

Biochemical and Histopathologic Changes in Liver of Albino Rats Exposed to 1% Dichlorvos Pesticide at Sub-Acute Period Liver toxicity of a Nigeriandichlorvors pesticide

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ABSTRACT

Background; The present work studied the effects of a Nigerian localdichlorvors (1%) pesticide with high patronage - on the biochemistry and histology of liver of white albino rats at sub-acute period. The use of dichlorvos pesticide at homes and in food preservation has gained acceptance due to ease availability and presumed cost effectiveness. There are little or no indices that demonstrateits toxic potential.

Aim; To establish the toxic potentials of the Nigerian local dichlorvors pesticide.

Materials and Methods; Forty clinically healthy adult (Wister strain) albino rats consisting of both sexes, weighing 150 to 210 g were randomly divided into treatment and control groups, each consisting of twenty rats. Each group was further divided into four experimental days of first, third, seventh and fourteenth days so that each experimental day has five treatment and five control rats. The pesticide solution at daily oral dose of 7.5 mg/kg was administered to the treatment groups for the assigned number of experimental days. At the end of these experimental periods, blood samples were taken for biochemical tests and liver biopsy for histology.

Statistics; Students' t - test

Results; There was significant increase in the levels of serum marker enzyme especially in early treatment days. The histopathology revealed generalized hepatic vascular congestion, mild periportal inflammation, lymphocytic infiltration and focal micro vesicular steatosis. These changes were days/dose dependent.

Conclussions: The Nierian local dichlorvos (1%) pesticide (*Otapiapia*) is hepatotoxic in laboratory animals, as such its use as pesticide and food preservation deserves re-evaluation.

KEY WORDS: Dichlorvos, Liver biochemistry, histopathology, Nigeria, Vector control, Food preservation.

INTRODUCTION

Toxicity in human is a threatening truth and much more than any disease caused by organism as toxic substances are everywhere in air, in water and in food. [1] Many compounds which are essential to use for human welfare are at the same time injurious when viewed from safety point. Some compounds are not directly used by humans but indirectly they enter human (through food chain) and induce injuries. Pesticides are examples of compounds that are used against various pests for human welfare but are also harmful to humans as they eventually find themselves within human body via food chain. Liver is the primary organ that handles toxic substances in the body and as such it suffers the hazardous effects of these substances first. According to Williams *et al*, [2] any change in liver systematic will definitely affect complete metabolism of an animal. It was shown that the levels of 'marker' enzymes in tissues and biological fluids may be altered following the administration of foreign agents and such alterations can be used to assess the assault inflicted on the tissue cellular system of experimental animals, hence, their use in this study. [3, 4, 5]

Dichlorvos (2,2-dichlorovinyl dimethylphosphate), an organophospate, is commonly formulated in varying concentration (1 - 10%) as *otapia-pia* in Nigeria and other countries in the tropical Africa. It is widely used to treat domestic animals and livestock for internal and external parasites, to control insects commercially and in homes, to protect crops from insect infestation. It was reported that *otapiapia* is used indiscriminately to preserve dried and

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smoked fish. The report went further to declare that the use of this chemical has cause serious damage to ecosystem –terrestrial as well as aquatic and consequently the flora and fauna of surrounding. [6] The continuous and indiscriminate domestic usage of *otapiapia* has exposed humans to varying degree of hazards which might cause debilitation or diseases in human. It is therefore pertinent to have a good understanding of its toxicity in humans and to understand the residual amount that gets to the body system as a result of its domestic utility. The following work explored the liver biochemical and histopathologic changes that resulted from exposure to sub-lethal doses of local dichlorvors pesticide (*otapiapia*) in wister albino rats so as to forecasts possible health hazards that could results from its use in food preservation and in vector control.

MATERIALS AND METHODS

Animals Used: Forty clinically healthy adult (Wister strain) albino rats consisting of both sexes, weighing 150 to 210 g were used. They were acclimatized for 1 week in the pharmacology research laboratory University of Maiduguri. They were fed with growers mesh and water adlibdum and hygienic environment maintained to prevent infection. Permission and grant were soughted from the University of Maiduguri. However, no ethical clearance was applied for due to the absence of such committee in the University at the time of study.

Reagents: a.Biochemical reagents: Aspartate Amino Transferase (RANDOX); Alamine Amino Ttansferase (RANDOX); Alkaline Phosphatase (RANDOX); Total Protein (RANDOX); and Albumin (RANDOX).

b. Histopathological reagents: Alcohol (BDH); Xylene(BDH); Formaldehyde (BDH); Paraffin Wax (ALEX WAX LTD); Haematoxylin (BIOTECH LTD); Eosin (BIOTECH); Slide (SAIL BRAND); Cover Slip (MARIENFELD); and Mountant DPX (BIOTECH).

c. A Nigerian local dichlorvos (1%) preparation (Otapiapia)

Experimental Design: Longitudinal panel study

Lethal Dose Determination: A modified Lorke's method was used to determine the LD_{50} of the *otapiapia* pesticide and a 30% of the LD_{50} was thereafter calculated as 7.5 mg/kg. This was adopted as the treatment dose.

