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Biochemical and Histomorphological Impact of a Potential Chlorate Pesticide Agent on Tissues of the Giant African Land Snail (*Archachatina marginata*) as Indication of Toxicity

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Abstract

Objective: Herbicides and pesticides are used in agricultural practice to mitigate activities of unwanted herbs and pests thereby boosting food production while exposing non-target organisms to their deleterious effects. This study investigated the biochemical and histological impact of a pesticide agent (potassium chlorate) on non-target giant African land snail (*Archachatina marginata*).

Methods: After laboratory acclimatization, thirty adult snails divided into six experimental groups of five snails each were exposed to 2 liters of distilled water (control), 1mg/L $KClO_3$, 2mg/L $KClO_3$, 4mg/L $KClO_3$, 8mg/L $KClO_3$ and 10mg/L $KClO_3$ respectively for 2 weeks after which edible foot region from each group were pooled together for analysis. Data was analyzed with descriptive statistics and results presented as standard deviation of mean. One sample T-Test was used to assess differences in mean values. Probability of $\leqslant 0.05$ was considered significant.

Results: Statistically significant differences were observed in mean values obtained for control and chlorate-exposed samples for alanine-aminotransferase ($P \leqslant 0.05$), alkaline-phosphatase ($P \leqslant 0.05$), gamma-glutamyltransferase ($P \leqslant 0.05$), urea ($P \leqslant 0.05$), total-bilirubin ($P \leqslant 0.05$), conjugated-bilirubin ($P \leqslant 0.05$), total-protein ($P \leqslant 0.05$), globulin ($P \leqslant 0.05$), cholesterol ($P \leqslant 0.05$) and glucose ($P \leqslant 0.05$) but not for aspartate-aminotransferase ($P>0.05$), unconjugated-bilirubin ($P>0.05$) and albumin ($P>0.05$). Shrinkage and degeneration of mucous secreting unicellular glands and hyaline fibers, inflammatory cells and necrotic materials enclosed in vacuoles, vacuole formation within oblique muscle fibers, splitting and atrophy of oblique muscle fibers were observed in the chlorate-exposed samples.

Conclusion: Biochemical and histological observations seen in this study are suggestive of liver and kidney dysfunction following exposure of the giant African land snail (*Archachatina marginata*) to the potassium chlorate pesticide agent. Caution should therefore be exercised in incorporating and adopting potassium chlorate as a proprietary pesticide agent.

Keywords: Histotoxicity, Pesticides, Oxidative enzymes, Liver dysfunction, Kidney dysfunction

1. Introduction

Nigeria is home to different species of land snails varying in size, color, adaptability and performance including Archachatina marginata, Achatina achatina, Achatina fulica, Limcolaria spp, [1- 4, 14]. Generally, the edible foot region of snails are reportedly rich in calcium, phosphorus, iron and all the amino acids required by man with low fat and saturated fatty acid content [5-14]. The giant African land snail (*Archachatina marginata*) is one of the largest known land snails widely distributed in the tropical and sub-tropical regions of the world [15]. It is one of the most important minor forest products in West Africa and Nigeria in particular where its production serves as cheap source of animal protein supply. The meat has a high protein content of about 83 - 93% [16-17], making it compare favorably with other conventional protein sources. In recent times, the activities of man including use of pesticides and other anthropogenic factors have led to a considerable decline in the wild snail population in Africa [18].

Pesticides are toxic chemical substances or mixtures of substances or biological agents that are intentionally discharged into the environment to mitigate populations of insects, weeds, rodents, fungi or other harmful pests [19]. The use of pesticides in combination with irrigation, application of fertilizer to soil and mechanization helps to reduce damage to crops and maintain food production in order to feed Africa's growing population. Conversely, continuous use of pesticides may be problematic mainly due to their toxicity and environmental impact [20-21]. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, because of the mode of application across entire agricultural fields [22]. Herbicides are the most widely used class of pesticides accounting for more than 60% of all pesticides applied in agriculture [23]. About 20 mechanisms of action, some sharing common target sites with mammalians have been elucidated [24]. Hence one of the main concerns about the use of herbicides and pesticides generally is their effects on non-target organisms. The chlorate anion (ClO_3-) and its salts (sodium chlorate, calcium chlorate, potassium chlorate and magnesium chlorate) are powerful oxidizers collectively known as chlorates having active ingredient status although, only sodium chlorate is currently registered for use as a herbicide [25-27]. The list of compounds for which toxic release inventory (TRI) reporting is required has never included a compound with "chlorate" in its name [28]. Considering the fact that picking of snails for consumption on farms that may have been sprayed with pesticides and other chemical products is a common practice in Southern Nigeria [29], this study therefore seeks to evaluate histochemical and histological impact of non-proprietary potassium chlorate on the edible foot region of the giant African land snail (*Archachatina marginata*).

