EFFECTS OF AGRO-PESTICIDES (BASUDYNE AND CARBOFURAN) ON LIVER DYSFUNCTION BIOMARKERS OF ALBINO WISTAR RATS.

BY

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ABSTRACT

The risks posed by pesticides to human health have been of public health concern. This study was aimed at determining the effect of oral administration of organophosphate and carbamate based agropesticides basudyne and carbofuran respectively on liver dysfunction biomarkers of albino wistar rats. The experiment was laid out in a complete randomized design (CRD). Each group was assigned a particular oral dose of the pesticides in the order 5000, 7000, 9000, 11000, 13000 and 15000mg/kg body weight. The control group was not exposed to the treatment. The rats were sacrificed within 24 hours after administration of the toxicants. Standard procedures outlined by the commercial kit producers were used to assay for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), globulin, total protein, albumin, and serum bilirubin. The results showed that the liver function enzymes were still within the standard accepted range ALT (10 - 55 p/L), AST (10 - 40 p/L), and ALP (45 - 115 p/L). Slight fluctuations at $P<0.05$ were recorded in the mean and standard deviation values of the liver dysfunction parameters as the agro-pesticide concentration increased across the groups. Bilirubin level increased from 0.68±0.04 to 1.07±0.04 as basudyne dose increased along the group. Carbofuran administered pesticide group had bilirubin and total protein mean and standard deviation values of 0.66±0.002 to 0.96±0.02 and 6.35±0.07 to 6.92±0.05 respectively. The liver dysfunction biomarkers were in safety range as the dose increased along the group. This study recommends that the use of the pesticides in agricultural processes such as storage of produce should be at a controlled rate which does not affect the non-target organisms.

INTRODUCTION

Food security in sub-Saharan Africa mostly depends upon improved food productivity through the use of sustainable agricultural practices and the reduction of post-harvest losses instigated by pests and diseases. Storage insect pests cause significant damage to stored grains. According to Okori *et al.* (2004), without pesticides, food would be more costly; because production would entail more labour and more intensive knowledgeable management

The UN Environment Programme (UNEP) and the World Health Organization (WHO) evaluated that each year, 3million farm workers in the developing world experience stark pesticide poisoning of which about 18,000 were fatal (Miller, 2004). Facts from India, disclosed that about 51% of the food materials are polluted with residues in contrast to 21% worldwide, of which 20% were above minimal risk level agreed by FAO standards (ANON, 1999). The contaminated food is usually not thrown away in many developing countries, but tends to enter the food chain again out of ignorance, innocence and rather importantly out of lack of affordability by the consumers. Absence of awareness of the consequence of pesticide- contaminated food could be one of the explanations for increased occurrences of cancers in the developing world. There have been many studies on determining

the ill effects of pesticide exposure (McCauley *et al.,2006).* Various inappropriate practices in the use of pesticides cause possible poisoning symptoms generally among farmers who do not wear protective clothing (Ntow *et al*.,2006).It is well known now that a significant fraction of pesticides are carcinogenic.

As the largest internal and major organ in the body whose functions include metabolizing, detoxifying and regeneration of cells, the liver secretes the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The levels of these enzymes in the plasma have over the years been used as indicators of toxicity. It is established that elevations in AST and ALT (aminotranferases) values indicate early diagnosis of hepatotoxicity and tissue damage (Kamal and Hessah, 2015). Hence, elevations in the levels of these liver enzymes beyond normal values are sure indicators of liver toxicity (Abdel *et al.,* 2014). ALT and AST are excellent markers for diagnostic purpose and they play a role in the conversion of amino acid to keto acid. AST was found in many tissues like liver, kidney, heart, brain and skeletal muscle. It is not a specific liver enzyme like ALT whose concentration in the liver is usually large. Serum ALP is a sensitive detector for intra-hepatic bile obstruction. In case of liver damage or necrosis, these enzymes are usually released into the serum, hence they are good indicators of liver integrity (Bhargava, 2015) and abnormal liver enzymes levels may signal liver damage or alteration in bile flow (Edoardo *et al.,* 2005). This study was aimed at determining the effect of oral administration of agro-pesticide (basudyne and carbofuran) on the liver dysfunction biomarkers of albino wistar rats.

MATERIALS AND METHODS

Test Organisms

A total number of 56 albino wistar rats of the order Rodentia and family Muridae were used a particular oral dose level of the respective pesticides (basudyne and for toxicity test studies of the agro-pesticides (Basudyne and Carbofuran)after 14 days acclimatization

Research Design.