Animal grouping: The rats were randomly separated into two groups of experimental (20 rats) and control (20 rats). Both groups were further randomly separated into four sub-groups (Grp I, II, III and IV) containing five rats each. The sub-groups of both experimental and control were assigned to experimental periods of 1, 3, 7 and 14 days so as to connote number of days through which treatment will be administered and also days on which investigational samples will be collected.

Administration of otapiapia: Rats in the experimental group were treated in the laboratory with 7.5mg/kg/day of *otapiapia* via intra-gastric intubation for their assigned experimental periods. Rats in the control group were treated with distilled water. These treatments were continued in the subgroups of both experimental and control groups for their corresponding assigned experimental periods such that subgroups I, II, III, and IV, were treated for 1, 3, 7 and 14 days respectively.

Sample and Data collection: On these experimental days, the rats were decapitated and blood samples obtained for biochemical analysis of liver enzymes. Liver enzymes specifically studied included serum alanine amino transferase (ALAT), serum aspartate amino transferase (ASAT), serum alkaline phosphates (ALKP), total protein (TP), albumin (ALB), total billirubin (TB) and conjugated billirubin (CB). Liver biopsy was taken to the histology department of University of Maiduguri Teaching Hospital for histological studies.

Data analysis and statistical procedures: The values of the liver enzymes were entered into a statistical software (Statistical Software for Social Sciences, version 16) and the mean values of the groups compared using Analysis of Variance (ANOVA).

RESULTS

Blood Chemistry: Table 1 shows the average mean effects after *otapiapia* treatment on liver biochemistry per treatment groups /duration as compared to the control groups. There were increases in serum level of some of the liver enzymes. The ALKP level in the experimental groups was higher than that of the control groups in all the treatment days with the first, third and seventh days being statistically significant(p = <0.01, p = <0.01, p = 0.01, respectively) by Welch's corrected t-test statistics. The third day treatment group exhibited the highest difference while the fourteenth day, the lowest. Similar trend of higher levels in experimental groups as compared to controls was observed with ASAT and TP, but statistically significant difference was only in the first(p = 0.01, p = 0.01) and third(p < 0.01, p = 0.08 respectively) days. This trend was not observed with ALB, TB and CB. The level of ALAT in the experimental group was higher than that of the control in the third day, and lower in the fourteenth day treatment groups. The differences in both cases were statistically significant.

Histopathology: Histopathological features of the control group showed normal hepatic parenchyma, while the treatment groups results showed generalized hepatic vascular congestion, mild periportal inflammation, lymphocytic infiltration and focal micro vesicular steatosis (Figures I, II III and IV). These changes were days/dose dependent.

Table 1 Mean Blood Level of liver Enzymes in Rats Treated with Oral Otapiapia as Compared to Controls (n = 5)

Liver					Number of Days of Treatment with Oral Otapiapia					
Enzymes		First Day	P value	Third Day	P value	Seventh Day	P value	Fourteenth	P value	
								Day		
ALKP (iu/L)	Experimental Group	187.0 ± 0.56	<0.01	197.0 ± 3.50	<0.01	94.0 ± 0.58	0.01	93.0 ± 1.35	0.07	
	Control Group	94.3 ± 0.33		95.4 ± 0.30		90.7 ± 0.63		89.7 ± 0.55		
ASAT (iu/L)	Experimental Group	93.6 ± 1.33	0.01	100.0 ± 2.00	< 0.01	84.3 ± 6.19	0.66	53.0 ± 11.50	0.09	
	Control Group	$\textbf{86.3} \pm \textbf{1.67}$		89.0 ± 0.07		80.9 ± 4.05		79.3 ± 5.05		
ALAT (iu/L)	Experimental Group	33.0 ± 1.15	0.31	33.0 ± 0.16	<0.01	30.0 ± 6.66	0.63	16.6 ± 0.88	< 0.01	
	Control Group	30.6 ± 1.86		29.4 ± 0.55		28.6 ± 0.64		25.6 ± 0.25		
TP (g/L)	Experimental Group	72.3 ± 0.33	0.01	69.3 ± 1.45	0.08	68.6 ± 1.20	0.35	66.6 ± 0.88	0.13	
	Control Group	65.0 ± 1.51		64.0 ± 2.11		64.0 ± 4.22		63.5 ± 1.54		
ALB (g/L)	Experimental Group	36.0 ± 0.58	0.67	38.6 ± 0.67	0.28	33.3 ± 1.86	0.53	35.6 ± 0.33	0.22	
	Control Group	35.6 ± 0.67		36.1 ± 1.88		35.0 ± 1.80		34.6 ± 0.64		
TB (µmol/L)	Experimental Group	3.0 ± 0.58	0.40	3.3 ± 0.33	0.79	3.6 ± 0.67	0.79	3.3 ± 0.33	0.91	
	Control Group	3.6 ± 0.33		3.7 ± 1.4		3.9 ± 0.85		3.6 ± 2.50		
CB (µmol/L)	Experimental Group	1.4 ± 0.67	0.88	1.0 ± 0.70	0.60	1.3 ± 1.05	0.94	1.0 ± 0.50	0.18	
	Control Group	1.3 ± 0.33		1.8 ± 1.25		1.2 ± 0.53		1.9 ± 0.36		

