameter like the Total Red Blood Cell Count, Haemoglobin Concentrations, Packed Cell Volume (PCV), Differential White Blood Cells, Total WBC, MCH, MCV and MCHC.

Measurement of Haematological Parameters

Haematological parameters were measured using Sysmex KX-21N automated haematology analyzer having standard calibration in line with the instructions of the manufacturer. Parameters measured were: RBC count, WBC count, differential WBC (lymphocyte count, eosinophils, basophils and monocytes) count, pcv, mcv, mch, mchc and platelet count.

Sample Collection

At the end of administration, the rats were sacrificed by administering chloroform as anesthesia. The rats were then dissected to harvest the liver which was then fixed immediately in 10% formal saline.

Tissue Processing

This is a chain reaction where the tissue was allowed to pass through different changes of chemical in order to prepare the tissues for subsequent treatment. The fixed tissues were subjected to secondary fixation, dehydration, clearing and impregnation steps. The process was aimed at production of ultra-section which are stained using the H&E stain

RESULT

TABLES

Table 1 ;shows the effect of Hensia crinata extract on DDVP induced toxicity in the different groups of administration. PCV is significant in group C with a p value of 0.009 while MCV is significant in group C and D with a P value of 0.018 and 0.017 respectively ,while MCHC is significant in group D with a P value of 0.007.

Table 2 ;shows the effect of Hensia crinata extract on DDVP induced toxicity in red cell indices in the different groups of administration. MCV is significant in group D with a P value of 0.017 ,while MCH is significant in group C with a P value of 0.031,MCHC is significant in group C and D with a P value of 0.018 and 0.007 respectively.

Table 3 ;shows the effect of Hensia crinata extract on DDVP induced toxicity in white cell parameters among the different groups of administration. WBC is significant in group B with a P value of 0.010 ,while NEUT is significant in group D with a P value of 0.031,LYMPH is significant in group D with a P value of 0.047.

HISTOLOGY PHOTOMICROGRAPH PLATES

Plate 4.1 Shows the morphology of the liver after staining with hematoxylin and eosin and viewed at x400 magnification. Sections showed normal slides with central vein (CV). Hepatocytes with intact sinusoidal space (S) consistent with normal liver histology.

Plate 4.2Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows congestion of the central vein (CV),areas of focal

necrosis (arrow) ,congestion of sinusoidal space with marked presence of Kupffer cells (K) and presence of councilman bodies(C) (X40) H&E.

Plate 4.3 Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows central vein congestion (CV) with areas of lobular necrosis(N)and presence of Kupffer cells (K)(X10,X40) H&E.

Plate 4.4Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows central vein congestion (C) with areas of necrosis(N), presence of Kupffer cells (K)(X10,X40).

Plate 4.5 Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows normal morphology of the liver, central vein (CV), hepatocytes with intact sinusoidal spaces (S) (X40)

Table 1

Comparative effect of *Hensia crinita* extract on DDVP induced toxicity in Group C, D and E

	Group A vs. B		Group A vs. C		Group A vs. D		Group A vs E	
Parameters	T-values	P-values	T-value	P-values	T-value	P-values	T-value	P-values
PCV	-0.385	0.710	-3.433	0.009*	0.099	0.923	-0.951	0.364
Hb	0.869	0.410	-0.584	0.575	1.323	0.218	0.110	0.910
PLT	1.278	0.237	-2.488	0.038	-2.000	0.077	-5.900	

0.568							
RBC	0.059	0.954	1.027	0.334	7.940	0.448	0.122
0.905							
MCV	-0.997	0.348	-2.962	0.018*	-2.908	0.017*	-1.848
0.094							
MCH	0.790	0.452	0.947	0.371	1.392	0.197	0.687
0.508							
MCHC	1.239	0.251	2.983	0.018	3.485	0.007*	1.854
0.093							
WBC	3.368	0.010*	0.182	0.860	0.674	0.517	1.667
0.126							
NEUT	0.676	0.518	0.147	0.887	-2.553	0.031*	0.327
0.751							
LYMPH	-0.730	0.486	-0.040*	0.969	2.301	0.047*	0.401
0.697							
MONO	-0.972	0.364	-0.365	0.724	0.548	0.597	-0.925
0.3777							
EOS	1.700	0.128	1.297	0.231	0.991	0.348	0.681
0.511							