The experiment was laid out in complete Randomized Design (CRD). The rats were randomly assigned to a control group and Six test groups (Grp 1, Grp2, Grp3, Grp4,Grp 5 and Grp 6).The respective test groups were assigned four albino wistar rats each. Each group was assigned carbofuran) and in the order: 5000, 7000, 9000, 11000, 13000, and 15000mg/kg body weight. The rats were sacrificed within 24.0hrs after administration of toxicant. Blood collection was done by cardiac puncture and introduced into K_3 EDTA and plain bottles for liver function tests.

DETERMINATION OF SOME BIOCHEMICAL PARAMETERS

Alanine aminotransferase (ALT) was determined according to the method of Reitman and Frankel, 1957 using Randox commercial kits and following procedures

outlined by the commercial kit producer, Randox Laboratories Limited, UK. Two test tubes were set up in a test tube rack and respectively labelled **blank** and **test** and 0.1ml of the sample (test serum) was introduced into the **test** test tube while 0.1ml of distilled water was pipetted into the **blank** test tube. 0.5ml of ALT reagent R_1 was then introduced into each of the tubes and mixed. The mixture was incubated for 30 minutes at 37^0C before the addition of 0.5ml of reagent R_2 to each of the tubes and also incubated at 20-25^0C for 20 minutes. 5ml of Sodium Hydroxide was added to each tube and allowed to stand for 5 minutes before absorbance was read against the reagent blank on a spectrophotometer (722N, Mindray, India) at 500nm. ALT activity in the serum was obtained by tracing the equivalent absorbance of the sample on the ALT absorbance chart and finding its corresponding ALT activity value in U/L.

For **Aspartate aminotransferase AST determination**, commercial kits were used and standard procedures as prescribed by the producer Randox Laboratories Limited, U.K were adopted. A blank test tube containing 0.1ml of distilled water was set up. A second test tube labelled "test" was made to contain 0.1ml of the test sample. AST reagent R_i (0.5ml) was added to each test tube, mixed and incubated at 37^0C for 30 minutes. After the incubation, 0.5ml of AST reagent R_2 was added to each test tube and allowed to stand for 20 minutes at 20-25^0C before 5.0ml of 0.4mol/ Litre NaOH was added to each and also allowed to stand for 5 minutes before reading absorbance against the reagent blank on a spectrophotometer (722N, Mindray India) at wavelength 546nm. AST activity in the serum was then obtained by finding an equivalent value of absorbance on the standard chart and obtaining its corresponding activity value in U/L.

Alkaline phosphatase (ALP) Activity was determined according to the method of King and Kind, (1957) using ALP commercial kits and following standard procedures outlined by the producer Teco Diagnostic, U.S.A. Three test tubes were set up and labelled test, control and standard respectively into which 0.5ml of Alkaline phosphatase substrate was added. 0.05ml (50pl) of the standard, control and sample (test serum) were added to the corresponding test tubes while distilled water was used for the control. The test tubes were incubated for 10 minutes at 37^0C before adding 2.5ml of alkaline phosphate colour developer at timed intervals. The mixtures were properly mixed before reading absorbance on a spectrophotometer at 590nm after zeroing with the reagent blank.

ALP value was calculated using the formular

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of standard}} \times 50$$

Where 50 *pl* is the standard ALP value

Serum bilirubin content was estimated using commercial kits and following standard protocols prescribed by the producer Randox Laboratories Limited, U.K. Two test tubes were set up and labelled blank and sample. To the sample test tube, 200 *pl* of Reagent 1 (0.17N hydrochloric acid), 50 *pl* of Reagent 2 (38.5mmol/L of sodium Nitrite) and 1000nl of Reagent 3 (0.52mmol/L sodium benzoate) were added and mixed properly while only 200ml of Reagent 1 and 1000nl of reagent R_3 were added to the blank test tube. The 2 test tubes were then incubated at 20-25^0C for 10 minutes, after which 100 *pl* of reagent 4 (1.9N sodium Hydroxide) was added to both test tubes. The test tubes were incubated for a further 30 minutes at 25^0C before reading absorbance on a spectrophotometer at 560nm after zeroing with blank. To obtain total bilirubin

concentration in mg/dl the formular below was used.