2. Materials and Methods

Test Animals and Laboratory Acclimatization:

Thirty giant African land snails (*Archachatina marginata*) purchased (within the month of May) from Omi-Adio market, Ibadan (Oyo state), Nigeria (Latitude: 7 ° 23'38" and Longitude: 3 ° 45'13"), within weight ranges of 43.31g to 45.43g were used for the experiments. The snails were allowed to acclimatize to laboratory conditions (temperature 25 ± 2 ℃, photoperiod 12hL/12hO, relative humidity 79 ± 2%) in a plastic terrarium (200cm x 80cm x 30cm) containing 2cm layer of moist humus soil substrate for 7 days prior to commencement of potassium chlorate treatment [15]. The terrarium was covered with a wire mesh for proper ventilation and prevention of escape. The terrarium was wet daily with distilled water and snails were fed on

fresh pawpaw leaves (carica papaya). Unconsumed food and fecal material were removed daily to prevent contamination.

Test Chemicals and Experimental Design:

Analar grade of potassium chlorate ($KClO_3$) as a representative of chlorate pesticide was used in this study. Solutions were prepared with distilled water. After the 7 days laboratory acclimatization, the snails were divided into 6 groups containing 5 animals each and maintained under existing laboratory conditions, however, snails in group 1 were exposed to soil and fresh pawpaw leaves wet with 2 liters of distilled water, snails in group 2 were exposed to soil and pawpaw leaves wet with 2 liters of 1mg/L potassium chlorate solution, snails in group 3 were exposed to soil and pawpaw leaves wet with 2 liters of 2mg/L potassium chlorate solution, snails in group 4 were exposed to soil and pawpaw leaves wet with 2 liters of 4mg/L potassium chlorate solution, snails in group 5 were exposed to soil and pawpaw leaves wet with 2 liters of 8mg/L potassium chlorate solution and snails in group 6 were exposed to soil and pawpaw leaves wet with 2 liters of 10mg/L potassium chlorate solution on a daily basis for two weeks. Unconsumed food and fecal material were removed daily to prevent contamination. At the end of the exposure period, snails were weighed and starved for two days after which the foot region was harvested for subsequent analyses [15].

Biochemical Study:

The harvested foot region from each experimental group were pooled together and homogenized in distilled water (50mg/mL). The homogenates were centrifuged at 8000 rpm for 1 minute at 5 ℃ in a refrigerated centrifuge. The deposits were discarded and the supernatant preserved at -4 ℃ until used for (i) evaluation of liver and kidney function which includes: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Urea, Total bilirubin (TB), Conjugated bilirubin (CB) and Unconjugated bilirubin (UB), Total protein (TP), Albumin (ALB) and Globulin (GLOB), Cholesterol (CHOL) and Glucose (GLU) (ii) evaluation of antioxidant enzyme activity: Gamma glutamyltransferase (GGT).

Histological Study:

The harvested foot region from each experimental group were fixed in Bouin's fluid for 48 hours and thereafter subjected to several rinses in distilled water. They were then dehydrated by passing sequentially through 70%, 80%, 90% and 100% ethanol for 1 hour each and cleared in 2 changes of xylene for 1 hour each. This was followed by impregnation in 2 changes of molten paraffin wax for 1 hour each and embedding in molten paraffin wax using Tissue-Tek stainless steel embedding moulds. Blocked out samples were then sectioned at a thickness of 5μm on Histoline Laboratories MR 2258 rotary microtome and stained by the hematoxylin and eosin method. Slides from each group were observed under the microscope and photomicrographs were taken.