Values where express as mean Standard Deviation (SD) with asterisk
(*) indicating significance that is $P<0.05$ in one-way analysis of variance (ANOVA)

Table 2.

p-values of red blood indices among the group

Parameters	group B	group C	group D	group E
MCV	0.348	0.18	0.017*	0.094.
MCH	0.452	0.031*	0.197	0.508
MCHC	0.251	0.018*	0.007*	0.093

Table 3 p-values of white blood cell parameters among the groups

Parameters	group B	group C	group D	group E
WBC	0.010*	0.860	0.517	0.126
NEUT	0.518	0.887	0.031*	0.751
LYMPH	0.486	0.969	0.047*	0.697

MONO	0.634	0.724	0.597	0.377
EOS	0.128	0.231	0.348	0.511

Table 4

p-values of haematological parameters among the group

Parameters	group B	group C	group D	group E
PCV	0.710	0.009*	0.923	0.364
Hb	0.410	0.575	0.218	0.910
PLT	0.237	0.038*	0.077	0.568

RBC	0.954	0.334	0.448	0.905 .

4.1 Plates

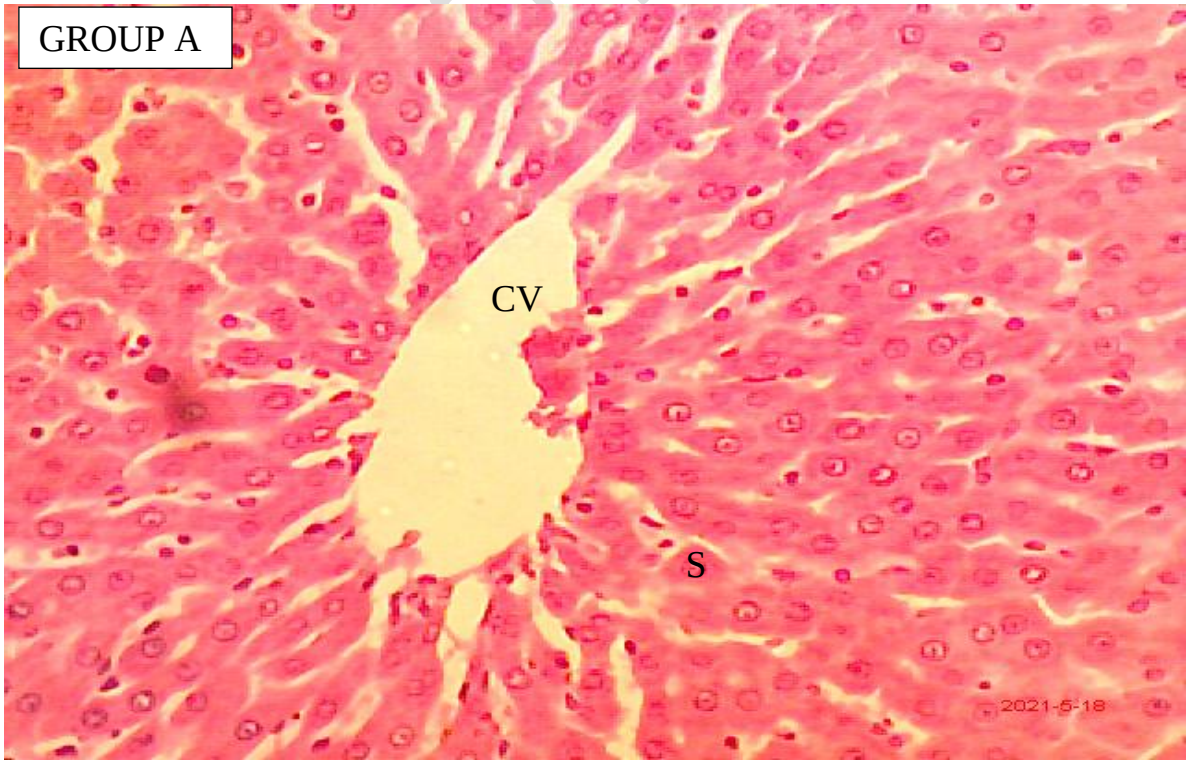

PLATE 4.1: Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows normal morphology of the liver,centralvein (CV),hepatocytes with intact sinusoidal space (S) (X10)H&E