Total Bilurubin =10.8 X Absorbance of Sample

RESULTS

Results obtained from the various tests are summarised in Tables 1 and 2 below

Tablel: EFFECT OF ACUTE ORGANOPHOSPHATE BASED PESTICIDE (BASUDYNE) TREATMENT ON LIVER DYSFUNCTION BIOMARKER

Dose	*Total Protein (g/dl)*	*Albumin (g/dl)*	*Globulin (g/dl)*	*AST p/L*	*ALT p/L*	*ALP p/L*	*Bilirubin mg/dl*
Control	6.87±0.08^{f}	3.98±0.06^{f}	2.97±0.02^{a}	15.25±2.99^{a}	11.00±2.58^{a}	65.78±0.23^{d}	0.67±0.03^{a}
5000mg/kg	6.64±0.03^{e}	3.21±0.03^{e}	3.43±0.05^{d}	23.50±2.89^{b}	24.00±2.94^{b}	60.95±0.04^{b}	0.68±0.04^{a}
7000mg/kg	6.51±0.04^{d}	3.05±0.04^{d}	3.30±1.29^{d}	25.50±1.29^{b}	35.00±2.16^{c}	61.32±0.07^{c}	0.76±0.02^{b}
9000mg/kg	6.22±0.03^{c}	3.02±0.02^{d}	3.19±0.02^{c}	31.25±3.30^{b}	43.50±3.87^{d}	60.52±0.36^{a}	0.89±0.03^{c}
11000mg/kg	6.15±0.04^{b}	2.96±0.04^{c}	3.15±0.03^{c}	47.75±0.05^{c}	51.25±4.15^{e}	68.49±0.18^{g}	0.96±0.02^{d}
13000mg/kg	6.11±0.02^{ab}	2.74±0.03^{b}	3.06±0.05^{b}	56.25±4.72^{d}	64.00±3.92^{f}	67.29±0.12^{f}	1.02±0.01^{e}
15000mg/kg	6.05±0.03^{a}	2.53±0.05^{a}	2.98±0.05^{a}	62.00±6.16^{d}	62.75±4, 79^{f}	66.67±0.21^{e}	1.07±0.04^{f}

Different alphabetical superscripts in the same column means there is a significant difference at P<0.05 between treatments according to Duncan test while same alphabetical superscripts in the same column means no significant difference at P<0.05 between treatments according to Duncan test

As the dose concentration increased from 5000mg/kg to 15000mg/kg, total protein level dwindled gradually from 6.64±0.03 (g/dl) for 5000mg/kg to 6.05±0.03 (g/dl) for 15000mg/kg dose. The control group for total protein remained 6.87±0.08 (g/dl) at P<0.05. A gradual increase in AST, ALT, ALP and Bilirubin was seen as the dose concentration increased from 5000mg/kg to 15000mg/kg. AST level rose from 23.50±2, 89 (p/L) to 62.00±6.16 (p/L) as against a mean ± standard deviation for the control group of 15.25±2.99 (p/L). ALT level and ALP level increased gradually as the dose concentration increased from 5000mg/kg to 15000mg/kg. They increased respectively from 24.00±2.94 (p/L) to 62.75±4.79 (p/L) for ALT and mean ± standard deviation of 60.95±0.04 (p/L) to 66.67±0.21 (p/L) for ALP. The control groups had a mean ± standard deviation of 11.00±2.58 (p/L) and 65.78±0.23 (p/L)

respectively at P<0.05. Also Bilirubin level increased as the dose concentration increased from 5000mg/kg to 15000mg/kg. Dose 5000mg/kg has a mean ± standard deviation of 0.68±0.49 mg/dl and this increase kept on occurring as the dose concentration increased. At 15000mg/kg 1.07±0.04 mg/dl mean ± standard deviation value was obtained at P<0.05 as against a control mean ± standard deviation of 0.67±0.03mg/dl. Albumin level decreased as the dose concentration increased from 5000mg/kg to 15000mg/kg. It decreased from a mean ± standard deviation of 3.21±0.03 g/dl to 2.53±0.05 g/dl as against a control group of 3.98±0.06 g/dl. Globulin levels also increased as the dose concentration was increased from 5000mg/dl to 15000mg/kg. 3.43±0.05 g/dl to 2.98±0.05 g/dl respectively. The control mean ± standard deviation at P<0.05 remained 2.97±0.02 g/dl.