Statistical Analysis:

The results were computed statistically using SPSS version 23.0. Data are expressed as Mean ± SD. One sample T-Test was used to check significance among means. Probability of $\leqslant 0.05$ was considered significant.

3. Results

Evaluation of Liver and Kidney Function:

To evaluate liver and kidney function, the levels of AST, ALT, ALP, UREA, TB, CB, UB, TP, ALB, GLOB, CHOL and GLU were determined in the extracts from the foot region of control and pesticide treated snails. It was generally observed that values for TP, ALB, AST, TB, CB, UB, UREA and GLU were higher in the pesticide treated than in the control snails; while values for GLOB, ALT, ALP and CHOL were lower in the pesticide treated than in the control snails (Table 1).

Evaluation of Antioxidant Enzyme Activity:

To evaluate antioxidant enzyme activity, GGT was determined in the extracts from the foot region of control and pesticide treated snails. Outcome of antioxidant enzyme activity show generally that pesticide treated snails had lower GGT value than control snails (Table 1).

Table 1: Biochemical changes (as Mean±S.D) in the foot of the giant African land snail (Archachatina marginata) exposed to different concentrations of $KClO_3$

Histo-chemical parameters	2 liters of distilled water	2 liters of 1mg/L $KClO_3$	2 liters of 2mg/L $KClO_3$	2liters of 4mg/L $KClO_3$	2 liters of 8mg/L $KClO_3$	2 liters of 10mg/L $KClO_3$	P-value
TP	0.67±0.03	0.81±0.01	0.28±0.02	0.49±0.01	0.19±0.01	0.30±0.01	0.01*
ALB	0.09±0.01	0.51±0.02	0.09±0.01	0.09±0.01	0.09±0.01	0.21±0.01	0.11
GLOB	0.59±0.01	0.29±0.02	0.19±0.01	0.38±0.03	0.09±0.01	0.09±0.01	0.03*
ALT	183.97±0.03	133.99±0.04	0.59±0.01	120.03±0.06	114.01±0.01	120.00±0.01	0.00*
AST	0.58±0.02	11.01±0.01	122.32±1.14	0.09±0.01	111.01±0.01	117.00±0.01	0.06
ALP	5103.02±0.03	4304.01±0.01	1328.02±0.03	765.01±0.01	538.01±0.01	496.01±0.01	0.05*
GGT	66.95±0.09	59.03±0.03	37.90±0.13	13.01±0.01	27.00±0.01	43.00±0.01	0.00*
TB	0.19±0.01	0.29±0.01	0.09±0.01	0.21±0.01	0.10±0.01	0.50±0.01	0.03*
CB	0.09±0.01	0.19±0.02	0.09±0.01	0.21±0.01	0.09±0.01	0.31±0.01	0.02*
UB	0.09±0.01	0.09±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.21±0.01	0.67
CHOL	53.91±0.03	34.02±0.02	14.01±0.01	29.01±0.01	31.00±0.01	29.01±0.01	0.00*
UREA	31.68±0.59	23.01±0.02	41.01±0.01	34.01±0.01	28.01±0.01	7.00±0.01	0.00*
GLU	20.98±0.03	39.02±0.02	34.01±0.01	27.01±0.01	40.01±0.01	38.00±0.00	0.00*

Note: Concentration is in mg/dl.

Key: TP= Total protein, ALB= Albumin, GLOB= Globulin, ALT= Alanine-aminotransferase, AST= Aspartate-aminotransferase, ALP= Alkaline- phosphatase, GGT= Gamma-glutamyl transferase, TB= Total bilirubin, CB= conjugated bilirubin, UB= Unconjugated bilirubin, CHOL= Cholesterol, UREA= Urea, GLU= Glucose.