All values are expressed in mean \pm S.E.M. P values are considered significant at < .05

TB= Total billirubin, CB= Conjugated billirubin, ALK.P=Alkaline phosphates, ALAT=Serum alanine amino transferase,

ASAT =Serum aspartate amino transferase, ALB = Albumin, TP = Total protein



Figure 1 Generalised hepatic vascular congestion with numerous necroinflammatory foci with lymphocytes infiltration of the 1 days treatment group. (x100 mag)



A photomicrograph showing hepatic congestion with mild periportal inflammation of the 3 days treatment group (x100Magnification)



A photomicrograph showing hepatic congestion with mild steatosis of the 7 days treatment group. (x100 mag)

DISCUSSION

The measurement of the activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis. [7]and to a reasonable extent, the toxicity of drugs including plant extract. [8] Tissue enzyme assay can also indicate tissue cellular damage long before structural damage can be picked up by conventional histological techniques. [9] Enzymes do not usually originate from the serum, but rather are derived from the disintegration, metabolism and turn-over of tissues and blood cells. Therefore, enzymes from diseased tissues and organs may become manifested in the serum resulting in increased activity. [10]

Consequently, in the present work marked increase in ALKP, ASAT and TP observed in the early days of treatment with *otapiapia* reflects hepatic tissue turn-over, as response by the body system towards overcoming stress induced by the test substance. Treatment for 14 days showed marked decrease in the enzymes levels when compared to the levels on the first, third and seventh days. This pattern of change in enzyme level is similar to the one produced in rats after treatment with isomers of nuvan, [11] with dichlorvos, [12] with aroclor 1260, [13] and with Azadiractaindica crude extract. [6] The changes in liver biochemistry was because it is a major site of metabolism and also primary site of detoxification and is therefore prone to various disorders as consequences of exposure to the toxins of extrinsic as well as intrinsic forms. [14] It is also site of biotransformation by which a toxic compound is transformed into less-harmful form to reduce toxicity. [15]

Alkalinephosphotase (ALKP) is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum, [17] it is therefore an ectoenzyme of the plasma membrane. [4] It is often used to assess the integrity of the plasma membrane. [3] ALKP is produced in the canaliculi and sinusoids membrane of the liver. Its level increase in liver diseases like cholestasis, metastases (infiltration in the liver). The significant rise in ALKP observed in the experimental group suggests the presence of cholestatic injury.

Amino transferases (transaminase) present in hepatocytes, leaks into the blood with liver cell damage. [8] The increase in transaminase activity in the liver is an indication of hepatocellular injury that occurs due to formation of reactive oxygen species and reactive intermediates after the treatment of the pesticide. [17] This increase transaminase activity leads to cellular damage and enzyme release from sinusoidal spaces to intraocular vein. [13] Aspartate aminotransferase is primarily found in the liver mitochondrial and cytoplasm. It is also found in heart, muscles, kidney and brain. Its serum level increases in hepatic necrosis, myocardial infarction and muscles injury. [11]In the present work, the significant rise observed in the level of ASAT in the early days of treatment may be attributable to such diseases of the liver, or of other organs where the transferase enzyme is located. However, in the later days of treatment, the rise in ASAT was not statistically significant, an observation that could be attributable to bodily response mechanism to the toxicant induced disease.

Alanine aminotransferase is a liver cytosol enzyme more specific to the liver so that a rise only occurs with liver disease. [18]A significant rise observed in third and fourteenth day signifies some degree of cytotoxicity.

Total proteins and albumin are plasma proteins that measure synthetic function of the liver. They help in maintaining blood osmotic pressure. The non-significant difference in the TP and ALB between control and experimental groups through most of the experimental periods suggests that there wasn't much depreciation of hepatic synthesis that could be attributed to *otapiapia*. However, the significant elevation of TP in the early days of treatment suggests a certain degree of depression of the synthetic function of the liver at that period.

Total billirubin and Conjugated Billirubin measures liver metabolic function. They are formed through breakdown of red blood cells by hepatocytes, used to access extent of hepatocellular damage. [1]The outcome of the present work suggests that the oral *otapiapia*did produced some significant hepatocellular damage in the experimental groups when compared with the control. The hepatocellular damage is however not significant statistically.

CONCLUSION

The Nigerian local dichlorvors pesticide (*otapiapia*) is hepatotoxic in laboratory animalsas such its use in preservation of food material and in vector control deserves re-evaluation to establish its safety indices.

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