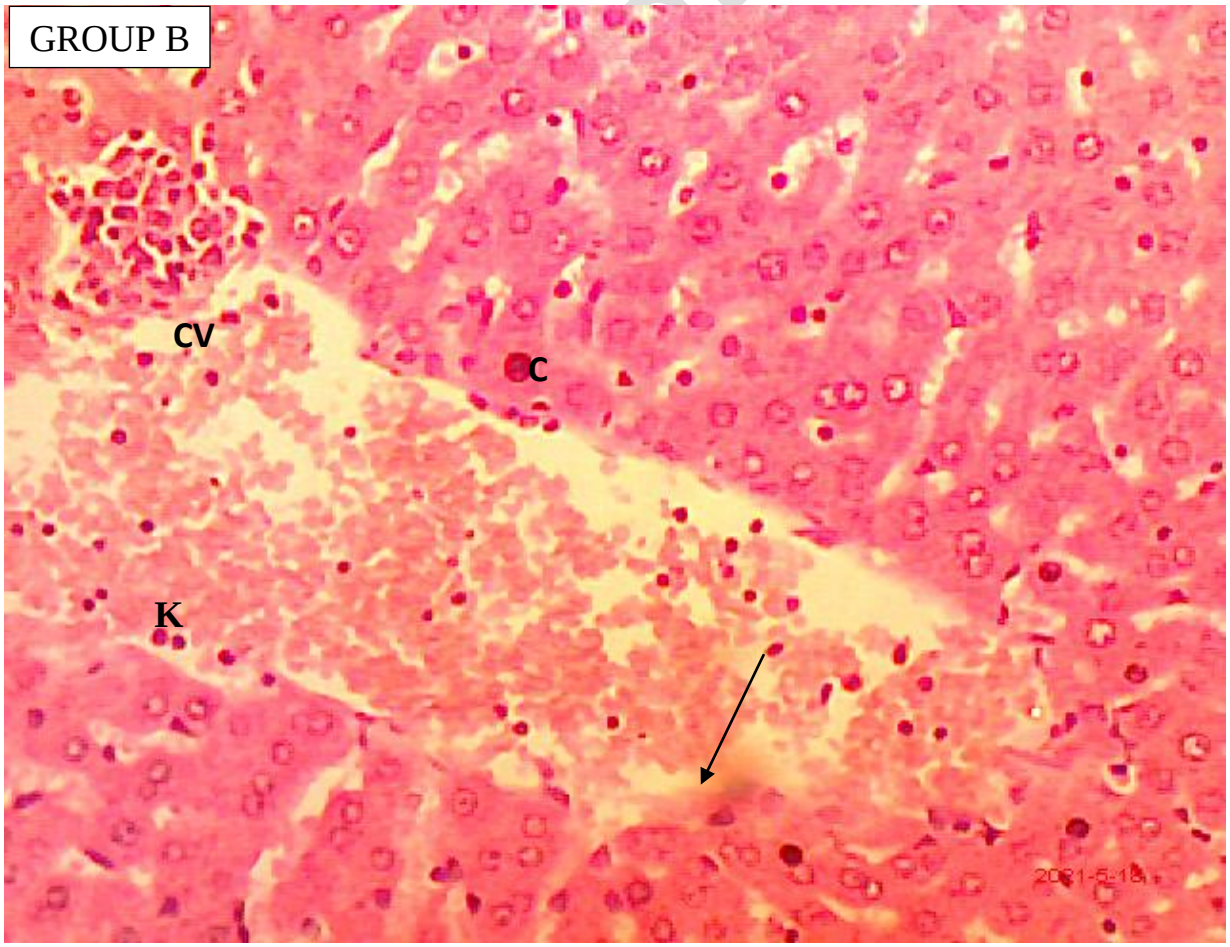

PLATE 4.2: Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows congestion of the central vein (CV),areas of focal

necrosis (arrow) ,congestion of sinusoidal space with marked presence of Kupffer cells (K) and presence of councilman bodies(C) (X40) H&E.