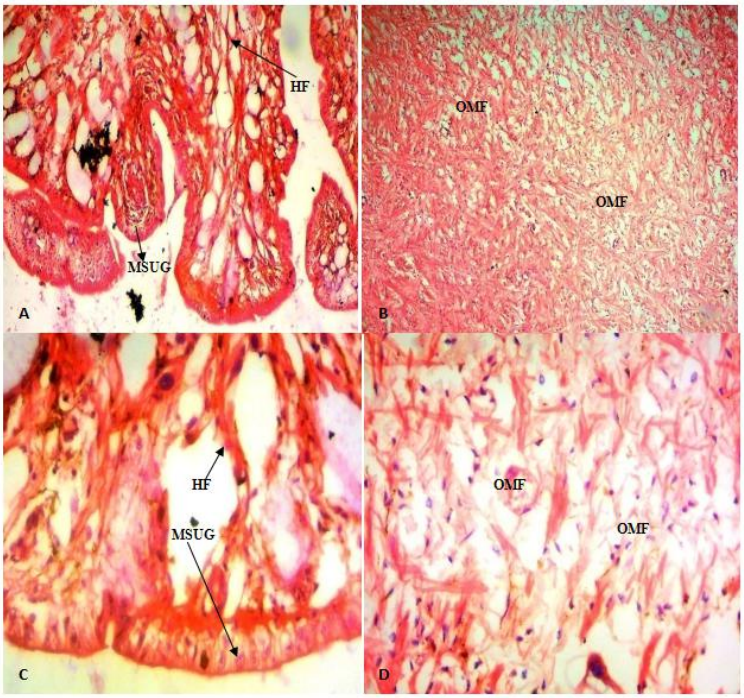

Figure 1: Histological sections of the foot of the giant African land snail (Archachatina marginata) in the control group after 2 weeks of exposure showing normal features: (A) hyaline fibers HF and mucous secreting unicellular glands MSUG x100; (B) oblique muscle fibers OMF x100; (C) hyaline fibers HF and mucous secreting unicellular glands MSUG x400; (D) oblique muscle fibers OMF x400