Table 2: EFFECT OF ACUTE CARBAMATE BASED PESTICIDE (CARBOFURAN) TREATMENT ON LIVER DYSFUNCTION BIOMARKERS

Dose	*Total Protein (g/dl)*	*Albumin (g/dl)*	*Globuli n (g/dl)*	*AST p/L*	*ALT p/L*	*ALP p/L*	*Bilirubin mg/dl*
Control	6.18±0.18 ^{a}	3.69± 0.04^{b}	2.33±0.09 ^{a}	19.75±3.86^{b}	13.50±1.29 ^{a}	63.73±0.05 ^{a}	0.69±0.11 ^{a}
5000mg/kg	6.79±0.05 ^{de}	3.84±0.09^{b}	2.88±0.07^{b}	15.25±1.71 ^{a}	19.00±1.83^{b}	66.36±0.05^{c}	0.66±0.02 ^{a}
7000mg/kg	6.61±0.07 ^{c}	3.02±0.52^{a}	3.36±0.05^{d}	22.25±2.22^{bc}	25.25±3.09^{cd}	65.15±0.03^{b}	0.88±0.02^{b}
9000mg/kg	6.35±0.07 ^{b}	3.17±0.03^{a}	3.22±0.09^{c}	25.75±1.7 ^{cd}	25.00±1.83^{cd}	67.82±0.02^{d}	0.89±0.04^{b}
11000mg/kg	6.70±0.05 ^{cd}	3.15±0.02^{a}	3.33±0.15^{cd}	34.75±2.22^{e}	23.50±2.08^{c}	70.63±0.02^{f}	0.88±0.02^{b}
13000mg/kg	6.90±0.03 ^{e}	3.27±0.04^{a}	3.65±0.08^{e}	28.25±1.71^{d}	27.25±0.96^{de}	69.66±0.02^{e}	0.88±0.03^{b}
15000mg/kg	6.92±0.05 ^{e}	3.25±0.04^{a}	3.78±0.08^{e}	34.00±2.94^{e}	29.50±1.91^{e}	73.27±0.01^{g}	0.96±0.02^{c}

Different alphabetical superscripts in the same column means there is a significant difference at P<0.05 between treatments according to Duncan test while same alphabetical superscripts in the same column means no significant difference at P<0.05 between treatments according to Duncan test

An increase in oral dosage of the carbamate based pesticide (carbofuran) from 5000mg/kg - 15000mg/kg increased globulin level from mean± standard deviation of 2.88±0.07 (g/dl) to 3.78±0.08 (g/dl) compared with the control group of 2.33±0.09 (g/dl) at P<0.05. This increase could also be seen in AST levels from mean ± standard deviation of 15.25±1.71 (p/L) to 34.00±2.94 (p/L) in comparison to the control group of mean ± standard deviation of 19.75±3.86 (p/L) to 29.50±1.91 (p/L) as the dosage concentration increased from 5000mg/kg to 15000mg/kg while the mean ± standard deviation of the control group remained 13.50±1.29. ALP level also increased from 66.35±0.05 (p/L) to 73.27±0.01 (p/L) compared with the mean ± standard deviation value of control of 63.73±0.05 (p/L). The total protein and bilirubin level increased. Total protein has a mean ± standard deviation of 6.79±0.03 (g/d) to 6.92±0.05 (g/dl) as the dosage increased from 5000mg/kg to 15000mg/kg. The control group remained 6.18±0.18 (g/dl) at P<0.05. Bilirubin values increased from 0.66±0.02 mg/dl to 0.96±0.02 mg/dl as against the control group value of 1.69±0.11mg/dl. A sharp decrease was seen in albumin level as the dose concentration increased from 3000mg/kg to 15000mg/kg. It reduced by mean ± standard deviation of 3.84±0.09 (g/dl) to 3.25±0.04 (g/dl) as against the mean ± standard deviation of 3.69±0.04 (g/dl) seen in the control group at P<0.05.

DISCUSSION

Liver enzymes Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations in the treated rats were only significantly raised following an increase in the dose concentration of the pesticides when compared with the control group. The elevations observed in AST and ALT values though they differed from those of the control, still fall within safety range. Liver function tests give information about the state of the liver, describing its functionality (albumin and lipid profile), cellular integrity (transaminases) and its link with biliary tract (ALP) (Ezejiofor *et al.*, 2013). Thapa and Anuj (2007) had reported that the standard range of accepted values for liver function tests, beyond which liver damage may be suspected is ALT (10 - 55 p/L), AST (10 - 40 p/L), and ALP (45 - 115 p/L). Kamal and Hessah (2015) corroborated this when they reported that rise in AST, ALT and ALP values beyond these limits indicate early diagnosis of hepatotoxicity and tissue damage. It has been reported that liver toxicity is associated with increase in various serum liver enzymes, resulting from damage to the hepatocytes. Elevation in AST can be associated with cell necrosis of many tissues. For example, pathology involving the skeletal or cardiac muscles and/or the hepatic parenchyma, allows for the leakage of large amounts of this enzyme into the blood (Cornelius, 1989). The levels of these enzymes in all treated rats remained within safety range.

CONCLUSION

The liver dysfunction biomarkers were in safety range as the dose increased along the group. Results of this study therefore suggest that the use of the pesticides in agricultural processes such as storage should be at a controlled rate that does not affect the nontarget organisms.

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