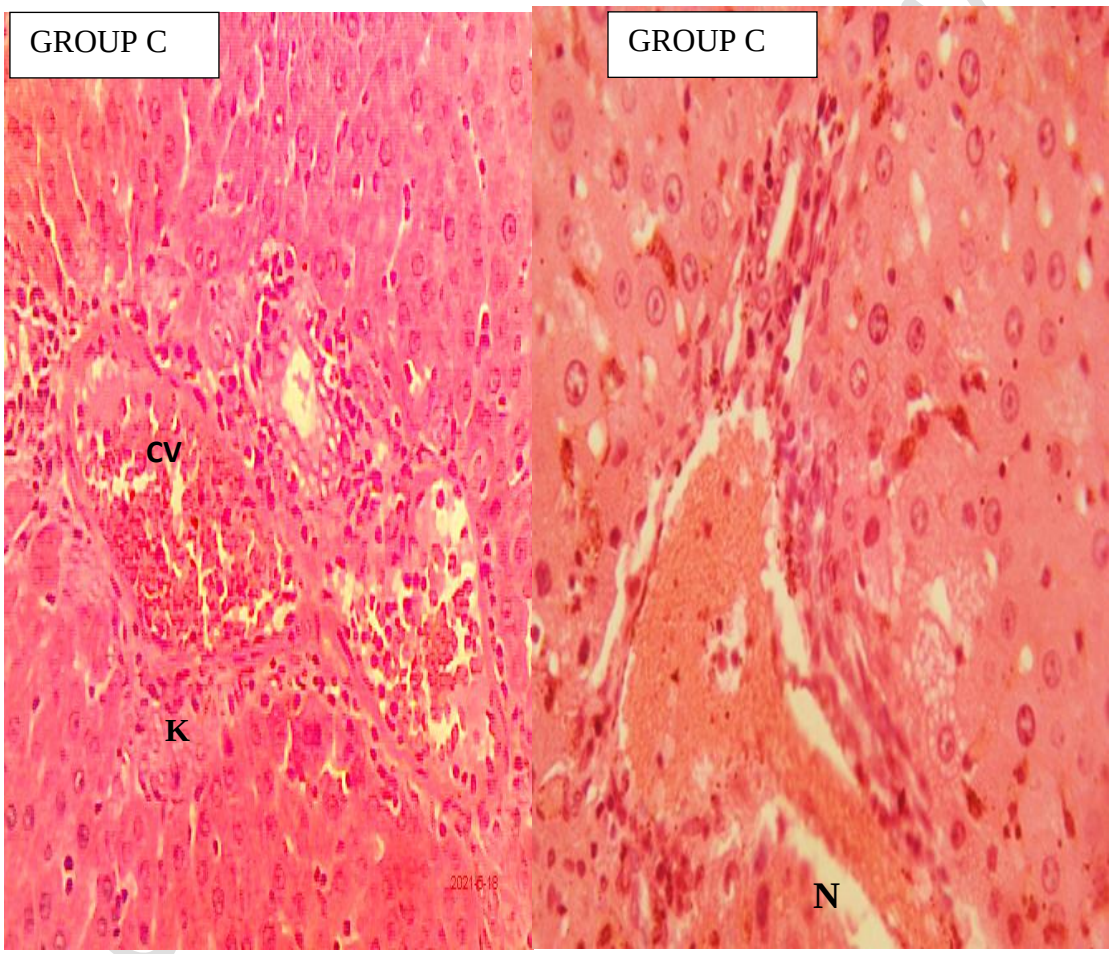

PLATE 4.3: Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows central vein congestion (CV) with areas of lobular necrosis(N)and presence of Kupffer cells (K)(X10,X40) H&E.