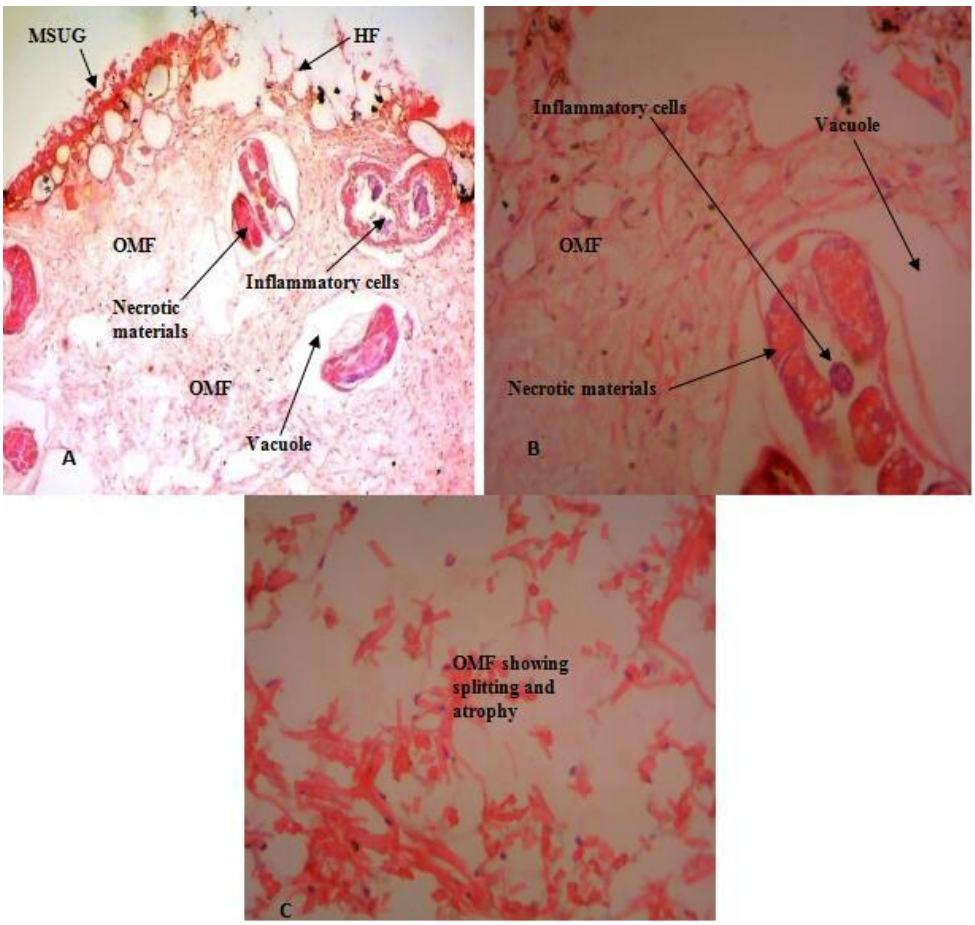

Figure 2: Histological sections of the foot of the giant African land snail (Archachatina marginata) in the pesticide treated groups after 2 weeks of exposure: (A) shrinkage and degeneration of mucous secreting unicellular glands MSUG and hyaline fibers HF, inflammatory cells enclosed in vacuoles with necrotic materials and vacuoles within oblique muscle fibers OMF x100; (B) vacuoles and necrotic areas within oblique muscle fibers OMF, inflammatory cells and necrotic materials enclosed in a vacuole within oblique muscle fibers OMF x400; (C) splitting and atrophy of oblique muscle fibers OMF x400

Assessment of Histological Alterations in the Foot Region:

To assess histological alterations in the foot region of snails exposed to control and experimental conditions, tissues were processed, sectioned, stained for general morphology and viewed microscopically. Representative control and pesticide treated sections of the foot region are shown in Figures 1 and 2 respectively.

4. Discussion

Evaluation of AST:

Extracts from the foot region of the giant African land snail (*Archachatina marginata*) exposed to control and pesticide-treatment conditions for two weeks were used to evaluate the activity of AST. Increased AST activity was observed in the pesticide-exposed snails. This increase was however not pesticide dose-dependent. As shown in Table 1, after two weeks of exposure, level of AST in the control snails was 0.58±0.02 mg/dl. With the exception of snails exposed to 4 mg of pesticide that had a value of 0.09±0.01 mg/dl, all the other pesticide-exposed snails had elevated values with snails exposed to 2 mg of pesticide being the highest at 122.32±1.14 mg/dl and snails exposed to 1 mg of pesticide being the lowest at 11.01±0.01 mg/dl. This agrees with previous reports in literature [30-31] which observed increased AST activity in land snails exposed to laboratory controlled pesticide induction and water snails harvested from a polluted lake respectively. Although statistical analysis of our result did not indicate significant difference ($P>0.05$), values

obtained however suggests possibility of liver dysfunction.

Evaluation of ALT:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and potassium chlorate treatment conditions for two weeks were used to evaluate ALT activity. As shown in Table 1, after two weeks of exposure, the level of ALT in the control snails was 183.97±0.03 mg/dl. However, all snails exposed to potassium chlorate exhibited none dose-dependent reduced ALT level with snails exposed to 2 mg of potassium chlorate being the lowest at 0.59±0.01 mg/dl and snails exposed to 1mg of potassium chlorate being the highest at 133.99±0.04 mg/dl. This is contrary to reported increased ALT activity in land snails exposed to laboratory controlled pesticide induction and water snails harvested from a polluted lake respectively [30-31]. Statistical analysis of our result indicates significant difference in mean of ALT level (P≤0.05). This is further indicative of possible liver dysfunction.

Evaluation of ALP:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and pesticide treatment conditions for two weeks were used to evaluate ALP activity. A dose-dependent specific pattern of response to pesticide treatment was observed. As shown in Table 1, after two weeks of exposure, value of ALP in the control snails was 5103.02±0.03 mg/dl. A progressive decline in the value of alkaline phosphatase as concentration of pesticide increased in the treated snails was observed with snails exposed to 1 mg of pesticide being the highest at 4304.01±0.01 mg/dl and snails exposed to 10 mg of pesticide being the lowest at 496.01±0.01 mg/dl. This is at variance with previous reports [30-31] which observed increased ALP activity in land snails exposed to laboratory controlled pesticide induction and water snails harvested from a polluted lake respectively. The difference in our values were statistically significant (P≤0.05) and suggestive of liver dysfunction.

Evaluation of Urea:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and pesticide treatment conditions for two weeks were used to evaluate the level of urea. As shown in Table 1, after two weeks of exposure, value of urea in control snails was 31.68±0.59 mg/dl. However, in the pesticide exposed snails, the pattern of response to pesticide treatment was observably ambiguous. Further observations show that snails exposed to 1 mg, 8 mg and 10 mg of pesticide exhibited reduced urea levels with those exposed to 10 mg of pesticide being the lowest at 7.00±0.01 mg/dl. On the other hand, snails exposed to 2 mg and 4 mg of pesticide exhibited elevated urea levels with those exposed to 2 mg of pesticide being the highest at 41.01±0.01 mg/dl. This is in contrast to a report [31] which observed unequivocal increase in urea level in water snails harvested from a polluted lake. The differences in our result were however statistically significant (P≤0.05) and points to possible liver dysfunction.

Evaluation of TB, CB and UB:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and pesticide treatment conditions for two weeks were used to evaluate the level of total bilirubin, unconjugated and conjugated bilirubin. No specific pattern of response to pesticide exposure was observed for the three parameters. As shown in Table 1, after two weeks of exposure, value of total bilirubin in the control snails was 0.19±0.01 mg/dl. In the pesticide-treated snails however, it was observed that snails exposed to 1 mg and 10 mg of pesticide had slightly elevated values at 0.29±0.01 mg/dl and 0.50±0.01 mg/dl respectively, while snails exposed to 2 mg and 8 mg of pesticide had slightly reduced values at 0.09±0.01 and 0.10±0.01 mg/dl respectively. Levels of CB and UB in the control snails were both 0.09±0.01 mg/dl. Similar to what was observed in the case of TB, snails exposed to pesticide treatments exhibited haphazard changes. This is in contrast to a report [31] which observed a general increase in the level of these parameters in water snails harvested from a polluted lake. Differences in our result in the case of TB and CB respectively were statistically significant (P≤0.05; P≤0.05) but not statistically significant in the case of UB (P>0.05). These results indicate possible liver dysfunction.

Evaluation of TP, Albumin and Globulin:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and potassium chlorate treatment conditions for two weeks were used to evaluate the level of total protein, albumin and globulin. No specific pattern of response to pesticide treatment was observed. As shown in Table 1, after two weeks of exposure, level of total protein in the control snails was 0.67±0.03 mg/dl. A slight elevation to 0.81±0.01 mg/dl was observed in snails exposed to 1 mg of potassium chlorate. It was further observed that the level of total protein in snails exposed to 2 mg, 4 mg, 8 mg and 10 mg of potassium chlorate respectively were lower than in the control snails. Similarly, no specific pattern of response to potassium chlorate treatment was observed for albumin. While the level of albumin in the control snails was 0.09±0.01 mg/dl, this was elevated to 0.51±0.02 mg/dl and 0.21±0.01 mg/dl in snails exposed to 1mg and 10 mg of potassium chlorate respectively. Conversely, it was observed that the level of albumin in snails exposed to 2 mg, 4 mg and 8 mg of potassium chlorate remained unchanged from that of the control snails. A specific pattern of response to pesticide treatment was observed for globulin. While the level of globulin in control snails was 0.59±0.01 mg/dl, a reduction in its level was observed in all the snails exposed to potassium chlorate with those exposed to 8 mg and 10 mg of potassium chlorate being the lowest at 0.09±0.01 mg/dl and snails exposed to 4 mg of potassium chlorate being the highest at 0.38±0.03 mg/dl. In a report [31], a general increase in the level of these parameters in water snails harvested from a polluted lake was observed. Another report [30], also observed an increase in the level of TP in land snails exposed to laboratory controlled pesticide induction. In our result, while differences in total protein and globulin values respectively were statistically significant ($P \leq 0.05$; $P \leq 0.05$), differences in the values obtained for albumin were not statistically significant ($P > 0.05$). The results obtained are however indicative of possible liver and kidney dysfunction.