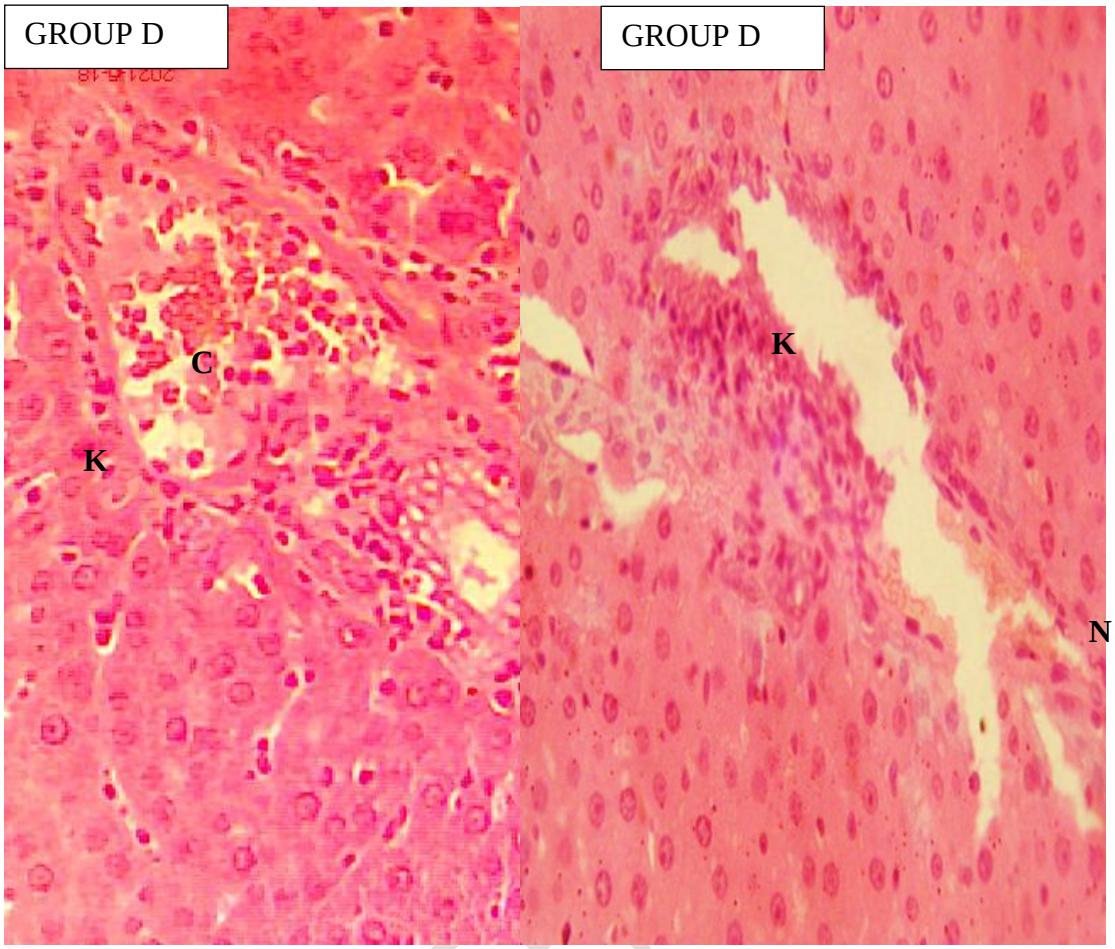

PLATE 4.4: Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows central vein congestion (C) with areas of necrosis(N), presence of Kupffer cells (K)(X10,X40).

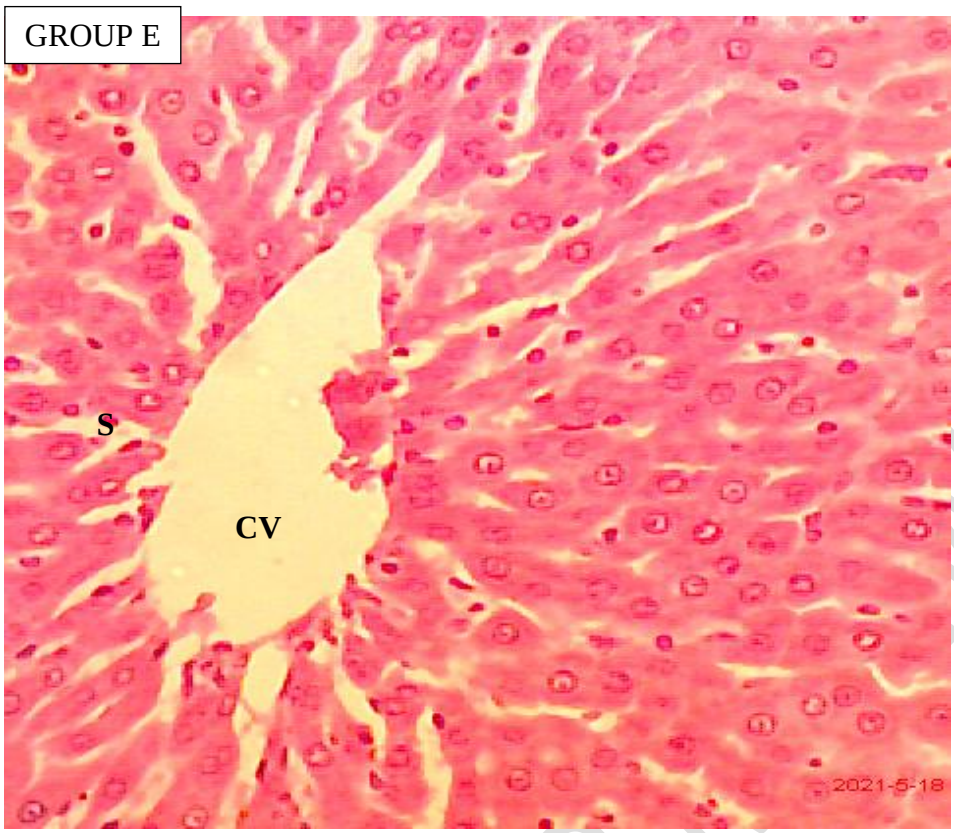

PLATE 4.5: Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows normal morphology of the liver, central vein (CV), hepatocytes with intact sinusoidal spaces (S) (X40)

Discussion

Heamatology

The findings from this study shows that mean cell volume (MCV) was statistically increased in Group B (rats induced with DDVP only), C (rats induced with DDVP and administered high dose of *Heinsia crinita* extract), & D (rats induced with DDVP and administered low dose of *Heinsia crinia* extract) as compared to the control group ($P<0.05$). Meanwhile, MCH and MCHC was statistically reduced in Group B, C & D compared to the control group. These findings agrees with that of Kingsley *et al.*, (2016) which also indicated that MCH and MCHC were lower than that of the

control. However statistically the MCV of Group D was significant (P <0.05) as well as the MCHC of Group C and D which were also significant (P <0.05). Looking closely at the table, it can be observed that there is always a slight difference between the mean of Group C and Group D for each Red Blood Cell indices. This is because they were administered different doses of treatment with that of Group C higher than Group D.

It was also observed that WBC was significantly decreased (P <0.05) in Group B. This findings does not corroborate with that of Onyinyechukwe *et al*., (2017) who observed in his study a significant increase (P <0.05) in WBC of rats administered Dichlorvos and Henshaw and Iwara ,(2018)) who attributed the increase in Total WBC of rats administered Dichlorvos to the rats defense mechanism in response to the invading xenobiotic (Dichlorvos). Monocyte was increased across the groups except in Group D which had a decrease compared to the control group. There was significant decrease (P <0.05) in Neutrophil, and Lymphocytes counts in Group D. However, the changes of monocyte and eosinophil counts were statistically insignificant across the groups B, C & D.

It was observed that there was an insignificant increase in PCV of Group B, a significant increase (P <0.05) in Group C, this finding does not agree with that of Henshaw and Iwara ,(2018)) and Brown *et al*., (2015). This increase in PCV may be due to dehydration (Holsworth *et al*., 2013) caused by dichlorvos and an insignificant decrease in Group D. A slight increase of Hb was observed in Group C, while a decrease was observed among Group B & D. Platelet count was increased in Groups B, C & D but statistically significant (P<0.05) in Group C. This is in agreement with

the work of Henshaw and Iwara ,(2018)) and Shakoori *et al*., (1992). There was no significant decrease in RBC among Groups B, C & D.

In Group E (rats treated with plant extract only) the mean values of PCV, RBC and Platelets were observed to increase in accordance to the study of Miikue-Yobe *et al*., (2015) though the increase was statistically insignificant. No change was observed in Haemoglobin concentration. This finding does not corroborate with the study of Miikue-Yobe *et al*., (2015) that reported a slight increase in Haemogblobin concentration. Total WBC count was insignificantly reduced. This finding does not agree with the findings of Miikue-Yobe *et al*., (2015) who in their study indicated a significant increase in total wbc count.

Histology

PLATE 4.2: Shows the morphology of the liver after the administration of the various treatments for 21 days. Slide shows congestion of the central vein, areas of focal necrosis (arrow), congestion of sinusoidal space with marked presence of Kupffer cells and presence of councilman bodies. This correlates with a study by (Romero, 2006) reported that acute exposure to dichlorvos (20 mg/kg/body weight) decreased the activity of hepatic glucokinase in rats.