Evaluation of Cholesterol:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and pesticide treatment conditions for two weeks were used to evaluate the level of cholesterol. As shown in Table 1, after two weeks of exposure, value of cholesterol in control snails was 53.91±0.03 mg/dl. However, in all the pesticide-exposed snails, a non-dose dependent specific pattern of response to pesticide treatment was observed. There was a general reduction in cholesterol level in the pesticide-exposed snails, with snails exposed to 2 mg of pesticide being the lowest at 14.01±0.01 mg/dl and snails exposed to 1 mg of pesticide being the highest at 34.02±0.02 mg/dl. This is at variance with a report [30] which observed increased cholesterol level in land snails exposed to laboratory controlled pesticide induction. The differences observed in our result were statistically significant ($P \leq 0.05$) and indicates possible liver dysfunction.

Evaluation of Glucose:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and pesticide treatment conditions for two weeks were used to evaluate the level of glucose. As shown in Table 1, after two weeks of exposure, the level of glucose in the control snails was 20.98±0.03 mg/dl. However, in the pesticide-exposed snails, a specific pattern of response to pesticide treatment was observed though not in a dose-dependent fashion. All the snails in the pesticide-exposed groups exhibited elevated glucose levels. It was further observed that snails exposed to 8 mg of pesticide had the highest glucose level at 40.01±0.01 mg/dl and those exposed to 4 mg of pesticide had the least at 27.01±0.01 mg/dl. This is in agreement with a report [31] which observed increased glucose level in water snails harvested from a polluted lake. The difference in our result was statistically significant ($P \leq 0.05$) and suggests possible liver dysfunction.

Evaluation of GGT:

Extracts from the foot region of the giant African land snail (Archachatina marginata) exposed to control and pesticide treatment conditions for two weeks were used to evaluate activity of GGT. A specific pattern of response to pesticide treatment was observed. As shown in Table 1, after two weeks of exposure, the level of GGT in the control snails was 66.95±0.09 mg/dl. A general reduction in its level was observed in all pesticide-exposed snails, with the lowest being 13.01±0.01 mg/dl in the snails exposed to 4 mg of

pesticide. This is contrary to a report [31] which observed a non-specific pattern of GGT activity in water snails harvested from a polluted lake. The differences observed in our result were statistically significant ($P \leq 0.05$) and indicates a possible liver dysfunction.

Histological Alterations in the Foot Region:

Figures 1 and 2 are histological sections of the foot region of the giant African land snail (Archachatina marginata) after two weeks of exposure to experimental conditions. While Figure 1 contains four representative photomicrographs (A-D) showing the normal features of the foot region in the control group, Figure 2 on the other hand contains three representative photomicrographs (A-C) showing the histological alterations observed in pesticide-exposed snails. Figure 1-A shows normal hyaline fibers and mucous secreting unicellular glands (mag. x100), while Figure 1-B shows normal oblique muscle fibers (mag. x100), furthermore Figure 1-C shows normal hyaline fibers and mucous secreting unicellular glands (mag. x400), and Figure 1-D shows normal oblique muscle fibers (mag. x400). In contrast, Figure 2-A shows shrinkage and degeneration of mucous secreting unicellular glands and hyaline fibers, inflammatory cells enclosed in vacuoles with necrotic materials and formation of vacuoles within oblique muscle fibers (mag. x100), while Figure 2-B shows vacuoles and necrotic areas within oblique muscle fibers, inflammatory cells and necrotic materials enclosed in a vacuole within oblique muscle fibers (mag. x400). Furthermore Figure 2-C shows splitting and atrophy of oblique muscle fibers (mag. x400). The observed histological derangements increased in severity in a dose-dependent pattern. This agrees with a report [31] which observed all the afore-mentioned histological derangements in water snails exposed to untreated waste from industrial, domestic and agricultural activities in a lake.

5. Conclusions

Going by the biochemical and histological observations made in this study, it is convenient to conclude that potassium-chlorate (a non-proprietary pesticide agent) produced deleterious toxic effects on non-target organisms such as the giant African land snail (Archachatina marginata) and this may be replicated over time in humans in the course of using such chemical formulations. In addition, consumption of such organisms should generate concern as this may predispose to serious public health hazards.

Competing Interests

The authors declare that there are no competing interests whatsoever.

Author's Contributions

Research concept and design, assembly, analysis and interpretation of raw data; statistical analysis and writing of article were done by KCO. Laboratory experiments were done by KCO, TGO and ASA. Critical revision and final approval of the article was done by KCO, TGO, ASA and TEA.

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