Plate 4.3 Represent animals in group C, shows the morphology of the liver after the administration Dichlorvos 35mg/120kg and Heinsia crinata as treatment for 21 days at (250mg/kg) Slide shows central vein congestion, with presence of lobular cells. Durkin and follansbee 2014, did a study which elucidates a possible mechanism by which chronic organophosphate exposure (dichlorvos 6 mg/kg for 12 weeks) causes liver dysfunction. This correlates with a study (Singh 2012.), Mitochondria, a

primary site of cellular energy generation and oxygen consumption represent a likely target for organophosphate poisoning and this agrees with my work.

Plate 4.4 Represent animals in group D, shows the morphology of the liver after administration Dichlorvos 35mg/120kg and Heinsia crinata as treatment for 21days at (250mg/kg) slide shows central vein congestion with area of necrosis as well as presence of Kupffer cells (Mgbeje *et al.*, 2016). These principles may also be responsible for mopping up cell damaging antioxidants thus restoring the integrity of the pancreatic B-cells with concomitant insulin production and restoration of the normal glycaemic state. This is not unprecedented. Histological studies in our laboratory has shown the regeneration of damaged tissues (kidney, pancreas, liver, testis) in diabetic animals on treatment with antidiabetic medicinal plant extracts and fractions (Edet *et al.*, 2013, Mgbeje *et al.*, 2016; Abdelhalim and Jarrar, 2012) At 10mg/kg. The appearance of hepatocytes degeneration and destruction leading to necrosis central vein congestion and present of kupffer cell in Wistar albino rats was investigated may be due to the generation of reactive oxygen species (ROS) generation by dichlorvos this agree with my work.

Plate 4.5 Represent animals in group E, shows the morphology of the liver after the administration of the various treatment for 21 days. Slide shows normal morphology of liver central morphology, hepatocyles with intact simusoidal spaces. Hensia crinita contains antioxidant principles (Mgbeje *et al.*, 2016), which are responsible for mopping up cell damaging antioxidants thus restoring the integrity of the pancreatic B-cells with concomitant insulin production and restoration of the normal glycaemic state. Antioxidant phytochemicals present in the leaf crude extract and fractions may also have led to regeneration of the damaged liver cells. This agrees with my work.

Considering P-value of < 0.05 as significant for groups B and D liver enzyme test showed significant changes for AST and albumin, ALT for group B and D respectively whereas group C and E showed no significant change P>0.05 Groups B showed significant changes in AST while group D showed significant changes in albumin and AST which group C and E groups (B,C,D and E) showed no significant change for total protein as shown in table 4.2. It is evident that change. Occurred in albumin (hyper albuminarmia) ALT (hepaticmia) AIT cheptic damage) in group D administered Hensia crinite extract and AST (Hepatic Damage) in group B administer Dichlorous. (Romero-Navarro, 2006) mitochondrial calcium uptake and its implications on the induction of liver enzymes and liver dysfunction in rodent model. Our results indicated decreased mitochondrial electron transfer activities of cytochrome oxidase along with altered mitochondrial complexes I and II activity.This decrease in the activities of electron transport complexes in turn affected the ATP synthesis and ATP levels adversely in the mitochondria isolated from dichlorvos (DDVP) treated rat liver.

5.2 CONCLUSION

The treatment of Dichlorvos (DDVP) induced toxicity with *Heinsia crinita* extract has little or no significant effect on the blood parameters which has been shown from the study. Meanwhile DDVP has effect on different blood parameters when used independently (decreased MCH, MCHC, WBC and Hb increasing MCV, RBC and Platelet count) while *Heinsia crinita* insignificantly increased MCV,PCV,RBC and platelet, insignificantly reducing MCH, MCHC, WBC, and Hb)or combined (DDVP and high dose of treatment significantly (P-<0.05) increased MCHC,PCV, and Platelet, reducing MCH, MCV, WBC and RBC) while (DDVP and low dose of

treatment significantly increased MCV, significantly reducing, MCHC, neutrophil and lymphocyte). Based on my findings, *Heinsia crinita* extract does not have any ameliorative effect on dichlorvos toxicity.

Similarly this study shows that oral administration of Dichlorvos induces hepatoxicity and inflammation of the liver histologically and biochemically. It also shows that the administration of Heinsia crinata at various concentrations (250mg/kg and 500mg/kg) has no effect on the liver.

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